

Understanding maize root physiology and whole plant response to nitrogen and hormone pulse

Findimila Dio Ishaya

Thesis submitted to the University of Nottingham for the degree of Doctor of Philosophy

July 2023

Abstract

Maize is widely grown for food, fodder, and biofuel. Its production requires high use of nitrogenous fertiliser for optimum productivity. But the total uptake of applied nitrogen fertiliser by cereal crops is believed to be less than 50 percent of the total amount applied. The remaining percentage constitutes environmental hazards to the environment and reduces the farmers profit margin. With this regard, several research has been conducted to address these problems, but with minimal success. It is not clear the real time depletion rate of applied nitrogen fertiliser at the background soil or adjacent to root types. Moreso, efforts to improve root uptake were faced with the challenge of how to successfully apply hormones to adults' plants growing on soil.

To solve the problem, this research aimed to determine the amount of applied nitrogen fertiliser available for uptake by maize roots, by finding the rate at which nitrate and ammonium from applied nitrogen deplete adjacent to root types and the background soil, in plants growing on low and high nitrogen background. It also aimed to determine the net uptake by maize crown and seminal roots during low and high nitrogen supply. Since the root uptake activities affects the shoot, the study examined the maize shoot response to root nitrogen dynamics during microdialysis at the short and long run. Maize has four root types which includes the primary and seminal roots (Embryonic roots), and the crown and brace roots (Post embryonic adventitious roots). The study explored the method of applying phytohormones to adult maize plants growing on soil and employed the best method to apply auxin to induce brace root emergence.

Maize plants were grown in soil inside rhizobox as low and high nitrogen plants in the glasshouse. At 21 days post germination, each rhizobox was opened and sprayed with one litre of either high or low nitrogen solution. Microdialysis technique was used to sample for available nitrate and ammonium ions at the background soil and adjacent to root types, at time sequence for up to 44 hours in the longest experiment out of three experiments. The samples were analysed using the calorimetric techniques. Stable isotopes of nitrogen ^{15}N were used to determine net influx by intact maize crown and seminal roots. PhotosynQ (Multispec), Dualex and SPAD were used to monitor shoot response to root nitrogen dynamics. Other shoot variables such as plant heights, leaf length and width for leaf area were measured weekly using tape and photographs for image analysis at the end of the experiment. Leaf numbers were counted weekly, shoots and roots fresh and dry weights were obtained at the end of each experiment. Allometric related techniques were used to obtain biomass partitioning patterns as influenced by nitrogen rates and resupply as well as the two ontogenetic phases. In order to explore the most effective method of applying hormones to adult maize plant growing on soil, gibberellins dissolved in water, and also in lanoline at 50µM and 100 µM and applied to scratched stem of maize

plants. The 100 µM in water became the earliest effective means and was then used to apply auxin to stem of adult maize at 8 fully expanded leaf stage.

Findings showed that ammonium rapidly disappeared from the soil surface as compared to nitrate. The occurrence was earlier during low nitrogen condition. Crown root captured more ammonium during perceived low external nitrogen while the seminal roots capture more nitrate during adequate nitrogen supply. In terms of shoot response, low nitrogen availability caused warmer canopy and enabled dissipation of excess captured solar radiation, but the quantum yield of the photosystem was not affected. The leaf area duration was higher during the onset of reproductive stage as compared to the vegetative stage while high nitrogen enabled allocation of more biomass to the stem as compared to leaves and roots in low N plants. The applied auxin enabled increased emergence of brace root whorls and number in both low and high nitrogen plants.

In summary, soil nitrogen varies with space and time even in controlled environment. This affect availability of nitrogen forms for plant uptake. The concentration of nitrogen and frequency of application influence the uptake rate of nitrogen forms by different maize root types. Maize plants on low nitrogen nutrition can photosynthesise at similar rates to plants growing on adequate nitrogen supply. Auxin can be applied exogenously on adult maize growing on soil to induce the emergence of brace roots. The implication is that soil test should be carried out before fertilizer application, maize plants can be grown on low nitrogen and supplied with a dose of high N intermittently to boast uptake. The maize crown roots can be optimised for low nitrogen nutrition and the emergence of brace roots can be enhanced by auxin.

Executive Summary

This research focused on the spatio-temporal dynamics of nitrogen forms in relation to real time depletion rates of applied nitrogen, at the background soil without visible roots and adjacent to maize root types. Measurements were carried out on a defined time sequence for up to 24 and 44 hours for the shortest and longest experiment respectively. The depletion rates adjacent to root types may not be the same as uptake rates by plant. So, the uptake capabilities of maize crown and seminal roots were determined after 24 or 44 hours of receiving pulses of nitrogen at low and high concentration. The roots nitrogen dynamics can affect the short-term metabolism and long-term morphology of plants. So, the shoot response was also determined to enable understanding of whole plant response to soil nitrogen dynamics. It is well known that phytohormones such as auxin influences transcriptions in relation to root developments in plants. But how this hormone can be successfully applied to adult maize grown on soil is unknown. To address this gap in knowledge, this study explored the most effective method, and applied auxin to maize plant growing on soil at the eight (8) fully expanded leave stage to induce brace root emergence.

The real time depletion rates of nitrate and ammonium ions from applied nitrogen was monitored in time sequence using a microdialysis technique in the glasshouse. The method utilises osmosis to allow for sampling of dissolved soil nutrient ions in real time with minimal soil disturbance. The obtained samples were analysed for nitrate and ammonium ion concentration using calorimetric techniques. The nitrogen uptake capabilities of maize crown and seminal roots were traced with stable isotopes. Key findings suggest ammonium rapidly disappeared from the soil surface as compared to nitrate especially during low nitrogen conditions, and ammonium was the preferred nitrogen form as compared to nitrate by intact maize roots during low nitrogen condition. In terms of root type uptake of nitrogen sources, the crown root captured more ammonium during perceived low nitrogen condition while the seminal roots capture more nitrate during adequate nitrogen condition. The shoot response from photosynQ suggest that the quantum yield of the photosystem II was not affected by low nitrogen condition and a high N pulse increased the photosynthetic efficiency of low N plants. The leaf area duration obtained from measured leaf length and width was higher during the reproductive stage than the vegetative stage regardless of nitrogen treatment. Meanwhile scratching of the stem lower nodes of adult plants and applying 100 µM of auxin in water can induce the emergence of maize brace roots.

Based on the findings from the real time depletion rates, root type uptake rates and whole plant response, it is recommended that the suitability of the rhizobox for nutrient related experiment should be investigated. The ratio of ammonium to nitrate in a combined fertiliser should be done according

to the soil type after soil testing. High density planting of maize varieties that have high affinity for ammonium is needed to increase uptake and minimise rapid ammonium loss. The maize crown roots can be the roots to be optimised for low nitrogen soils. Maize should be grown on low nitrogen and be given a pulse of high nitrogen to improve uptake since the photosynthesis rate is not affected by low soil nitrogen, and a pulse of high N can immediately increase the photosynthetic efficiency for photosystem II. Auxin inducement of the emergence of maize brace roots offers opportunity of manipulating maize root architecture to improve capture of soil resources. Researchers can employ auxin application methods in the study of genes and other molecular players in mutants that are involved in brace roots development for the optimisation of root function.

Further work proposed include factorial experiments involving water and nitrogen rates to monitor depletion rates of N sources, quantity, and quality of sapflow. The same factorial design could be used to study the interaction between nitrogen rate and auxin on brace root emergence and growth.

Publications from the work

[Ishaya FD and Rasmussen A (2020) Advances in understanding plant root growth regulators. Chapter IN: *Gregory, P. J. (ed.), Understanding and improving crop root function, Burleigh Dodds Science Publishing, Cambridge, UK (ISBN: 978 1 78676 360 0).*

Rasmussen A, Dobrijevic DP, Ola A, Ishaya FD, Lovelock CE (2019) Aerial root physiology: Reaching for the sky or down to earth? Annual Plant Reviews, Issue 3. https//doi.org/10.1002/9781119312994.apr0668. Contribution: I searched for relevant literature in relation to maize roots physiology.

Papers in preparation for publishing:

Exogenous application of auxin (IAA) at maize stem nodes increased brace root whorls and number. To be published in Plant biology.

The use of rhizobox for root nutrient study could be a paradox. To be published in Plants direct.

Soil nitrogen fluxes and net uptake by intact maize crown and seminal roots shows differences in uptake rates of ammonium and nitrate. To be published in plant direct.

Acknowledgements:

First and foremost, I would like to express my sincere gratitude to God for the start and completion of this PhD journey. A big thank you to the University of Nottingham Vice-Chancellors Scholarship for Research Excellence (International), for offering me the opportunity to fulfil my dream of earning a world class PhD by funding my PhD tuition. I like to appreciate TetFund Nigeria, for the 3 years stipends offered in support of my PhD. I must appreciate the Rank prize for their financial assistance after Covid lockdown. A big appreciation to Dr Saidu Umaru Jen, a father and an academic mentor for the encouragement and support to advance in my academic career.

A special thanks to Dr Amanda Rasmussen (my main and only supervisor) for giving me the opportunity to work on this project, providing valuable guidance and feedback, and challenging me to grow as a scientist. Indeed, you have offered unlimited support and unconditional guidance during my PhD journey. I would like to extend my thanks to all members of AR lab for being awesome. Your wilful assistance during research, and feedback from writing retreats, these made my PhD journey less stressful. I hope we continue in this manner to build solid teamwork attitude and make the world a better place for all.

I must thank Prof Erik Murchie for assisting me with PhotosynQ to take measurements when our lab photosynQ was unavailable for use. I would also like to extend my thanks to Dr Dylan Jones and Dr Daren Wells for assisting to produce a 3 dimensional quantitative and qualitative anatomy of the brace roots through Laser Ablation Tomography. In addition, I would like to mention Dr Mark Meacham and the SB glasshouse team for technical support in times of need while working in the glasshouse. Mr John Alcock and Matthew Tovey from the SB farm office for assisting with the storage and supply of sugar beet topsoil used for my last experiment.

My special appreciation to my examiners Prof Miriam Gifford (external examiner), Prof Debbie Sparks and Dr Alexandra Burgess (internal examiners) for taking time to read through the thesis and giving constructive feedback to enable me advance in the field of crop research.

I want to thank all members of academic staff and post graduate research students in Gateway building and school of Biosciences at Sutton Bonington, for being a great. You people were always there for discussions about anything that I was unsure of.

Finally, but most importantly, I would like to thank my husband Mr Ishaya Albert, Children: Gilead Ishaya, David Ishaya and Serah Ishaya for making all these years much more enjoyable and keeping me sane throughout the whole process. Thank you for your love, prayers, and support, you are greatly treasured in my heart. I love you greatly!!!

Dedication:

I dedicate this PhD to my father Mr Christopher Jiniya Dio for the good upbringing and for laying the foundation for my academic growth. But whose PhD research in Geological sciences was disrupted twice by his supervisor incapacitation in 1996, and again 2017 due to sight impairment.

Table of Contents

Abstract……

Executive summary……I

Publications……III

Acknowledgement……IV

Dedication……V

Table of content……VI

Table of Figures……XII

Table of tables……XVII

Thesis structure……XVIII

Chapter 1……1

1.1. Introduction……2
1.2. Plant nitrogen balances……4
1.2.1. Forms of inorganic nitrogen fertilizer, uptake and transport in plants……4
1.2.1.1. Nitrate and ammonium uptake……4
1.2.1.2. Nitrate and ammonium assimilation……6
1.2.1.3. The concept of nitrogen use efficiency (NUE)……8
1.3. Plant root and root system architecture……8
1.3.1. The maize root system……9
1.3.1.1. Different areas of maize roots absorb different mineral ions……11
1.3.1.2. Cell types that form the maize individual root……11
1.3.1.3. The maize crown root……14
1.3.1.4. The maize brace root……16
1.3.1.5. The maize primary and seminal root……17
1.3.1.6. Nitrogen uptake by maize root types……19
1.3.1.7. Root traits affecting nitrogen uptake and utilization efficiency……19
1.3.2. The role of hormones in the control of root architecture by nutrient signals……20
1.3.3. Recently discovered master regulators of molecules……24

1.4. Research Aims and Objectives……25
1.5. References……26

Chapter 2……34

2.1. Abstract……35

2.2. Introduction……36

2.3. Methods……38

2.3.1. Experimental design……38

2.3.2. Plant material and growth conditions for all the 3 microdialysis experiment……44

2.3.3. Microdialysis for sampling soil nutrient ions……47

2.3.4. Nitrate and ammonium assay……50

2.3.5. Measuring roots nitrogen uptake capacity……51

2.3.6. Data analysis……54

2.4. Results……55

2.4.1. Nitrogen depletion at the background and adjacent to roots as uncovered by microdialysis……55

2.4.2. Net effect of nitrate and ammonium uptake by intact maize crown and seminal roots……70

2.5. Discussion……76

2.5.1. The spatiotemporal dynamics of nitrogen forms in relation to soil background and root types……77

2.5.2. The net effect of nitrate and ammonium uptake by intact maize roots……80

2.5.3. Extend of genetic variation in crown roots traits……83

2.5.4. Conclusion ……85

2.5.5. References……87

Chapter 3……91

3.1. Abstract……92

3.2. Introduction……93

3.3. Methods……95

3.3.1. Above the ground plant measurement……95

3.3.2. Measurements of chlorophyll and other photosynthetic parameters……96

3.3.3. Comparison of Dualex and PhotosynQ readings for photosynthetic apparatus……97

3.3.4. Data analysis……98

3.4. Results……98

3.4.1. Short-term effect of nitrogen on maize shoot……98

3.4.2. Long-term effect of nitrogen on maize shoot……113

3.5. Discussion……128

3.5.1. Soil nitrogen level and the chlorophyll content of maize leaves…… *128*

3.5.2. Soil nitrogen level and maize leaf temperature differential……129

3.5.3. Soil nitrogen level and photoprotection (ϕNPQt)……130

3.5.4. Nitrogen availability and the relative allocation of energy……131

3.5.5. Nitrogen availability and the linear electron flux……131

3.5.6. Nitrogen availability and the efficiency of Photosystem II……133

3.5.7. Nitrogen availability and flavonoid contents of maize leaves……133

3.5.8. Nitrogen availability and the relative growth rates of maize plants……134

3.5.9. Nitrogen availability and estimated net assimilation rate of maize leaves……135

3.5.10. Nitrogen availability, Ontogeny, and the leaf area duration……136

3.6. Conclusion……137

3.7. References……139

Chapter 4……144

4.1. Abstract……145

4.2. Introduction……146

4.3. Methods.....147

4.3.1. Root and shoot photographs for image analysis.....147

4.3.2. Fresh and dry weights (Biomass).....147

4.3.3. Root to shoot ratio.....148

4.3.4. Calculation of biomass allocation to functional plant organs.....148

4.3.5. Calculation of the components of leaf anatomical traits.....149

4.3.6. Data analysis.....149

4.4. Results.....149

4.4.1. Biomass analysis of maize plants after 5 weeks of high N-pulse.....149

4.4.2. Biomass partitioning to organs during the two ontogenetic phases.....155

4.5. Discussions.....161

4.5.1. The root-to-shoot ratio.....161

4.5.2. Percentage biomass allocation to functional organ.....162

4.5.3. Moisture contents of shoots and roots.....164

4.5.4. Nitrogen uptake during maize reproductive phase.....165

4.5.5. Nitrogen rates and the structural characteristics of maize leaves.....166

4.6. Conclusion.....168

4.7. References.....168

Chapter 5.....173

5.1. Abstract.....174

5.2. Introduction.....175

5.3. Methods.....177

5.3.1. Gibberellins (GA) trial.....178

5.3.1.1. Experimental design.....178

5.3.1.2. Plant material and growth conditions……178

5.3.1.3. Shoot and root measurement……181

5.3.2. Auxin (IAA) trial……181

5.3.2.1 Experimental design for auxin trial……181

5.3.2.2. Plant material and growth conditions……183

5.3.2.3. Shoot measurement……186

5.3.2.4. Final harvest……186

5.3.3. Data analysis……186

5.4. Results……187

5.4.1. Effects of gibberellins on maize heights and brace root number……187

5.4.2. Effects of auxin on maize shoot and brace root number……

5.5. Discussion……198

5.5.1. The importance of the new phytohormone treatment method uncovered……198

5.5.2. Auxin and shoot growth parameters of maize……200

5.5.3. The influence of auxin (IAA) on brace root whorls and number……202

5.5.4. Optimizing maize root system architecture for low nitrogen soils……208

5.6. Conclusion……211

5.7. References……211

Chapter 6……217

6.1. Abstract……218

6.2. Introduction……219

6.3. Summary of discussion in relation to research aims……222

6.4. Comparison of the performance of the two maize lines used in the study……224

6.5. Contribution to knowledge……225

6.6. Wider implication of the study……..225

6.7. Future work…….226

6.8. Conclusion……227

6.9. References……229

Table of Figures

Chapter 1

Figure 1.1. Different maize root types and the various nutrient ion absorption site……..page 10

Figure 1.2. Cross sectional representation of maize crown root cells…….page 13

Figure 1.3. The molecular and hormonal pathways of crown and brace root development in maize……page 18

Figure 1.4. Summary of how nitrogen availability promotes or supresses root initiation and growth in maize……page 23

Chapter 2

Figure 2.0.A. Experimental design for high nitrogen resupply experiment ……page 39

Figure 2.0.B. Experimental design for normal nitrogen resupply experiment…….page 40

Figure 2.0.C. Experimental design for factorial niotrogen resupply experiment……page 42

Figure 2.1. Growth conditions……..Page 46

Figure 2.2.A. Microdialysis set-up……page 48

Figure 2.2.B. Probe positioning during microdialysis……page 49

Figure 2.3.A. Stable isotope uptake experiment immediately after 44 hours of soil nutrient sampling……..page 51

Figure 2.3.B. Stable isotope uptake experiment of control plant……page 52

Figure 2.4. Soil nitrate availability rapidly decreases in rhizobox soils……page 57

Figure 2.5. Soil Ammonium availability rapidly decreases in rhizobox soils……page 58

Figure 2.6. Soil nitrate and ammonium availability rapidly decreases in rhizobox soils. The graph is the summary of Figure 2.4. and Figure 2.5……page 59

Figure 2.7. Soil ammonium rapidly disappeared from rhizobox surface within one hour of spraying low N solution on the rhizosphere of low N plants (A), and high N solution on high N plants (B)……Page 63

Figure 2.8. Soil ammonium rapidly disappeared from rhizobox surface irrespective of nitrogen background and re-supply while nitrate availability to roots varied with nitrogen background and nitrogen re-supply over time……page 65

Figure 2.9. Nitrate and ammonium uptake rates were influenced by nitrogen background and concentration of N re-supply after 24hrs (Figure A) and 44hrs (Figure B)……page 71

Figure 2.10. Nitrate and ammonium uptake rate by maize crown and seminal roots were influenced by nitrogen background……..page 73

Figure 2.11. Nitrate and ammonium uptake by maize crown and seminal roots showed a weak tendency to correlate with their root diameter after a switched nitrogen re-supply…..page 75

Figure 2.12. Summary of methods and findings in Chapter 2……page 86

Chapter 3

Figure 3.0. The correlation chart used to predict the values of chlorophyll measured with Dualex from the photosynQ readings and vice versa ……page 97

Figure 3.1. Chlorophyll related parameters were greatly influenced by nitrogen background and high nitrogen resupply……page 99

Figure 3.2.A. Opening of rhizobox increased the maize leaf temperature differential……page 102

Figure 3.2.B. Nitrogen background and the concentration of nitrogen supplied influenced the maize leaf temperature differential……page 102

Figure 3.3.A. The opening of rhizobox and spraying with N solution did not cause immediate change in ϕNPQ……..page 104

Figure 3.3.B. Nitrogen background influenced the rate of ϕNPQ in 3 days……..page104

Figure 3.4.A. High nitrogen condition increased the thermal dissipating mechanism of leaves (ϕPhiNO) regardless of nitrogen background……page 106

Figure 3.4.B. Nitrogen background and re-supply slightly influenced the partitioning of energy within three days of root-soil nitrogen interaction……..page 106

Figure 3.5. The linear electron flux was not affected by growth condition but slightly affected by the concentration of nutrients resupplied……page 108

Figure 3.6. Nitrogen background and re-supply did not affect the efficiency of the photosystem II shortly after root soil nutrient interaction (A) or within the 3 days of root-soil nutrient sampling……..page 110

Figure 3.7. Nitrogen stress increased the flavonoid contents of maize leaves……page 112

Figure 3.8. The influence of nitrogen background and nitrogen pulse on major shoot growth parameters over time……page 114

Figure 3.9. The effects of nitrogen background and nitrogen pulse on major shoot growth parameters during the vegetative and onset of reproductive stage……page 117

Figure 3.10. The effects of nitrogen background and nitrogen pulse on major shoot growth parameters during the vegetative and onset of reproductive stage……page 118

Figure 3.11. The rate of increase in leaf area of all measurable leaves at vegetative and reproductive stages, following nutrient pulse application……page 121

Figure 3.12. Maize plants expand their middle and upper canopy leaves during high nitrogen availability, especially at the onset of reproduction……page 123

Figure 3.13. Chlorophyll values of all measurable leaves at the vegetative and reproductive stage, following nitrogen pulse application……page 126

Figure 3.14. Chlorophyll contents of lower leaves in low nitrogen plants decreased over time while adequate nitrogen supply maintained leave chlorophyll content……page 127

Figure 3.15. Summary of findings from Chapter 3……page 138

Chapter 4

Figure 4.1. Nitrogen resupply did not affect the root : shoot ratio or moisture contents. ……page 151

Figure 4.2. Low nitrogen background increased the stem biomass while high N resupply reduced the leaf biomass as compared to the unsprayed plants regardless of nitrogen background. ……page 153

Figure 4.3. Nitrogen background and high nitrogen pulse influenced the anatomical properties of maize assimilatory systems……page 155

Figure 4.4. Nitrogen background and stage of development affected the shoot and roots respective fresh and dry weights……..page 157

Figure 4.5. Nitrogen stress increased the root:shoot of maize plants during their vegetative stage……..page 158

Figure 4.6. Effects of limited nitrogen supply on biomass partitioning……page 160

Chapter 5

Figure 5.1. Experimental design for gibberellin (GA) treatment……page 179

Figure 5.2. Plant height a week after GA treatment……..page 180

Figure 5.3. Experimental design and layout of auxin trial……..page 182

Figure 5.4. Growth condition for the hormone trial……..page 184

Figure 5.5. Degree of scratching the lower stem node for IAA application……page 185

Figure 5.6. The percentage increase in plant height was influence by treatment method in low N plants……..page 188

Figure 5.7. The number of brace roots per whorls was not influenced by nitrogen background or treatment application method……..page 190

Figure 5.8. Auxin did not influence maize shoot growth irrespective of nitrogen background……..page 192

Figure 5.9. Nitrogen background and auxin did not significantly influence nitrogen balance index and flavonoid levels, but auxin changed chlorophyll level over time……..page 192

Figure 5.10. There is no effect of auxin on leaf area duration and the plants percentage shoot dry matter……page 195

Figure 5.11. Auxin increased the total number and whorls of brace roots in both high and low nitrogen plants……..page 196

Figure 5.12. The 3-Dimensional qualitative and quantitative view of the internal structure of maize brace roots after emergence……page 197

Figure 5.13. Differentially expressed IAA/Aux genes in root types of B73 inbred lines……..page 205

Figure 5.14. Optimized root architecture for improved nutrient uptake……page 210

Table of Tables

Chapter 2

Table 2.1. Nutrient resupply regimes during microdialysis.……page 43

Table 2.2. Treatment, types, and number of excised roots from each plant after ^{15}N uptake of intact maize roots……page 53

Table 2.3. Depletion rates of nitrogen forms at other sampling times compared to 0, 3 and 24 hours sampling time after high N resupply (represented in Figure 2.4, 2.5, and 2.6)……page 60

Table 2.4. Statistical comparison of the depletion rates of nitrate and ammonium between probes adjacent to crown roots and seminal roots, and the background soil at each sampling time after high nitrogen resupply (represented in Figure 2.4, 2.5, and 2.6)……page 61

Table 2.5. Significant differences in ammonium depletion rates at 0, 3, and 24 hrs each, as compared to other sampling times after factorial nitrogen resupply (represented in Figure 2.8, green panel (A, B, C, D))……page 66

Table 2.6. No significant difference in nitrate depletion rates at 3 hrs and 24 hrs each, as compared to other sampling time across root types and background after factorial nitrogen resupply (represented in Figure 2.8 orange panel (E, F, G, I))……page 68

Table 2.7. Statistical comparison of nitrate and ammonium depletion rates between probes adjacent to crown roots and seminal roots, and the background soil (far from roots) at each sampling time after factorial nitrogen supply (represented in Figure 2.8)……page 69

Chapter 3

Table 3.1. The significant differences in relative growth rates, net assimilation rates and leaf area duration over time, as influenced by nitrogen background and nitrogen resupply at 21 days post germination (represented in Figure 3.8)……page 115

Table 3.2. Statistical differences of 4^{th} – 13^{th} leaves of all plants as compared to the 8^{th}leaves of sprayed low N plants during the high nitrogen resupply experiment, in terms of leaf area duration and chlorophyl levels of leaves at each position on the stem nodes (Leaf area duration: Figure 3.11; Chlorophyll: Figure 3.13)……page 124

Thesis Structure

This thesis is composed of a mixture of papers in 'paper format' to be submitted as manuscript for publication.

Chapter 1 is a summary of the literature reviewed.

Chapter 2 explores the spatiotemporal dynamics of nitrogen forms at the background soil and adjacent to maize root types growing on low and high nitrogen background. It also measures the net nitrogen uptake by intact maize crown and seminal roots.

Chapter 3 describes the short-and-long term response of maize shoot including photosynthetic and growth parameters in relation to the roots and soil nutrients dynamics.

Chapter 4 investigates allocation of biomass to above the ground plant parts and below the ground plant parts using biomass data.

Chapter 5 explores the method of exogenous application of hormones to adult maize plants growing on soil and applied auxin successfully for the inducement of brace root emergence.

Chapter 6 discusses the project in a wider context of implications of findings.

Chapter 1

Introduction and Literature review

1.1. Introduction

Global human population is expected to rise to 9.8 billion by 2050, this will invariably cause increase in demand for food, fuel and fibre worldwide (Khosla, 2018). Thus, the sustainability of agricultural systems for a growing population is of huge concern (Daryanto et al., 2017).

Maize is a widely grown cereal crop for food, fodder, and biofuel (Gu et al., 2013). Its production requires high quantity of nitrogen fertiliser for optimum crop performance (Garnett et al., 2015). Global maize production was projected to be nearly 1.03 billion tonnes in 2017-2018 (STATISTICAL, 2018). But maize production is projected to decline by 3.8% consequence of climate change, if left unabated (Daryanto et al., 2017; Ramirez-Cabral et al., 2017). The unabated situation can cause intense hardship for peasant farmers since maize is central to their diets and the main stay of their traditional economy (Ramirez-Cabral et al., 2017). Only about 40-50% of the applied nitrogen fertiliser is taken up by cereals (Garnett et al., 2013), showing a pressing need to improve the uptake of nitrogen fertiliser. Therefore, any improvement in nitrogen uptake and use efficiency will bring significant monetary and environmental benefit.

The green revolution of the 1960s paved the way for food sufficiency using synthetic fertiliser, improved seeds through breeding programmes, irrigation facilities, farm machinery and crop protection measures (Powlson et al., 2010; Singh, 2000). This approach was geared toward increasing yield without many regards to the sustainability of production (Powlson et al., 2010). For instance, over 100 million tons of nitrogen fertiliser are used globally and annually in attempt to optimize crop performance with half being used on cereals (Garnett et al., 2015; Lupini et al., 2016). It was also reported that global total nitrogen fertilizer consumption grew from 112.5 million tons in 2015 to 118.2 million tons in 2019 (Sandhu et al., 2021) and it was predicted that the fertiliser consumption will double from 112 million tons in 2015 to about 236 million tons by 2050 (Sandhu et al., 2021). A remedy to the situation is required because there is no direct relationship between nitrogen applied and crop yield (Briat et al., 2020; Inman et al., 2005; Sandhu et al., 2021). The nitrogen fertiliser recovery of maize was reported to be 37% of the applied nitrogen fertiliser (Davies et al., 2020; Sandhu et al., 2021). Thus, amount to a waste of a greater percentage of the applied resource.

Excess soil nitrogen is loss through leaching, volatilization, denitrification, and surface run-off (Liu et al., 2014) causing eutrophication of water bodies, ground water contamination, soil acidification, soil biodiversity loss and build-up of chemical toxins affecting humans and animals (Hirel et al., 2007; Khosla, 2018; Li et al., 2015). Intensive resource input farming worldwide contributed to soil depletion, water scarcities and high level of greenhouse emissions resulting to climate change (Gastal. et al., 2015).

Changing the narratives requires an integrated approach in nitrogen management for improved nitrogen use efficiency. Indices for nitrogen use efficiency includes agronomy efficiency which is the increase in grain yield per unit nitrogen applied (Davies et al., 2020; Fageria, 2014; Sandhu et al., 2021). The physiological efficiency implying the biological yield including grains and straw obtained per unit of N captured by both grain and straw (Fageria, 2014; Sandhu et al., 2021). But nitrogen use efficiency is associated with soil N availability and the way plants utilised captured nitrogen, which is governed by ontogeny (processes and mechanisms relating to the lifetime development of an organism) (Masclaux-Daubresse et al., 2010). The interaction between local and systemic pathways offers understanding of plants responses to nitrogen availability (Nacry et al., 2013). Plant nitrogen use efficiency includes the fundamental uptake of N sources, reduction to useable forms, assimilation into amino acids, transfer of N metabolites and remobilization to reproductive tissues (Kant, 2018; Sandhu et al., 2021). Therefore, nitrogen use efficiency involves efficient capture of N sources and efficient utilisation of plant use of the captured nitrogen sources (Sandhu et al., 2021) .

Nitrate and ammonium are the predominant nitrogen source in agricultural lands (Dechorgnat et al., 2018; Hachiya and Sakakibara, 2017). The estimation of soil nitrate and ammonium could be a strategy to diagnose nitrogen status and resulting plant performance (Sandhu et al., 2021; Sharma et al., 2016). But most of the nitrogen nutrient uptake studies in maize are carried-out in hydroponics for maximum of 3 weeks only (Dechorgnat et al., 2018; Garnett et al., 2013; Jackson and Volk, 1992; Tian et al., 2008). And some studies focussed on the molecular aspect(Jackson and Volk, 1995; Yu et al., 2016a; Yu et al., 2015a; Yu et al., 2016b; Yu et al., 2015b) . There are fewer maize studies that considers both the root and shoot response to varying soil nutrient availability. According to literature, there are no studies on the real time dynamics of nitrogen depletion rates adjacent root types or how the shoot responds immediately to the rhizosphere change in nutrient availability. Also, it is unclear how to apply hormones on adult maize plants growing on soil. This undermines research in maize brace roots because the root emerges late between the period of transition from vegetative to reproductive stage, and this knowledge gap can limit selection of desirable root traits for plant breeding (Paez-Garcia et al., 2015), improvement of fertilizer and irrigation techniques as well as timing of resource applications for improved crop yield with minimal production cost. Measuring the physiological mechanisms of root nitrogen uptake and spatio-temporal nitrogen fluxes in soil solution is an effective approach towards improving nitrogen use efficiency. This is because a complementary approach in the improvement of maize germplasm for nitrogen use efficiency can be by increasing the nitrogen uptake efficiency and/or utilization efficiency (Sandhu et al., 2021; Wortmann et al., 2011), of which nitrogen utilization efficiency depends on the nitrogen uptake efficiency of plant roots (Andrews et al., 2013).

1.2. Plant Nitrogen Balances

Nitrogen is an important macronutrient required for plant growth. It is a primary constituent of nucleic acids, amino acids, and protein. Nitrogen is also important as a signalling molecule affecting diverse plant processes including lateral root growth, resistance to biotic and abiotic stress, regulation of seed germination and mediation of hormone signalling (Lupini et al.,2016; Dechorgnat et al., 2018). Nitrogen management in crops can be divided into two main phases occurring either successively or overlapping in different species. The first phase is the vegetative phase, where young developing roots and leaves serves as sink organs for the assimilation of inorganic N and the synthesis of amino acids obtained from captured N before flowering. These are later broken down through nitrate assimilatory pathway and are further utilised as building blocks of plant architecture and different aspect of photosynthesis (Hirel et al., 2007). Remobilization of accumulated N occurs after flowering. Both shoot and leaves act as source of nitrogen providing amino acid through hydrolysis of protein and are transferred to reproductive and storage organs such as seeds, bulbs or trunks (Hirel et al., 2007).

1.2.1. *Forms of inorganic nitrogen fertilisers, uptake and transport in plants*

Nitrates and ammonium are the two main forms of inorganic fertiliser used for enhancing crop yield (Plett et al., 2018). But nitrate is the predominant form used in most agricultural soil (Garnett et al., 2015; Plett et al., 2018) with varied concentration range of 0.5 to 10mM (Lupini et al., 2016). Nitrate is highly mobile in soil; thus, uptake depends more on root capacity (the capability of root to capture soil resource) than morphology (structure and form of the root) (Garnett et al., 2015). Ammonium being a less mobile ion in the soil as compared to nitrate (Garnett et al., 2015), is the preferred nitrogen source to support cell division in the meristem (Taiz and Zeiger, 2006). Its absorption consumes less energy than nitrate. This makes it easily available to the meristematic tissues which are mostly carbohydrate deficient (Taiz and Zeiger, 2006). Knowledge is limited regarding the genetic variability of maize uptake of nitrate or ammonium (Garnett et al., 2015).

1.2.1.1. Nitrate and ammonium uptake and transport

To cope with the spatial and temporal fluctuations of soil N, plants evolved very flexible and dynamic transport systems to acquire it (Lupini et al., 2016; Plett et al., 2018). This is achieved through two physiological mechanisms, the low affinity transport system (LATS) at higher nitrate concentration (>250µM) and the high affinity transport system (HATS) when nitrate concentration is low (<250µM) (Miller and Chapman, 2011; Hawkesford et al., 2012; Dechorgnat et al., 2018; Plett et al., 2018). However, Lupini et al., (2016) gave 3 classifications as: a constitutive ((cHATS) when no nitrogen is applied), an inducible ((iHATS) during nitrogen supply) high affinity transport systems (up to 500µM NO_3^-), and a low –affinity transport system (LATS) (higher than 500µM NO_3^-).

Nitrate transporters are divided into four classes: the Nitrate Transporter 1 family (NRT/NPF), Nitrate transporter 2 Family (NRT2/NAR2), CHLORIDE CHANNEL FAMILY (CLC), and the SLOW ACTION ANION CHANNEL-ASSOCIATED HOMOLOGUES (SLAC/SLAH) but only NRT1 and NRT 2 families are notably involved in root nitrate uptake (Lupini et al., 2016; Gu et al., 2018). NRT1 controls nitrate uptake in LATS range while NRT 2 contribute most to HATS activity (Garnett et al., 2015; Dechorgnat et al., 2018) with NRT1.1/NRT1.2 (NPF6.3/NPF4.6) and NRT2.1/NRT2.2/NRT2.4/NRT2.5 mainly arbitrating NO_3^- uptake (Plett et al., 2018). It was reported that HATS specifically NRT2.1 and NRT2.2 respond to plant nitrogen demand and contribute the bulk of total uptake at high NO_3^- concentration (>250µM) questioning the abilities of each uptake system (Garnett et al., 2013; Garnett et al., 2015; Plett et al., 2018).

NRT1.1(NPF6.3/CHL1) which is mainly expressed in the epidermis of Arabidopsis young root tips, is NO_3^- inducible and encodes a dual affinity nitrate transporter consisting of both LATS and HATS roles (Lupini et al., 2016; Plett et al., 2018). Its dual affinity for high and low nitrate depends on its phosphorylation status at the theonine101 residue. The phosphorylated type mediates auxin transport and NO_3^- uptake during low N (Han et al.,2017; Shahzad and Amtmann, 2017; Kant, 2018; Zhao et al., 2018). NRT1.1 can sense external NO_3^- availability and trigger the NO_3^- signalling network (Plett et al., 2018). In maize, ZmNPF6.3 has four putative homologs which are ZmNPF6.4 (ZmNRT1.1A), ZmNPF6.6 (ZmNRT1.1B), ZmNPF6.7 (ZmNRT1.1C) and ZmNPF6.5 (ZmNRT1.1D) (Plett et al., 2010; Dechorgnat et al., 2018). Each of these was shown to have different tissue specificity and transcriptional responses to nitrogen availability (Plett et al., 2018).

In contrast to NRT1.1, NRT1.2 (NPF4.6) is localized in both young root tips and mature regions of Arabidopsis root and is constitutively expressed (Plett et al., 2018). One orthologue of NRT1.2 has been identified for each sequenced cereal species, suggesting the possibility of conserved evolutionary roles (Plett et al., 2018). It was reported that ZmNRT1.2 did not show much difference in transcript levels in plants grown in low and high NO_3^- until the late reproductive phase where expression levels differ between treatments (Garnett et al., 2013; Plett et al., 2018).

NRT2.1 (NRT3.1/NAR2)and NRT2.2 were suggested to be a product of gene duplication as studies showed that they share 90.4% sequence identity and are localised in tandem on chromosome 1(Plett et al., 2018) in respective of the above similarities, AtNRT2.1 was shown to be the predominant part of the HATS under diverse circumstances with AtNRT2.2 contributing only little (Lupini et al., 2016; Plett et al., 2018). Nonetheless, the transcript level of AtNRT2.2 increased contributing more to HATS when AtNRT 2.1 was knocked out. Similar mechanisms were also recorded in cereals (Plett et al., 2018). In most angiosperms, NRT2.1 transports nitrates in company of a partner, NRT2.1

(NRT3.1/NAR2)(Lupini et al., 2016; Gu et al., 2018). For example, OsNRT2 family genes (OsNRT2.1, OsNRT2.2, OsNRT2.3, OsNRT2.5) partner with (OsNAR 2.1) to control rice nitrate acquisition at various nitrate concentrations (Gu et al., 2018).

AtNRT2.4 is expressed in both epidermis of lateral roots and shoot tissues with affinity for nitrate during NO_3^- starvation, implying a role in both root and shoot during NO_3^- deficiency (Plett et al., 2018). AtNRT2.5 is in the epidermis and cortex of roots at the root hair zone and is activated during NO_3^- stress but is repressed byNO_3^- in both Arabidopsis and maize (Plett et al., 2018).

The Ammonium uptake and transport are mediated by the proteins of the ammonium transporter/methylammonium permease/rhesus (AMT/MEP/Rh) family, either via NH_4^+ uniport or NH_3^+ symport in AMT subfamily or the AMT2/MEP subfamily which include NH_3^+ channel Am+B from *E.coli* and the mep 1-3 transporter from yeast. AMT1 family is the major entry pathway of root uptake of ammonium ions (Gu et al., 2013; Hawkesford et al., 2012; Miller A.J., 2011). The ammonium transport system also consists of the LATs and HATS system (Garnett et al., 2015; Dechorgnat et al., 2018). At low external concentration (HATS), members of the AMT gene family are involved (Dechorgnat et al., 2018). Presently there is no known ammonium LATS activity in plants, although recent identification of AMF1 proteins in soybean and yeast suggests a promising candidate for this transport characteristic (Dechorgnat et al., 2018). A reduction in the internal pool of glutamate and/or associated metabolites are one of the signals for activating AtAMT1 and AtNRT2 genes during low nitrate availability (Gu, et al., 2018). High-affinity ammonium uptake capacity in roots increased under N limitation and rapidly declined when sources are reapplied in Arabidopsis, as concomitant changes in cytoplasmic glutamine levels are thought to regulate AtAMT gene expression by negative feedback mechanism (Gu et al., 2013). The scholars showed that the main contributor to maize root high-affinity ammonium transport were most likely ZmAMT1.1A and ZmAMT1.3.

1.2.1.2. Nitrate and ammonium assimilation

Internal nitrate transport across plasma membrane of epidermal and cortical cell of the root takes place against a steep electrochemical potential gradient. Since the nitrate ion is negatively charged, it must overcome the negative plasma membrane potential concentration gradients of which metabolic energy is inevitable for nitrogen influx (Forde, 2000; Hawkesford *et al*., 2012). The metabolic cost of absorption and assimilation of NO_3^- is higher than that of NH_4^+ because of the reduction reaction of NO_3^- to NO_2^- and then to NH_4^+ by nitrate reductase and nitrite reductase respectively. And is assimilated into organic N through the GOGAT cycle (Forde, 2000). But NO_3^- was shown to be the main source of nitrogen for most angiosperms (Taylor and Bloom, 1998; Forde 2000).

As soon as nitrate and ammonium ion enter the roots, they are assimilated directly or stored intracellularly within the vacuole and or cytoplasm of root cells. They can also be radially transported to the xylem through symplastic and apoplastic pathways (Dechorgnat et al., 2018). Increased root sucrose may improve uptake efficiency of both ammonium and nitrate through increased expression of HATS (White, 2012). Nitrate taken up into the root system through the symplast moves across the different cell types into apoplast of the xylem for long transport via the transpiration stream to the leaves (Hawkesford *et al*., 2012). In contrast to ammonium, which is usually assimilated into organic compounds in the roots, nitrate is distributed throughout the plant through storage in the vacuole of roots and shoot organs where it can be retrieved (Hawkesford *et al*., 2012). Storage of nitrate is mediated by chloride channels (Miller and Chapman, 2011) and in the night when nitrate is not metabolized by nitrate reductase. The stored nitrate is depleted within 12-48h of nitrate starvation. Similarly, the diurnal uptake and ammonium (expression of AtAMT1:3) also increases during the day at the end of the light period, showing that nitrogen uptake is regulated by C supply (Hawkesford et al., 2012). Aquaporins are involved in ammonia transport (Miller and Chapman, 2011).

Two separate experiments that examined N fluxes in rice and maize seedlings (Colmer and Bloom, 1998) found that NH_4^+ uptake was consistently higher than NO_3^- in the apical zone of root when both NH_4^+ and NO_3^- were available. Utilization of NH_4^+ in the meristematic zone would be more efficient energetically, as a whole root system study shows that 25% and 14% of root carbon catabolism takes place during absorption and assimilation of NO_3^- and NH_4^+ respectively. Whether this true in terms of genotypic differences within species, remained to be established. A study by Garnett *et al*., 2015, compared the response of 27 maize genotypes to varying nitrate and ammonia levels. Genotypic responses were assessed based on biomass traits and the activities of nitrates and ammonia high affinity transport system. Findings shows great variation among genotype ability to maintain biomass with decreased nitrogen N supply. Garnett and colleagues (2015) reported that maize lines that maintained biomass during limited N supply maintained net N uptake with no change in size of the root relative to the shoot. While the rate of nitrate and ammonium acquisition increased with reduced N in such lines. These studies concluded that transporters play a vital role in enhancing N utilization efficiency and genotypic variation in ability to capture N at low N, providing a scope for improving N utilization efficiency in maize and to better understand the N uptake systems in cereals.

1.2.2. The Concept of Nitrogen Use Efficiency

Nitrogen use efficiency is the ratio between total biomass of output (e.g., grain yield) and the N input (N supplied as fertilizer and /or residual nitrogen present in soil) (Hawkesford *et al*., 2012). Nitrogen use efficiency consist of nitrogen uptake efficiency (the capacity to remove N from the soil as nitrate and ammonia ions by plants) and nitrogen utilization efficiency (the ability to use absorbed nitrogen to produce grain yield). NUE is also defined as the grain yield per unit of N but it is debated if plants could produce large biomass per unit N without converting the acquired nitrogen to seed production. Thus, low nitrogen use efficiency in grain is said to occur (Han *et al*., 2015).

Nitrogen use efficiency is subject to genetic variation and phenotypic plasticity, given that heterogeneity of nutrients exists in soils. Thus, it is crucial to study a defined genetic population under different N supply with the interactions between N uptake and water availability, macro nutrients and micronutrients in other to determine the contribution of N level from genetic and other environmental effects on plant yield (Han *et al*., 2015). Modifying root system to enhance nitrate acquisition from sub soil could have negative effects on the uptake efficiency of phosphate. Thus, trade-off must be considered (Han *et al*., 2015). This is because phosphate is usually found in the topsoil while nitrogen sources such as nitrate may move down the soil profile due to leaching.

1.3. Plants root and root system architecture

Plant roots regulate morphological traits and encourage adaptation to soil environmental condition by modifying rhizosphere processes through physiological activities including exudation of organic acids, phosphatase signalling substances, and pronto release and redox changes (Jacoby et al., 2017; Shen et al., 2013). The root system architecture is the overall spatial arrangement of individual part of the root system and is important in determining how plant can effectively access soil resources (Lynch et al., 2012; Shahzad and Amtmann, 2017). Root architecture respond to the environment by changing growth pattern (Lynch et al., 2012; Shahzad and Amtmann, 2017), and can be used to predict yield under soil resource scarcity. It is indicative of plant fitness to acquire water, nutrient and anchorage which determines crop production and sustainability (Lynch et al., 2012). Following this, one can deduce that root growth and rhizosphere processes could be manipulated to achieve improved nutrient and water use efficiency for enhanced crop productivity.

The efficiency of root/rhizosphere in terms of improved nutrient mobilization, acquisition and utilization may be fully exploited by manipulating root growth (root development and size, root system architecture and distribution and regulation of rhizosphere processes (rhizosphere acidification, organic anion and acid phosphatase exudation, localized application of nutrients, rhizosphere interaction and use of efficient genotypes). In addition, optimizing root zone management

to synchronize root growth and soil nutrient supply and demand in cropping systems could be achieved (Gastal. et al., 2015).

It is important to note that root growth is affected by supply of assimilates from the shoot and the soil physical conditions such as moisture supply, pH, temperature, aeration, and soil resistance (Ober. and M.A.J. Parry 2011). While the various function of roots is well known, the mechanisms controlling root types, branching and functions is not completely understood.

1.3.1. The maize root system

The maize root system (Figure 1.1) comprises of embryonic and postembryonic components. The embryonic root system consists of single primary root and a variable number of seminal roots formed at the scutellar node and are important for seedling vigour during early development. The postembryonic root system consists of roots formed from consecutive shoot nodes (nodal roots or crown root) and lateral roots which are initiated in the pericycle of all root types (Hochholdinger *et al*, 2004; Saengwilai et al., 2014; Yu et al., 2015; Hochholdinger et al., 2018; Ju et al., 2018). Maize roots are made up of different tissues and cell-types, each providing distinct diverse gene expression trend related to their individual roles (Hochholdinger and Tuberosa, 2009). The different cell types include the vasculature, pericycle, endodermis, cortex, epidermis, proximal meristem, quiescent centre, distal meristem and the root cap (Hochholdinger et al., 2004). The cross-sectional root anatomy of the maize crown root (Figure 1.2) showed the structure and localization of some of the cell types.

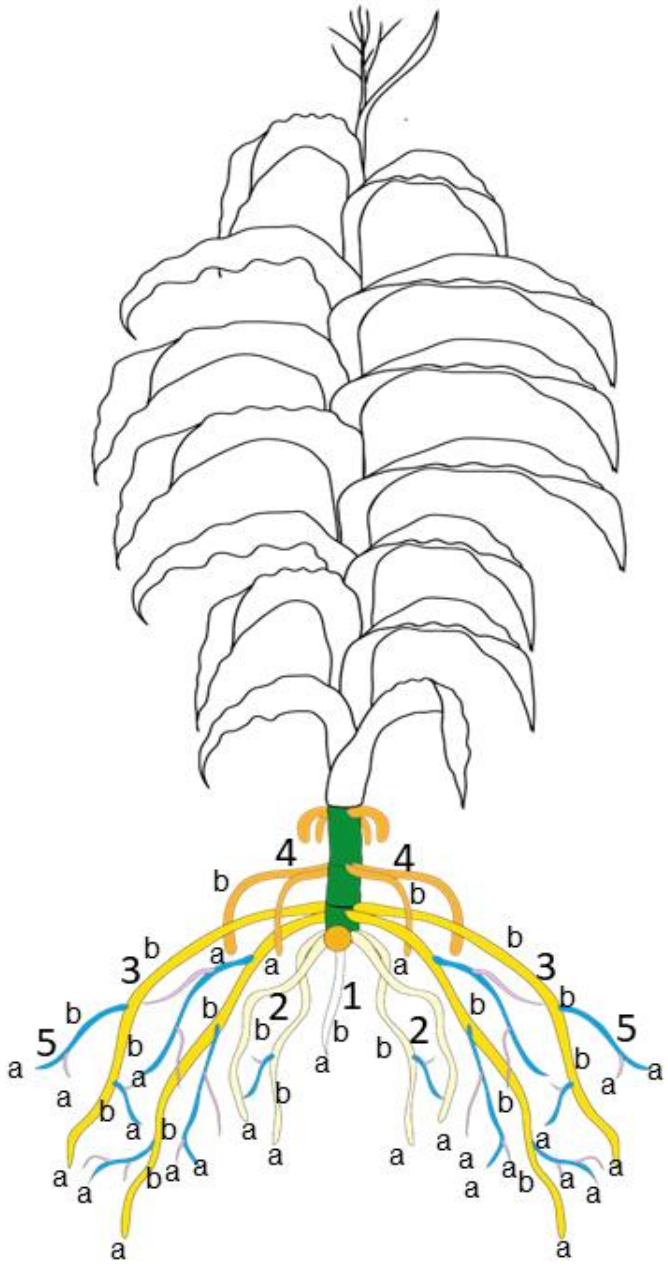


Figure 1.1. Different maize root types and the various nutrient ion absorption site. Numbers represents the root types as follows: (1) primary root, (2) seminal roots, (3) crown roots, (4) brace roots, and (5) lateral roots. Nutrient ion absorbing site: (a) represent 0-40mm from the root tips and (b) represents the basal parts of the roots. The apical areas (a) absorbs iron, nitrate, ammonium, potassium, and phosphate more rapidly. The basal region (b) also absorbs iron, nitrate, ammonium, and phosphorus at a slower rates as compared to the apical region (a). These are based on the findings of Lupinni et al., 2016; Taiz and Zeiger, 2006; Colmer and Bloom, 1998.

1.3.1.1. Different areas of maize root absorb different mineral ions

Previous studies demonstrated high spatial and temporal variability in net nitrate uptake rate along the root axis of several crop species (Lupini et al., 2016). It was reported that maize roots region closed to root tips (0-40mm) show greater ability to capture nitrate than the basal ones (Lupini et al., 2016). Root absorption of calcium in barley appears to be restricted to the apical region. Studies shows that irons may be absorbed at the apical region or over the entire root surface as in maize, also potassium, nitrate, ammonia and phosphate can be taken up at all locations of the root surface freely but the elongation zone has a higher rate of potassium and nitrate absorption in maize (Taiz and Zeiger, 2006). Colmer and Bloom, 1998, reported that the root apex absorbs ammonia more rapidly than the elongation zone in maize and rice. Taken together, suggests distinct functional specialization of root cells in resource capture among species. The nutrient absorption site of the maize roots is illustrated on Figure 1.1. However, it is not clear if the regional differences in the capture of different ions were similar or different among root types in maize and other plants.

1.3.1.2. Various cell types of the maize root

The root apical meristem produces cells that differentiate to form primary tissues such as the epidermis, cortex, endodermis, and various tissues in the stele including xylem, phloem, parenchyma, sclerenchyma and collenchyma (Lynch et al., 2012). The root apical meristem integrates all the cell types within the roots (Mira et al., 2020). The stem cell niche of the root apical meristem is made up of two distinct stem cells, the slowly dividing portion referred to as the quiescent centre (QC), and the rapidly dividing sides of the cell located at above (proximal meristem) and below (distal meristem) end of the quiescent centre (Iijima et al., 2008; Mira et al., 2020). The quiescent centre relies on the basipetal transport of auxin for the creation of auxin gradient within the quiescent centre and the establishment of a highly oxidized ascorbate and glutathione environment (Mira et al., 2020). It is important to note that the survival of the root system depends on the viability of the quiescent centre (Mira et al., 2020). However, it is unclear if nitrogen availability determines the degree for which the slow cell division takes place in the quiescent centre.

The vasculature known as the vascular systems that runs through the entire plants from the root, stem and leaves are critical paths for the supply of water, mineral nutrients and organic substances (Zhang et al., 2018). The vasculature also functions to give anchorage for the maize plant (Zhang et al., 2018). However, not much is known on the effect of nitrogen rates on the functional specificity of the vasculature.

The pericycles surrounds the vascular tissues and is encircled by the inner cortical layer known as the endodermis (Beeckman and De Smet, 2014). The cells are heterogenous, non-vascular tissues located at the xylem and phloem poles (Beeckman and De Smet, 2014), and are marked by differences in size, proteins and gene expression (Beeckman and De Smet, 2014). The pericycle responds to changes in external nutrient concentration to initiate the development lateral root via the xylem pole in *A. thaliana* or the phloem pole in maize and other members of the grass family (G. Viana et al., 2022; Yu et al., 2016b).

Furthermore, the endodermis surrounds the vascular tissues and forms apoplasmic diffusion barrier (Figure 1.2) to modulate the translocation of mineral ions, water and hormones in and out of the vasculature within the stele (Doblas et al., 2017). The stele allows the transport of water, nutrients and photosynthates, while the cortical parenchyma represents the ground tissues of the roots which has metabolic function, but also transport minerals into the stele (Hochholdinger et al., 2018a). In maize, the endodermal cells contribute to lateral root development and forms the epidermis and the root cap, but the pericycle cells are the principal source of tissues for lateral roots (Bellini et al., 2014).

The cortex transport water and nutrients absorbed by the root epidermis (Figure 1.2) into the vasculature including starch, resins, and essential oils, the cells also store photo assimilates in the form of starch (Kim et al., 2022). Interestingly, the cortical cells determine the fate of the epidermal cells (Kim et al., 2022). But it is unclear if nitrogen availability exerts greater influence on the determination of the epidermal cell fate. Moreso, the molecular mechanisms for the specification of outer cell layers such as the epidermis, exodermis and sclerenchyma are not fully understood (G. Viana et al., 2022).

Next is the root cap that functions to protects the root apical meristem and enhanced penetration into the soil by production of mucilage (Iijima et al., 2008; Lynch et al., 2012). The root cap also play significant roles in diverting pathogens from the root axes and secreting enzymes and compounds in other to enhance nutrient availability (Lynch et al., 2012).

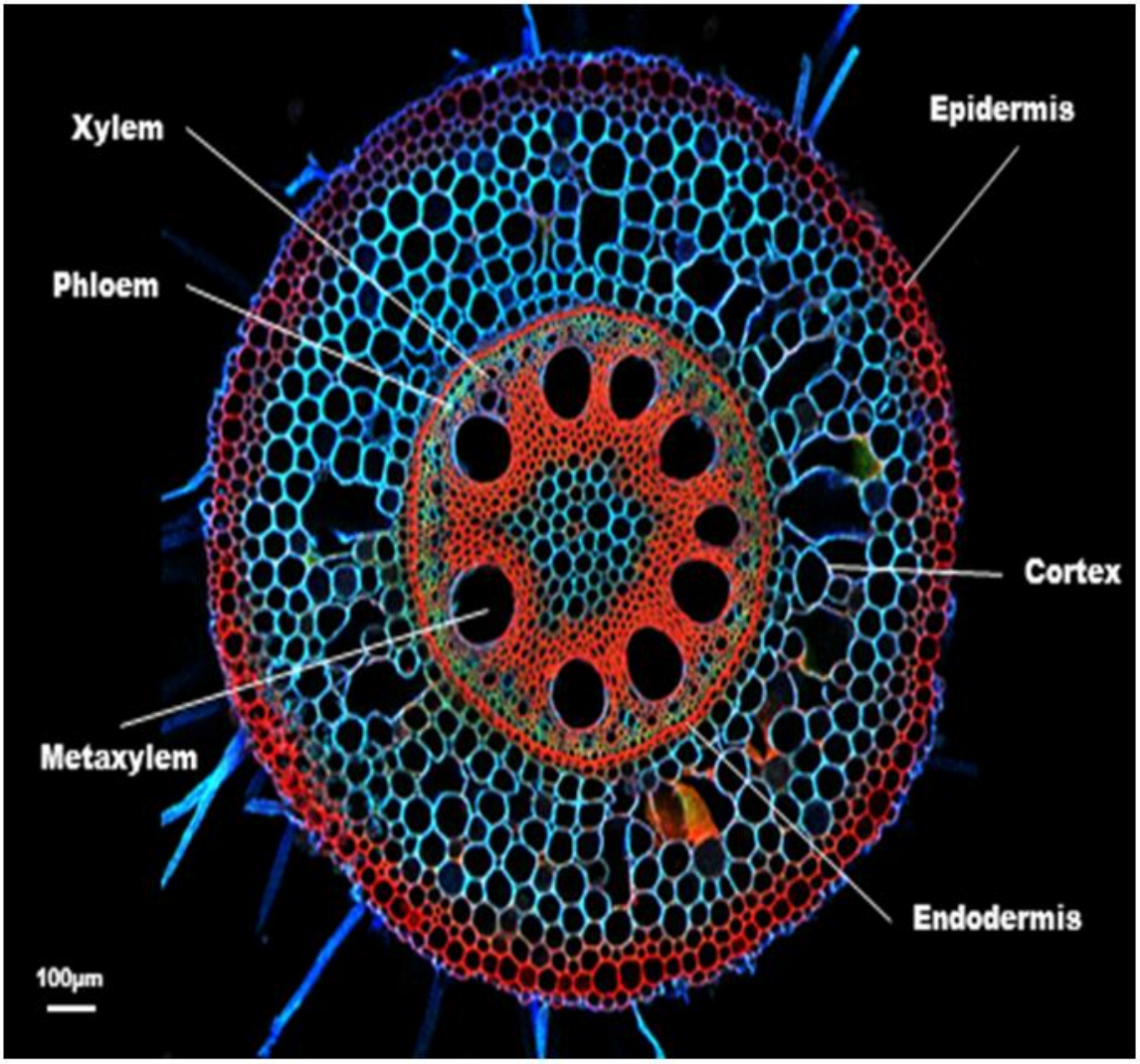


Figure 1.2. Cross-sectional representation of the maize crown root cells. The central area known as the stele, houses the metaxylem vessels which are surrounded by xylem (orange/red) for water and mineral ion transport, phloem (blue) for transport of carbohydrates and phytohormones. The stele is outwardly encircled by a layer of endodermal (red) and then several layers of cortical cells aerenchyma. The outer cells, exodermis (red) and epidermis (blue) act to retain water and form root hairs for uptake of mineral ions. Image and description adapted from: https://www.nottingham.ac.uk/hiddenhalf/crop/maize.aspx.

The root hairs represent key sites for nutrient absorption. The root hairs develop as long extensions from the root epidermal cells, after initial formation of a bulge at the distal end of the epidermal cell (George and Brown, 2021). Root hairs and exopolysaccharides produced by roots and microbes enhances soil root interactions and modify water content and movement (Marschner and Rengel, 2012).Although the transporters and influx systems for nitrate, ammonium, phosphate, potassium, urea, calcium, peptides and sulphate were reportedly found in root hairs (George and Brown, 2021), root hairs specifically act to capture immobile mineral elements like potassium and phosphorus as they increase in volume of the soil they mine (Datta et al., 2011). While it is known that the relative number of Arabidopsis hair cells and non-hair cells differs with nitrate and phosphate availability (Datta et al., 2011), it is unclear if variation exists in maize or Arabidopsis root hair cells as a result of differences in the ratio of nitrate and ammonium in soil or growing medium.

1.3.1.3. Maize crown root

The crown root is central feature of maize root architecture and dominates nutrient and water uptake during the vegetative growth after the first few weeks and remains essential during reproductive development (Hochholdinger et al., 2004; Saengwilai et al., 2014; Yu et al., 2014). Crown root in maize originate from the root stem base and ranges from 5 to 50 during fertile (adequately balanced nutrient availability) conditions but nutrient deficiency causes spatial dispersion of crown roots for sufficient soil exploration (Saengwilai et al., 2014; Xuan and Beeckman, 2021). Saengwilai and colleagues showed that low crown root number improves N acquisition by enhancing deep soil exploration. The diameter of crown root of the third whorl and subsequent nodes is much larger than that of the primary and seminal roots, and these roots are thus a greater sink for photosynthates (Saengwilai et al., 2014). Different inbred lines of maize have varying number, growth rate, and angle of growth in crown roots. During an intensive field phenotyping of maize, researchers identified crown root traits and trait integration associated with plant growth and nutrient acquisition(York and Lynch, 2015).

The developmental regulation of crown root in maize is like in rice (G. Viana et al., 2022). Auxin transport is required for the establishment of auxin maxima and increased concentration at the vascular bundles and ground meristem cells adjacent to the peripheral vascular cylinder, and triggers cell dedifferentiation and the formation of crown root primordium(Bellini et al., 2014; G. Viana et al., 2022; Xuan and Beeckman, 2021). The polar auxin transport of PIN1 genes for the creation of auxin gradient was shown to be regulated by *CRL4/OsGNOM1* in rice (G. Viana et al., 2022; Jung and McCouch, 2013). Disruption of the role of *CRL4/OsGNOM1* will prevent the initiation of crown root (Jung and McCouch, 2013). The crown root primordia initials are produced from periclinal divisions of parenchyma cells (Jung and McCouch, 2013). This influences the arrangement pattern of

differentiated epidermis/endodermis initials central cylinder cells and root cap initials (Jung and McCouch, 2013). It is important to understand that the crown root can develop from other cell types adjacent to the vascular tissue, including phloem and xylem cells, pericycle cells, cambium cells near the phloem poles (Bellini et al., 2014; Xuan et al., 2021), but not from true roots as shown in Figure 1.2.

The maize and rice homologue of the Arabidopsis *SHR* and *SCR* genes, *GRAS* transcription factors were also shown to be critical for the radial patterning required for crown roots development (Jung and McCouch, 2013). The monogenic maize mutant rootless concerning crown and seminal roots (rtcs) lacks crown roots but only primary roots with their laterals (Hetz et al., 1996). Another monogenic maize mutant exhibiting lesser developmental defects in phenotypes is the lateral rootless 1 (*lrt1*) which does not develop crown roots at the coleoptile nodes or any lateral on embryonic roots (Hoccholdinger and Felix, 1998; Jung and McCoucly, 2013). The myriad influence of nitrate or ammonium availability on the activities of the genes regulating the development of crown roots is yet to be established. But an increased crown root diameter was reported in nitrogen limited soils (Wang et al., 2021).

Crown roots that emerged because of drought provides an essential mechanism for water conservation across grass species (Sebastian et al., 2016). Reasonable phenotypic variation exists in maize for the above trait and can be an essential target of breeding to enhance drought tolerance (Sebastian et al., 2014). However, only little is known on how crown roots respond to water stress (Sebastian *et al*., 2016). Crown roots of *Zea mays* and *Setaria italica* (relatives of teosite and *S. viridis* respectively) appear to be less sensitive to moisture stress than the ancestral species, probably influenced indirectly by human selection. Enhanced water status of maize mutants lacking crown roots suggest that stronger suppression of crown roots may be beneficial to crop productivity under moisture stress (Sebastian *et al*., 2016). Sebastian *et al*., (2016), deduced that the crown root region is the major site for sensing water availability and that suppressing it can dramatically stop the post-emergence crown root development while keeping the root system in its prior primary root dominated state. The existing wide differences in the sensitivity of crown roots to moisture deficit in inbred of maize implies that breeders might have unwittingly chosen for various response strategies based on the water availability kinetics of the environmental conditions where they were chosen. Interestingly, sorghum that usually abolish crown root growth completely during moisture stress is known to show reasonable drought tolerance (Sebastian *et al*., 2016). Studies discovered a negative correlation between higher crown root density and nitrogen use efficiency but found that low crown root number enhances nitrogen acquisition from low nitrogen soils (Saengwilai *et al*., 2014).

1.3.1.3. The maize brace root

The mechanism for the development of brace roots is still poorly understood as compared to crown roots in maize (G. Viana et al., 2022). Crown root formation in maize were shown to be governed by auxin as crown roots in rice (Majer et al., 2012). Studies on transgenic rice seedlings overexpressing the auxin biosynthesis gene *OsYUCCA1*, showed that more crown roots and shorter seminal roots were produced after increased in auxin contents and auxin signalling *in vivo* (Xuan and Beeckman, 2021). In rice, *Osadventitious rootless1/Oscrown rootless1 (Osarl1/Oscrl1*) is mutant lacking in crown roots (Xuan and Beeckman, 2021). Its mutant phenotype shows the loss of function of gene encoding an *OsASSMMETRICLEAVES2 (AS2)/LATERAL ORGAN BOUNDARIE* domain transcription factor which is under the coordination of auxin and directly targeted *OsARF1* (Xuan and Beeckman, 2021). But rice does not have brace roots. Here, this study discussed the genes, mutants and collaborators involved in the development of brace roots which may or may not involve crown roots (Figure 1.3). The *LATERAL ORGAN BOUNDARY DOMAIN (LBD*) transcription factor mutant known as the *rootless concerning crown and seminal roots (rtcs)* is deficient in the initiation of seminal, crown and brace roots, and controls the development of brace roots independent of the shoot meristem (Hetz et al., 1996; Hostetler et al., 2021; Sparks, 2023). The expression of *RCTS1* is induced by auxin like other stem-borne roots and the lack of root primordia in *rcts1* showed that auxin is critical in the induction, initiation, emergence, and growth of adventitious roots in maize (Hostetler et al., 2021). *RTCS* and *RTCL* are *LBD* transcription factors and orthologs of *CRL1* and *ARL,* implying that initiation mechanism of adventitious roots in maize and rice is conserved (Viana et al., 2022). The *RTCS* and *RTC*L were shown to bind to the *LBD* motif in the promoter of auxin response factors *ZmARF* 34 to trigger downstream genes (G. Viana et al., 2022; Majer et al., 2012) .

Recently, *AP2/ERF (APETALA2/ETHYLENE RESPONSE FACTOR) ZmRAP2.*7 was identified as a controller of brace roots of which its expression was said to be 10-fold higher in the nodes with brace roots than nodes without brace roots (Li et al., 2019). However, the downstream genes of *ZmRAP2.7* and the manner of their regulation with regards to the development of brace roots is unknown (Li et al., 2019). The *corngrass1* mutant was reported to increase expression of ZmRAP2.7 and have a display 6-fold rise in brace root number in relation to the wild type and crown roots (Cigan et al., 2007; G. Viana et al., 2022; Li et al., 2019). But more detailed study is needed to understand the role of the genes in the auxin pathway. It was proposed that an investigation should be launched to know if *ZmRAP2.7* was involved in the pathway of *ZmRTCS* and the upstream regulation of *ZmARF35* in modulating the development of adventitious roots (Li et al., 2019). Also, the relationship between ZmRAP2.7 and the response regulator (RR) of cytokinin signalling in relation to nitrogen availability needed to be studied.

Another auxin regulated gene involved in organ sizes is the (*argos8*) that prevent root emergence but not formation of primordia in maize nodal roots (Hostetler et al., 2021). The over expression of *argos8* was shown to reduce ethylene sensitivity leading to delayed brace root emergence (G. Viana et al., 2022). The manner of its involvement in the auxin-ethylene pathway needs to be studied. This is because ethylene was reported to synergistically work with auxin in the development of lateral roots (Alarcón et al., 2013).

The BIG EMBRYO1 (BIGE1) was shown to regulate the initiation and the size of lateral organ in maize (G. Viana et al., 2022; Suzuki et al., 2015). The mutation leads to enlargement of embryo scutellum, rapid leaf formation, and brace root formation at higher stem nodes that are not seen in the wild type (G. Viana et al., 2022; Suzuki et al., 2015). Furthermore, the ZmCCT10 (CO, CONSTANS and CO-LIKE, and TIMING OF CAB1) protein family modulate photoperiod response. Higher transcript abundance of ZmCCT10 was said to prolong the vegetative phase accompanied with phenotype change (G. Viana et al., 2022). Transgenic plants of ZmCCT10 were shown to produce brace roots up to the 37th nodes while the wild type is only up to the 6th node (G. Viana et al., 2022). By and large, the transcriptional regulation of brace root formation requires more studies at the molecular and the physiological level, to broaden our understanding of the activities of these genes.

1.3.1.4. The maize primary and seminal roots

Crown roots are a major structural investment of root biomass and constitute the basic structural framework from which lateral roots initiate. Crown root location in the soil is important in influencing the foraging of soil area by lateral roots and root symbionts (Gao and Lynch, 2016).

The lateral roots increase the absorbing surface area of all the maize main root system (Hochholdinger et al., 2018a). It isn't clear if the formation and development of lateral roots and crown roots shares a similar regulatory network (Xuan and Beeckman, 2021).

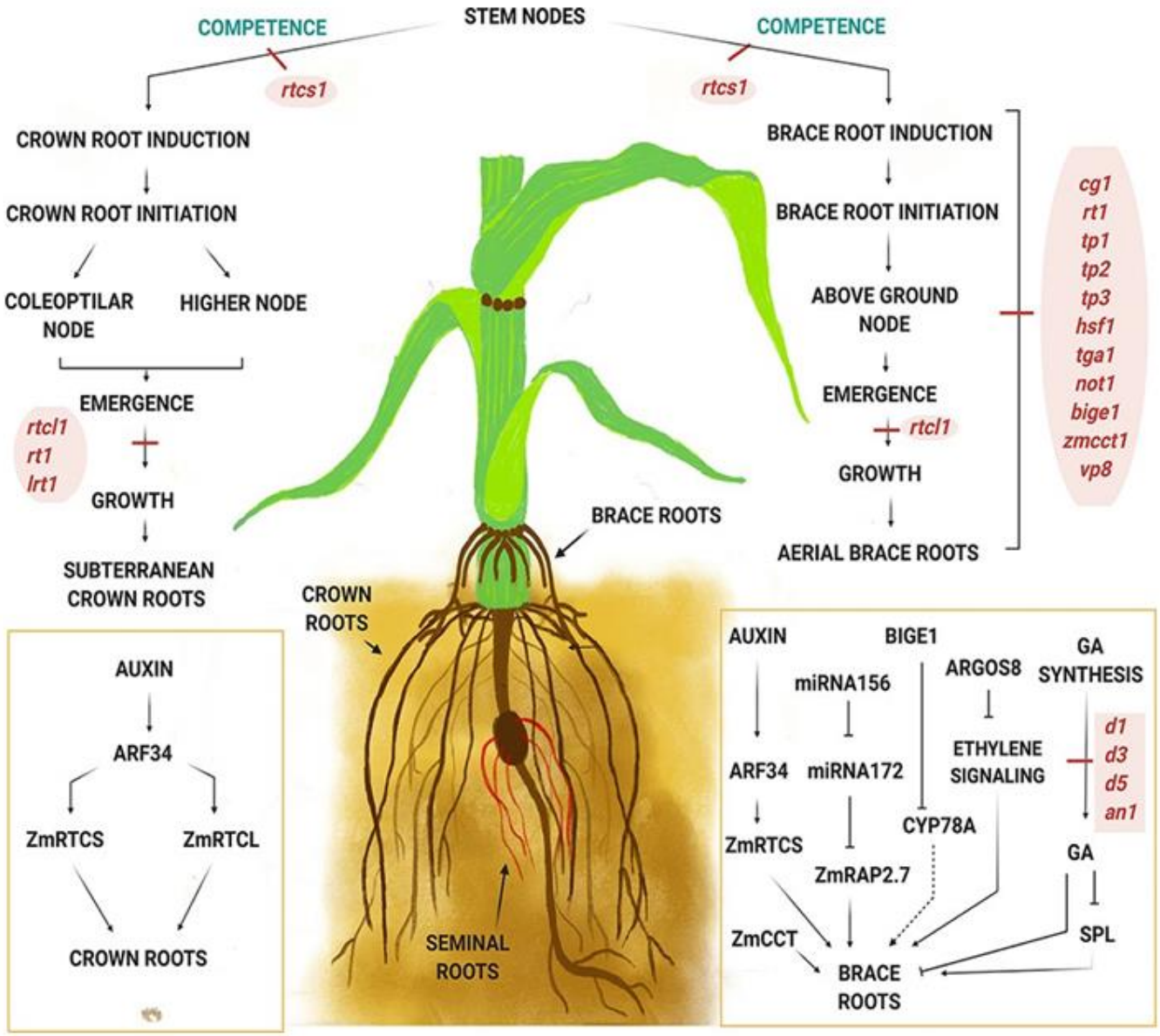


Figure 1.3. The molecular and hormonal pathways of crown and brace root development in maize. Arrows indicate positive regulation, and lines with a flat head represent negative regulation. (Reproduced from Singh et al., 2022, *Plant and Cell Physiology*, Volume 63, Issue 12, December 2022, Pages 1806–1813, https://doi.org/10.1093/pcp/pcac084

1.3.1.5. Nitrogen uptake by maize root types

The root uptake efficiency depends on the plants intrinsic properties including root depth, degree of branching and entire proliferation, root angle as an indicator of root speed, and the activities of the plasma membrane transporters (Hawkesford et al., 2021). Maize roots respond differently when exposed to patchy nutrients. Primary roots capture more nitrogen at higher levels than seminal roots. Crown roots capture more nitrogen under even distribution of low nitrogen than under uniform distribution of high nitrogen which is the opposite for primary and seminal roots (Yu *et al.,* 2014; Steffens and Rasmussen, 2015). However, among the root types, primary and crown roots have higher nitrogen capture ability when local high nitrogen is applied but seminal roots have the highest nitrogen capture ability under low local nitrogen levels (Yu *et al.,* 2014). Recently, Brackin *et al*., (2017) utilized microdialysis to sample flux rates of nitrates and ammonium ion in maize roots discovered that nitrate flux rate was higher in probes closest to roots and lower when placed far from the roots whereas ammonium was lower in probes closest to the roots and much more present far from the roots. The scholars attributed this to mass flow pull of negatively charge nitrate towards the roots during transpiration than the 10 times less mobile nature of positively charged ammonium ions.

1.3.1.6. Root Traits affecting nitrogen uptake and utilization efficiency

Nitrogen uptake efficiency is affected by the ability of the root to capture soil nitrogen and depends on physiological traits such as root architecture and the density of nitrate and ammonium transporters at the plasma membrane of the roots. The ability of the root to capture nitrogen depends on the degree of which root reaches its acquisition area and is determined by complex root morphology (Hawkesford *et al*., 2012; Shahzad and Amtmann, 2017). Primary root traits affecting N uptake includes root axis number, rooting depth and rooting density. Foulkes *et al*., (2009), and Hawkesford *et al*., (2012), reported that a prolific root system (densely configurated root system comprising of large number of lateral roots on each root type) has higher nutrient capture efficiency than sparse system (fewer roots with a smaller number of lateral roots on all root type), debating that inter-root-competition acts as a barrier to optimum root length density. Reduced crown root eases nitrate uptake in maize, most likely due to decreased competition for internal and external resource among roots of same plants (Saengwilai *et al.*, 2014). Previous studies (York *et al*, 2015) comparing leading maize lines over a century related reduced crown roots formation with improved nitrogen acquisition.

The uptake of nitrate and ammonium into plants roots is mediated by transport proteins located in the plasma membrane of the epidermal and cortical root cells (Hawkesford *et al*., 2012, Lynch *et al*., 2012). The acquisition of low nitrogen from soils requires high rooting density because the expression of nitrate and ammonia transporters are controlled by supply and demand of N (Hawkesford *et al*.,

2012) and means that higher threshold levels of down-regulation of transporter genes may allow much influx to induce increased N assimilation and decrease efflux to improve uptake efficiency.

Physiological traits that affect nitrogen utilization efficiency in crops are effects of N on carbohydrate partitioning, N storage and N remobilization from senescent tissues. N harvesting index, which is the minimum N% in the grain at harvest, determines the amount of grain that can be produced per unit absorption (Mei *et al*., 2015). Bertin and Gallais, (2000) found a close correlation between nitrogen uptake efficiency and NUE at high N supply and nitrogen utilization efficiency and NUE at low N supply. Studies show that maize hybrids with similar NUE differed in uptake and utilization efficiency. Moll *et al*., (1982) found that nitrogen utilization efficiency contributed to variation in NUE at low applied N while nitrogen uptake efficiency contributed to variation in NUE when higher amount of N was applied.

Nitrogen uptake capacity of maize and sorghum is affected positively by delayed senescence which allow photosynthetic activity to be prolonged. This enables sorghum to assimilate more carbon and utilize more nitrogen biomass production whereas in maize, yield become higher (Hirel *et al*., 2007). Knowledge of the regulatory processes controlling the stay-green trait is still limited in terms of the stay-green trait (a secondary trait that enables crop plants to maintain their green leaves and photosynthetic capacity for a longer time after anthesis, especially during unfavourable condition) is important in terms of NUE and yield (Hirel *et al*, 2007).

1.3.2. Role of hormones in the control of root architecture by nutrient signals

Phytohormones enhance nitrogen uptake by regulating the activities of nitrogen transporters and by modulating root morphology. Hormonal signals trigger root hairs to respond to nutrient availability by specific nutrient pathways (López-Bucio et al., 2003). Plant growth regulators determine most of the root system traits such as growth of primary, lateral root, and formation of root hair (Himanen et al., 2002).

1.3.2.1. Auxin:

Auxin local accumulation in the root epidermal cells of *A. thalina* initiates lateral root formation. Once a root is formed, a gradient of auxin concentration develops and is highest at the tips (promoting the production of new cells at the meristems) and lowest in the region of differentiation (promoting the elongation and differentiation of root cells) (Bouguyon, et al., 2016). The radial positioning and reactivation of lateral root phloem pole pericycle cells in maize is regulated by auxin response maxima (Hochholdinger et al., 2018b; Yu et al., 2016a). It was suggested that *OsPIN* family genes are involved in root formation and elongation in rice. They genes inhibit auxin transport from shoot to root

depending on reduced expression of *OsPIN* genes in low N and low P modulated lateral root formation and seminal root elongation (Huwei et al., 2018). Inhibition of auxin transport slows down lateral root growth but does not affects lateral root formation (Motte and Beeckman, 2019).

Auxin also stimulates the formation of adventitious roots in many species (Rasmussen et al., 2015). Early organogenesis of adventitious roots (AR) involved IAA gradient and accumulation in specific cells through polar auxin transport, biosynthesis and conjugation (Gonin et al., 2019). Auxin response factor 34 (*ARF 34*)binds to LBD elements (5'-GCGGCG-3') in their promoter to express the *rtcs* (defective in crown root initiation and also seminal root initiation) and *rtcl* (*rtc*-like) genes to control shoot–borne roots in maize(Hochholdinger et al., 2018b). Although ARFs regulates several genes transcriptionally, some LATERAL ORGAN BOUNDARIES-DOMAIN/ASSYMETRIC LEVELS2-LIKE (LBD/ASL) genes are more important during lateral root initiation. The rice ARF7/ARF19 orthologue, OsARF16 regulates the LBD16-related to OsLBD3-2/CROWN ROOTLESS (CRL) that operates in lateral root initiation (Motte and Beeckman, 2019). Disruption of auxin transport represses or alters founder cell priming and cell division, resulting in reducing AR formation (Gonin et al., 2019).

1.3.2.2. Cytokinin:

Cytokinin is involved in nitrate foraging by orchestrating preferential development of lateral root in areas of nitrate availability to maximise uptake (Figure 1.4.) (Gu et al., 2018; Pavlů et al., 2018). Shoot synthesized ip-type cytokinin negatively control the elongation of embryonic primary roots and formation of post embryonic lateral roots by antagonizing auxin in the root meristem during early embryogenesis (Gu et al., 2018). Cytokinin controls root apical meristem size in part by promoting cell differentiation in the transition /elongation zones (Kieber and Schaller, 2018).

1.3.2.3. Abscisic acid:

Abscisic acid (ABA) controls lateral root repression in response to very high (>50mM) nitrate supply (O'Brien et al., 2016). ABI_2 (aPP2C Phosphatase controlled by ABA) regulates NRT1.1 (Nitrate transporter 1.1, a dual affinity transporter of nitrate usually at high concentration) dependent nitrate regulation of gene expression (NRT2.1) possibly through interaction with CIPK23/CBL1 (Calcineurin B-like interacting protein kinase 23) that phosphorylates NRT1.1. Consequently, a direct connection between ABA and nitrate signalling was found but it was not known if ABA really function in long-distance signals (Bellegarde et al., 2017). The nitrate transporter NPF3, an influx carrier expressed in root endodermis can transport ABA and GA. Thus, there is known to be an interaction between ABA and GA (O'Brien et al., 2016).

Strigolactones are relatively new class of hormones that regulate to RSA and also involved in Pi (inorganic phosphate) uptake (Nath and Tuteja, 2016; Rasmussen et al., 2012). It was suggested that the strigolactone pathway (signalling and biosynthesis) is involved in rice root elongation under low N and low P conditions (Huwei et al., 2018). Strigolactones signalling genes are needed for NO induced elongation of seminal roots in response to low N and low P in rice (Huwei et al., 2018). Strigolactone is reported to modulate auxin transport for the control of root formation and elongation by either altering auxin biosynthesis or polar auxin transport (Figure 1.4) (Rasmussen et al., 2013, Huwei et al., 2018). Their signalling is needed for NO mediated suppression of polar auxin transport during low N and low P conditions in rice. Also, OsPIN1b is involved in the NO and SL induced elongation of seminal root under low P and low N conditions (Huwei et al., 2018).

In Arabidopsis, strigolactones regulates auxin level in a way that depends on the auxin status of the plant. In rice, strigolactones obviously decrease auxin transport under low N and low P increases seminal root length (Huwei et al., 2018). The effects of NO and strigolactones on auxin transport involves regulation of PIN proteins (Huwei et al., 2018). Rasmussen et al., 2015, discovered that strigolactones negatively control the formation of adventitious roots. But repressing strigolactones promoted adventitious roots in several crop species.

1.3.2.4. Nitric Oxide (NO):

Nitric Oxide (NO) is a signalling molecule and is involved in the root organogenesis, growth, and development when nitrate is available (Sun et al., 2018). The two NO production pathways in plants are NO synthase-like (NOS-Like) and nitrate reductase (NR). NR pathway controls NO generation and nitrate concentration in roots and influences the production of NO by mediating NR activities (Sami et al., 2018, Sun et al., 2018). They regulate low N and low P induced seminal root elongation by minimizing auxin levels. NO activates lateral root formation and seminal root elongation by modulating auxin transport in the presence of nitrate. Nitrate influences lateral root formation by increasing the number of lateral root primordia and root elongation by increasing root meristem activities (Sun et al., 2018). NO negatively modulates auxin polar movement and decreased auxin levels in the root tip (Huwei et al., 2018). The levels of nitrate and nitrite are crucial determinants of NR-induced NO generation (Sun et al., 2018). Manoli et al., 2016, found that the NO controlled root apex responses to nitrate by controlling auxin in maize (Figure 1.4). NO induce root development in cucumber, lateral root formation in tomatoes (Sami et al., 2018). Implying complex crosstalk between auxin and NO in controlling root growth.

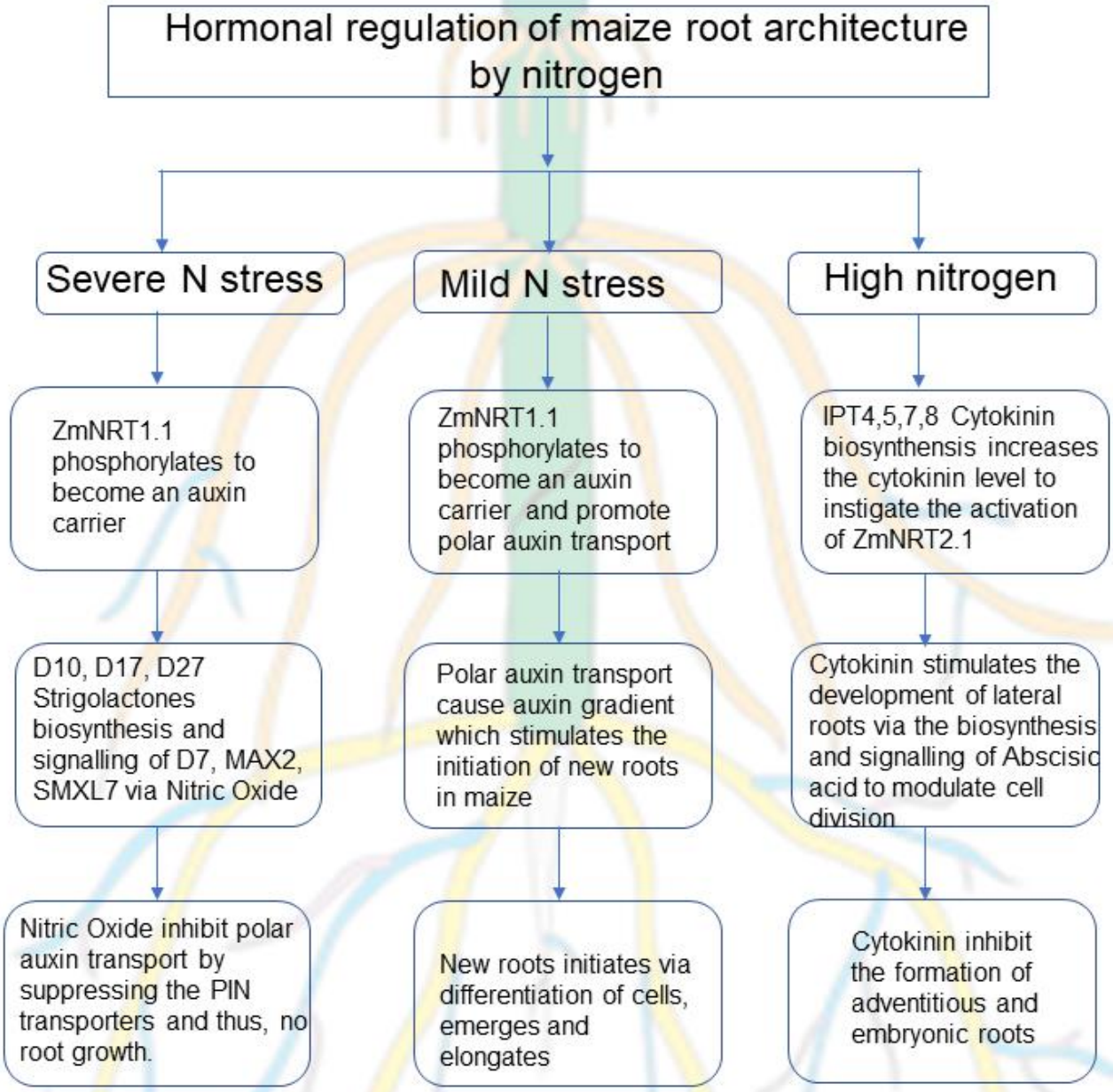


Figure 1. 3. Summary of how nitrogen availability promotes or suppreses root initiation and growth in maize. These are based on Gu et al., 2018; Ishaya and Rasmussen, 2021 and Sun et al., 2018.

1.3.3. Recently discovered master regulators of molecules

Recent molecular studies showed that all the plant growth processes mentioned above are orchestrated by a so-called target of rapamycin (TOR) kinase, an atypical Ser/Thr protein kinase evolutionarily conserved in plants, yeast, and animal (Meng et al., 2022; Sakr et al., 2018; Song et al., 2021; Sun et al., 2020). TOR kinase is implicated for the sensing and coordinating internal nitrogen and carbon concentration, solar radiation, hormone, and stress signalling to drive plant growth (Meng et al., 2022; Sakr et al., 2018; Song et al., 2021; Sun et al., 2020). TOR kinase was reported to control immediate plant growth and development by integrating the upstream signals through the glycolysis and mitochondrial bioenergetics-dependent transport (Meng et al., 2022). Other shoot associated growth promoting role of TOR kinase include the upregulation of genes related to chloroplast development and photosynthesis, activation of WUSCHEL (a home domain transcription factor responsible for the maintenance of stem cells (WUS)) at the shoot apex during light-cytokinin signalling and photosynthesis-driven sugar signalling, (Meng et al., 2022; Sakr et al., 2018). Nitrogen nutrition (nitrate, ammonium, and multiple versions of amino acids) is critical for the upstream signalling of TOR kinase activity alongside glucose and auxin (Meng et al., 2022; Sakuraba et al., 2018; Wu et al., 2019). During low nitrogen levels that is beyond detection in both shoot and roots apex, the elevated concentration of ammonium and amino acids in the root apex could maintain high TOR kinase activity in the root apex to promote cell proliferation and rapid root growth (Meng et al., 2022). TOR kinase was said to phosphorylate and stabilize the auxin efflux carrier PIN-formed 2 (PIN 2) to regulate auxin gradient distribution in the root elongation zones (Meng et al., 2022). How far TOR kinase signalling can be activated by nitrogen and their metabolites is linked with the time required to generate signalling molecules, possibly through the integration of mitochondrial bioenergetics to activate TOR kinase (Wu et al., 2019). This shows that the quantum yield of photosystem II during photochemistry may be directly linked with the level of TOR kinase. Plants have evolved a process to regulate TOR kinase activities base on the uneven signalling or translocation of nitrogen, sugar, auxin in response to diverse environmental conditions and developmental stages which gives the plant the sense of spatial domain (Meng et al., 2022).

This PhD aims to understand how maize root physiology in relation to real time soil nitrogen availability affects the whole plant response, and to explore the most effective method to successfully apply auxin to adult maize plants growing in soil. This will enable optimisation of maize root architecture, identification of essential traits for improved nitrogen use efficiency, and incorporation of the traits into commercial maize germplasm. Since maize has different root types including primary, seminal both embryonic, crown and brace both postembryonic, it may be possible to improve capture of applied fertiliser or to reduce the amount applied through optimised root architecture.

1.4. The research aims and objectives

- To determine the spatio-temporal dynamics of nitrogen in soil during maize growth and to measure nitrogen uptake efficiency of maize root types during suboptimal and adequate nitrogen availability (Chapter 2).
- To determine whole plant response to soil nitrogen fluxes after nutrient resupply at the short and long run (Chapter 3).
- To examine the allometric functional relationship between the above the ground plant parts and the below the ground parts as influenced by nitrogen availability and ontogenetic phase (Chapter 4).
- To explore effective method of stem node hormone application in adult maize plants growing in soil. And to determine if exogenous auxins can influence the emergence of brace roots in in low and high nitrogen plants (Chapter 5).

Methodology

To achieve this, microdialysis which is a novel technique for sampling solutes via diffusion with minimal soil disturbance was used (Brackin et al., 2017; Brackin et al., 2015; Buckley et al., 2017; Shaw et al., 2014). Its suitability lies in its ability to assess soil nutrient availability at micro scales (Buckley et al., 2017). The concentration of molecules in the dialysate shows the concentration of solute and the rate at which these molecules diffuse through the soil matrixes. This reflects the spatio temporal dynamics of solutes over time (Shaw et al., 2014). More so, it allows for simultaneous efflux of compounds from the dialysate and influx of solutes from the soil, hence depicting a pseudo-rhizosphere (McKay Fletcher et al., 2021). Using microdialysis will help mitigate several problems associated with the conventional soil sampling methods including destructive bulk soil sampling, sample clean up, and sample degradation (Brackin et al., 2017; Buckley et al., 2017). The openable window rhizoboxes were used to enable assessment of the roots of intact growing plants during microdialysis. Rhizoboxes have been used to study root growths in relation to nutrient availability (Schmidt et al., 2020) and crops root to shoot biomass partitioning (Maskova and Klimes, 2019). For the real time shoot responses, the PhotosynQ Multispec was used to examine the quantum yield of the photosystem II and the photosynthetic efficiency among other useful physiological variables, of maize plants during the process of microdialysis. This is to understand the variation of the photosynthetic component as a reflection of the rhizosphere situation in real time. The Dualex was used to take measurements including the nitrogen balance index, chlorophyll level and flavonoid for each of the experiment. The SPAD was used once as a backup during the first microdiaysis experiment. Other growth parameters

including plant height, leaf number, leaf length and width as well as the fresh and dry weight of plants were determined to understand long term response to soil nitrogen dynamics and the allometric relationship of functional equilibrium resulting from nitrogen nutrition and changes in ontogenetic phases. For the hormone application method, I used lanolin and water to apply gibberellins on plants stem which I scratched with sandpaper, this enabled immediate increase in plant heights.

1.5. References:

Alarcón, M. V., Lloret, P. G., and Salguero, J. (2013). Auxin–ethylene interaction in transversal and longitudinal growth in maize primary root. *Botany* **91**, 680-685.

Andrews, M., Raven, J. A., and Lea, P. J. (2013). Do plants need nitrate? The mechanisms by which nitrogen form affects plants: Do plants need nitrate? *Annals of applied biology* **163**, 174-199.

Beeckman, T., and De Smet, I. (2014). Pericycle. *Current biology* **24**, R378-R379.

Bellini, C., Pacurar, D. I., and Perrone, I. (2014). Adventitious roots and lateral roots: similarities and differences. *Annual review of plant biology* **65**, 639-666.

Brackin, R., Atkinson, B. S., Sturrock, C. J., and Rasmussen, A. (2017). Roots-eye view: Using microdialysis and microCT to non-destructively map root nutrient depletion and accumulation zones. *Plant, cell and environment* **40**, 3135-3142.

Brackin, R., Näsholm, T., Robinson, N., Guillou, S., Vinall, K., Lakshmanan, P., Schmidt, S., and Inselsbacher, E. (2015). Nitrogen fluxes at the root-soil interface show a mismatch of nitrogen fertilizer supply and sugarcane root uptake capacity. *Scientific Reports* **5**, 15727.

Briat, J.-F., Gojon, A., Plassard, C., Rouached, H., and Lemaire, G. (2020). Reappraisal of the central role of soil nutrient availability in nutrient management in light of recent advances in plant nutrition at crop and molecular levels. *European journal of agronomy* **116**, 126069.

Buckley, S., Brackin, R., Näsholm, T., Schmidt, S., and Jämtgård, S. (2017). Improving in situ recovery of soil nitrogen using the microdialysis technique. *Soil biology & biochemistry* **114**, 93-103.

Cigan, A. M., Saeteurn, K., Hake, S., and Chuck, G. (2007). The heterochronic maize mutant Corngrass1 results from overexpression of a tandem microRNA. *Nature genetics* **39**, 544-549.

Daryanto, S., Wang, L., and Jacinthe, P.-A. (2017). Global synthesis of drought effects on cereal, legume, tuber and root crops production: A review. *Agricultural water management* **179**, 18-33.

Datta, S., Kim, C. M., Pernas, M., Pires, N. D., Proust, H., Tam, T., Vijayakumar, P., and Dolan, L. (2011). Root hairs: development, growth and evolution at the plant-soil interface. *Plant and soil* **346**, 1-14.

Davies, B., Coulter, J. A., and Pagliari, P. H. (2020). Timing and rate of nitrogen fertilization influence maize yield and nitrogen use efficiency. *PloS one* **15**, e0233674-e0233674.

Dechorgnat, J., Francis, K., Dhugga, K. S., Rafalski, J. A., Tyerman, S. D., and Kaiser, B. N. (2018). Root Ideotype Influences Nitrogen Transport and Assimilation in Maize. *Frontiers in Plant Science* **9**, undefined-undefined.

Doblas, V. G., Geldner, N., and Barberon, M. (2017). The endodermis, a tightly controlled barrier for nutrients. *Current opinion in plant biology* **39**, 136-143.

Fageria, N. K. (2014). "Nitrogen Management in Crop Production," First edition/Ed. CRC Press, Boca Raton.

G. Viana, W., Scharwies, J. D., and Dinneny, J. R. (2022). Deconstructing the root system of grasses through an exploration of development, anatomy and function. *Plant, cell and environment* **45**, 602-619.

Gao, Y., and Lynch, J. P. (2016). Reduced crown root number improves water acquisition under water deficit stress in maize (Zea mays L.). *Journal of experimental botany* **67**, 4545-4557.

Garnett, T., Conn, V., Plett, D., Conn, S., Zanghellini, J., Mackenzie, N., Enju, A., Francis, K., Holtham, L., Roessner, U., Boughton, B., Bacic, A., Shirley, N., Rafalski, A., Dhugga, K., Tester, M., and Kaiser, B. N. (2013). The response of the maize nitrate transport system to nitrogen demand and supply across the lifecycle. *New Phytologist* **198**, 82-94.

Garnett, T., Plett, D., Conn, V., Conn, S., Rabie, H., Rafalski, J. A., Dhugga, K., Tester, M. A., and Kaiser, B. N. (2015). Variation for N Uptake System in Maize: Genotypic Response to N Supply. *Frontiers in plant science* **6**, 936.

Gastal., F., Lemaire., G., Durand., J.-L., and Louarn., G. (2015). Quantifying crop responses to nitrogen and avenues to improve nitrogen-use efficiency. *In* "Crop Physiology

Applications for Genetic Improvement and Agronomy" (V. O. S. a. D. F. Calderini, ed.), pp. 161-206. Academic Press, United Kingdom.

Gonin, M., Bergougnoux, V., Nguyen, T. D., Gantet, P., and Champion, A. (2019). What makes adventitious roots? *Plants (Basel)* **8**, 240.

Gu, J., Li, Z., Mao, Y., Struik, P. C., Zhang, H., Liu, L., Wang, Z., and Yang, J. (2018). Roles of nitrogen and cytokinin signals in root and shoot communications in maximizing of plant productivity and their agronomic applications. *Plant science (Limerick)* **274**, 320-331.

Gu, R., Duan, F., An, X., Zhang, F., Von Wirén, N., and Yuan, L. (2013). Characterization of AMT-mediated high-affinity ammonium uptake in roots of maize (Zea mays L.). *Plant and cell physiology* **54**, 1515-1524.

Hachiya, T., and Sakakibara, H. (2017). Interactions between nitrate and ammonium in their uptake, allocation, assimilation, and signaling in plants. *Journal of experimental botany* **68**, 2501-2512.

Hawkesford, M., Horst, W., Kichey, T., Lambers, H., Schjoerring, J., Moller I.S., and White, P. (2012). Functions of Micronutrients. (P. e. Marschiner, ed.), Vol. 3, pp. 135-189. Academic Press, Mineral Nutrition of Higher Plants.

Hetz, W., Hochholdinger, F., Schwall, M., and Feix, G. (1996). Isolation and characterization of rtcs, a maize mutant deficient in the formation of nodal roots. *The Plant journal : for cell and molecular biology* **10**, 845-857.

Himanen, K., Boucheron, E., Vanneste, S., de Almeida Engler, J., Inzé, D., and Beeckman, T. (2002). Auxin-Mediated Cell Cycle Activation during Early Lateral Root Initiation. *The Plant cell* **14**, 2339-2351.

Hirel, B., Le Gouis, J., Ney, B., and Gallais, A. (2007). challenge of improving nitrogen use efficiency in crop plants: towards a more central role for genetic variability and quantitative genetics within integrated approaches. *Journal of experimental botany* **58**, 2369-2387.

Hochholdinger, F., Marcon, C., Baldauf, J. A., Yu, P., and Frey, F. P. (2018a). Proteomics of maize root development. *Frontiers in plant science* **9**, 143-143.

Hochholdinger, F., Park, W. J., Sauer, M., and Woll, K. (2004). From weeds to crops: genetic analysis of root development in cereals. *Trends in plant science* **9**, 42-48.

Hochholdinger, F., Yu, P., and Marcon, C. (2018b). Genetic Control of Root System Development in Maize. *Trends in Plant Science* **23**, 79-88.

Hostetler, A. N., Khangura, R. S., Dilkes, B. P., and Sparks, E. E. (2021). Bracing for sustainable agriculture: the development and function of brace roots in members of Poaceae. *Current opinion in plant biology* **59**, 101985-101985.

Huwei, S., Jinyuan, T., Yang, B., Mengmeng, H., Jiajing, L., Xinni, C., Xuhong, Z., Le, L., Xiaonan, X., Koichi, Y., Quanzhi, Z., Guohua, X., and Yali, Z. (2018). OsPIN1b is Involved in Rice Seminal Root Elongation by Regulating Root Apical Meristem Activity in Response to Low Nitrogen and Phosphate. *Scientific Reports* **8**, 1-11.

Iijima, M., Morita, S., and Barlow, P. W. (2008). Structure and function of the root cap. *Plant production science* **11**, 17-27.

Inman, D., Khosla, R., Westfall, D. G., and Reich, R. (2005). Nitrogen uptake across site specific management zones in irrigated corn production systems. *Agronomy journal* **97**, 169-176.

Jackson, W. A., and Volk, R. J. (1992). Nitrate and ammonium uptake by maize: adaptation during relief from nitrogen suppression. *The New phytologist* **122**, 439-446.

Jackson, W. A., and Volk, R. J. (1995). Attributes of the Nitrogen Uptake Systems of Maize (Zea mays L.): Maximal Suppression by Exposure to Both Nitrate and Ammonium. *The New phytologist* **130**, 327-335.

Jacoby, R., Peukert, M., Succurro, A., Koprivova, A., and Kopriva, S. (2017). The Role of Soil Microorganisms in Plant Mineral Nutrition-Current Knowledge and Future Directions. *Frontiers in plant science* **8**, 1617-1617.

Jung, J. K. H., and McCouch, S. (2013). Getting to the roots of it: Genetic and hormonal control of root architecture. *Frontiers in plant science* **4**, 186-186.

Kant, S. (2018). Understanding nitrate uptake, signaling and remobilisation for improving plant nitrogen use efficiency. *Seminars in Cell and Developmental Biology* **74**, 89-96.

Khosla, R. (2018). Science Breakthroughs 2030: Transforming Food and Agriculture Research. *CSANews (Madison, Wis.)* **63**, 14-15.

Kieber, J. J., and Schaller, G. E. (2018). Cytokinin signaling in plant development. *Development* **145**, dev149344.

Kim, H., Jang, J., Seomun, S., Yoon, Y., and Jang, G. (2022). Division of cortical cells is regulated by auxin in Arabidopsis roots. *Frontiers in plant science* **13**, 953225-953225.

Li, J., Chen, F., Li, Y., Li, P., Wang, Y., Mi, G., and Yuan, L. (2019). ZmRAP2.7, an AP2 Transcription Factor, Is Involved in Maize Brace Roots Development. *Frontiers in plant science* **10**, 820-820.

Li, P., Chen, F., Cai, H., Liu, J., Pan, Q., Liu, Z., Gu, R., Mi, G., Zhang, F., and Yuan, L. (2015). A genetic relationship between nitrogen use efficiency and seedling root traits in maize as revealed by QTL analysis. *Journal of experimental botany* **66**, 3175-3188.

Liu, C.-W., Sung, Y., Chen, B.-C., and Lai, H.-Y. (2014). Effects of nitrogen fertilizers on the growth and nitrate content of lettuce (Lactuca sativa L.). *International journal of environmental research and public health* **11**, 4427-4440.

López-Bucio, J., Cruz-Ramírez, A., and Herrera-Estrella, L. (2003). The role of nutrient availability in regulating root architecture. *Current opinion in plant biology* **6**, 280-287.

Lupini, A., Mercati, F., Araniti, F., Miller, A. J., Sunseri, F., and Abenavoli, M. R. (2016). NAR2.1/NRT2.1 functional interaction with NO3- and H+ fluxes in high-affinity nitrate transport in maize root regions. *Plant Physiology and Biochemistry* **102**, 107-114.

Lynch, J., Marschner, P., and Rengel, Z. (2012). Effects of Internal and External Factors on Root Growth and Development. *In* "Marschner's Mineral Nutrition for Higher Plants" (P. Marschner, ed.). Accademic Press, United Kingdom.

Majer, C., Xu, C., Berendzen, K. W., and Hochholdinger, F. (2012). Molecular interactions of ROOTLESS CONCERNING CROWN AND SEMINAL ROOTS, a LOB domain protein regulating shoot-borne root initiation in maize (Zea mays L.). *Philosophical Transactions of the Royal Society B: Biological Sciences* **367**, 1542-1551.

Marschner, P., and Rengel, Z. (2012). Nutrients availability in soils. *In* "Marschners Mineral Nutrition for Higher Plants" (P. Marschner, ed.), pp. 315-328. Academic Press, United Kingdom.

Masclaux-Daubresse, C., Daniel-Vedele, F., Dechorgnat, J., Chardon, F., Gaufichon, L., and Suzuki, A. (2010). Nitrogen uptake, assimilation and remobilization in plants: challenges for sustainable and productive agriculture. *Annals of botany* **105**, 1141-1157.

Maskova, T., and Klimes, A. (2019). The Effect of Rhizoboxes on Plant Growth and Root: Shoot Biomass Partitioning. *Frontiers in plant science* **10**, 1693-1693.

McKay Fletcher, D. M., Shaw, R., Sánchez-Rodríguez, A. R., Daly, K. R., van Veelen, A., Jones, D. L., and Roose, T. (2021). Quantifying citrate-enhanced phosphate root uptake using microdialysis. *Plant and soil* **461**, 69-89.

Meng, Y., Zhang, N., Li, J., Shen, X., Sheen, J., and Xiong, Y. (2022). TOR kinase, a GPS in the complex nutrient and hormonal signaling networks to guide plant growth and development. *Journal of experimental botany*.

Miller A.J., a. C. N. (2011). Transporters involved in Nitrogen Uptake annd Movement. (M. a. B. P. e. Hawkesford, ed.). Willey-Blackwell Publishers, UK.

Mira, M. M., El-Khateeb, E. A., Gaafar, R. M., Igamberdiev, A. U., Hill, R. D., and Stasolla, C. (2020). Stem cell fate in hypoxic root apical meristems is influenced by phytoglobin expression. *Journal of experimental botany* **71**, 1350-1362.

Motte, H., and Beeckman, T. (2019). The evolution of root branching: increasing the level of plasticity. *Journal of Experimental Botany* **70**, 785-793.

Nacry, P., Bouguyon, E., and Gojon, A. (2013). Nitrogen acquisition by roots: physiological and developmental mechanisms ensuring plant adaptation to a fluctuating resource. *Plant and Soil* **370**, 1-29.

Nath, M., and Tuteja, N. (2016). NPKS uptake, sensing, and signaling and miRNAs in plant nutrient stress. *Protoplasma* **253**, 767-786.

O'Brien, José a., Vega, A., Bouguyon, E., Krouk, G., Gojon, A., Coruzzi, G., and Gutiérrez, Rodrigo a. (2016). Nitrate Transport, Sensing, and Responses in Plants. *Molecular Plant* **9**, 837-856.

Ober., E., and M.A.J. Parry (2011). Drought and implications for nutrition. *In* "The molecular and physiological bases of nutrient use efficiency in crops." (H. M. a. B. P. (eds), ed.). Willey-Blackwell Publishers.

Paez-Garcia, A., Motes, C. M., Scheible, W.-R., Chen, R., Blancaflor, E. B., and Monteros, M. J. (2015). Root Traits and Phenotyping Strategies for Plant Improvement. *Plants* **4**, 334-355.

Pavlů, J., Novák, J., Koukalová, V., Luklová, M., Brzobohatý, B., and Černý, M. (2018). Cytokinin at the Crossroads of Abiotic Stress Signalling Pathways. *International journal of molecular sciences* **19**.

Powlson, D. S., Jenkinson, D. S., Johnston, A. E., Poulton, P. R., Glendining, M. J., and Goulding, K. W. T. (2010). Comments on "Synthetic Nitrogen Fertilizers Deplete Soil Nitrogen: A Global Dilemma for Sustainable Cereal Production," by R.L. Mulvaney, S.A. Khan, and T.R. Ellsworth in the Journal of Environmental Quality 2009 38:2295–2314. *Journal of environmental quality* **39**, 749-752.

Ramirez-Cabral, N. Y. Z., Kumar, L., and Shabani, F. (2017). Global alterations in areas of suitability for maize production from climate change and using a mechanistic species distribution model (CLIMEX). *Scientific reports* **7**, 5910-13.

Rasmussen, A., Hosseini, S. A., Hajirezaei, M.-R., Druege, U., and Geelen, D. (2015). Adventitious rooting declines with the vegetative to reproductive switch and involves a changed auxin homeostasis. *Journal of Experimental Botany* **66**, 1437-1452.

Rasmussen, A., Mason, M. G., De Cuyper, C., Brewer, P. B., Herold, S., Agusti, J., Geelen, D., Greb, T., Goormachtig, S., Beeckman, T., and Beveridge, C. A. (2012). Strigolactones suppress adventitious rooting in Arabidopsis and pea. *Plant physiology* **158**, 1976.

Saengwilai, P., Tian, X., and Lynch, J. P. (2014). Low Crown Root Number Enhances Nitrogen Acquisition from Low-Nitrogen Soils in Maize. *Plant physiology (Bethesda)* **166**, 581-589.

Sakr, S., Wang, M., Dédaldéchamp, F., Perez-Garcia, M.-D., Ogé, L., Hamama, L., and Atanassova, R. (2018). The Sugar-Signaling Hub: Overview of Regulators and Interaction with the Hormonal and Metabolic Network. *International journal of molecular sciences* **19**, 2506.

Sakuraba, Y., Kanno, S., Mabuchi, A., Monda, K., Iba, K., and Yanagisawa, S. (2018). A phytochrome-B-mediated regulatory mechanism of phosphorus acquisition. *Nature plants* **4**, 1089.

Sandhu, N., Sethi, M., Kumar, A., Dang, D., Singh, J., and Chhuneja, P. (2021). Biochemical and Genetic Approaches Improving Nitrogen Use Efficiency in Cereal Crops: A Review. *Frontiers in plant science* **12**, 657629-657629.

Schmidt, J. E., Poret-Peterson, A., Lowry, C. J., and Gaudin, A. C. M. (2020). Has agricultural intensification impacted maize root traits and rhizosphere interactions related to organic N acquisition? *AoB plants* **12**, plaa026-plaa026.

Sebastian, J., Yee, M.-C., Viana, W. G., Álvarez, R. R., Feldman, M., Priest, H. D., Trontin, C., Lee, T., Jiang, H., Baxter, I., Mockler, T. C., Hochholdinger, F., Brutnell, T. P., and Dinneny, J. R. (2016). Grasses suppress shoot-borne roots to conserve water during drought. *Proceedings of the National Academy of Sciences - PNAS* **113**, 8861-8866.

Shahzad, Z., and Amtmann, A. (2017). Food for thought: how nutrients regulate root system architecture. *Current Opinion in Plant Biology* **39**, 80-87.

Sharma, L. K., Bu, H., Franzen, D. W., and Denton, A. (2016). Use of corn height measured with an acoustic sensor improves yield estimation with ground based active optical sensors. *Computers and electronics in agriculture* **124**, 254-262.

Shaw, R., Williams, A. P., and Jones, D. L. (2014). Assessing Soil Nitrogen Availability using Microdialysis-Derived Diffusive Flux Measurements. *Soil Science Society of America journal* **78**, 1797-1803.

Shen, C., Wang, S., Zhang, S., Xu, Y., Qian, Q., Qi, Y., and Jiang, D. A. (2013). OsARF16, a transcription factor, is required for auxin and phosphate starvation response in rice (Oryza sativa L. *Plant, Cell & Environment* **36**, 607-620.

Singh, R. B. (2000). Environmental consequences of agricultural development: a case study from the Green Revolution state of Haryana, India. *Agriculture, ecosystems & environment* **82**, 97-103.

Song, Y., Alyafei, M. S., Masmoudi, K., Jaleel, A., and Ren, M. (2021). Contributions of TOR Signaling on Photosynthesis. *International journal of molecular sciences* **22**, 8959.

Sparks, E. E. (2023). Maize plants and the brace roots that support them. *The New phytologist* **237**, 48-52.

Sun, X., Chen, H., Wang, P., Chen, F., Yuan, L., and Mi, G. (2020). Low nitrogen induces root elongation via auxin-induced acid growth and auxin-regulated target of rapamycin (TOR) pathway in maize. *Journal of plant physiology* **254**, 153281.

Suzuki, M., Sato, Y., Wu, S., Kang, B.-H., and McCarty, D. R. (2015). Conserved Functions of the MATE Transporter BIG EMBRYO1 in Regulation of Lateral Organ Size and Initiation Rate. *The Plant cell* **27**, 2288-2300.

Taiz, L., and Zeiger, E. (2006). "Plant physiology,," fourth edition,/Ed. Sinauer associate inc publishers., USA.

Tian, Q., Chen, F., Liu, J., Zhang, F., and Mi, G. (2008). Inhibition of maize root growth by high nitrate supply is correlated with reduced IAA levels in roots. *Journal of plant physiology* **165**, 942-951.

Wang, H., Tang, X., Yang, X., Fan, Y., Xu, Y., Li, P., Xu, C., and Yang, Z. (2021). Exploiting natural variation in crown root traits via genome-wide association studies in maize. *BMC plant biology* **21**, 1-346.

Wortmann, C. S., Tarkalson, D. D., Shapiro, C. A., Dobermann, A. R., Ferguson, R. B., Hergert, G. W., and Walters, D. (2011). Nitrogen Use Efficiency of Irrigated Corn for Three Cropping Systems in Nebraska. *Agronomy journal* **103**, 76-84.

Wu, Y., Shi, L., Li, L., Fu, L., Liu, Y., Xiong, Y., and Sheen, J. (2019). Integration of nutrient, energy, light, and hormone signalling via TOR in plants. *Journal of experimental botany* **70**, 2227-2238.

Xuan, W., and Beeckman, T. (2021). The development of crop root architecture and optimization of nutrition acquisition: the case of rice. *In* "Understandimg and improving crop oot function" (P. Gregory, ed.), pp. 33-53. Burleigh dodd, United Kingdom.

York, L. M., and Lynch, J. P. (2015). Intensive field phenotyping of maize (Zea mays L.) root crowns identifies phenes and phene integration associated with plant growth and nitrogen acquisition. *Journal of experimental botany* **66**, 5493-5505.

Yu, P., Baldauf, J. A., Lithio, A., Marcon, C., Nettleton, D., Li, C., and Hochholdinger, F. (2016a). Root Type-Specific Reprogramming of Maize Pericycle Transcriptomes by Local High Nitrate Results in Disparate Lateral Root Branching Patterns. *Plant physiology (Bethesda)* **170**, 1783-1798.

Yu, P., Eggert, K., von Wirén, N., Li, C., and Hochholdinger, F. (2015a). Cell Type-Specific Gene Expression Analyses by RNA Sequencing Reveal Local High Nitrate-Triggered Lateral Root Initiation in Shoot-Borne Roots of Maize by Modulating Auxin-Related Cell Cycle Regulation. *Plant physiology (Bethesda)* **169**, 690-704.

Yu, P., Gutjahr, C., Li, C., and Hochholdinger, F. (2016b). Genetic Control of Lateral Root Formation in Cereals. *Trends in Plant Science* **21**, 951-961.

Yu, P., Hochholdinger, F., and Li, C. (2015b). Root-type-specific plasticity in response to localized high nitrate supply in maize (Zea mays). *Annals of Botany* **116**, 751-762.

Yu, P., Li, X., Yuan, L., and Li, C. (2014). A novel morphological response of maize (Zea mays) adult roots to heterogeneous nitrate supply revealed by a split-root experiment. *Physiologia plantarum* **150**, 133-144.

Zhang, Y., Ma, L., Pan, X., Wang, J., Guo, X., and Du, J. (2018). Micron-scale Phenotyping Techniques of Maize Vascular Bundles Based on X-ray Microcomputed Tomography. *Journal of visualized experiments*.

Chapter 2

Spatio-temporal Dynamics of N forms and Nitrogen Balances of Maize Root Type

2.1. Abstract

As described in the literature review, not much is known about differences between maize root types for nitrogen uptake or how that changes the rhizosphere nutrient availability.

To address this gap in knowledge, maize plants were grown as high and low nitrogen plants in the glasshouse inside openable window rhizoboxes containing sand-compost low N soil. Then one litre of either low or high N solution were sprayed on the exposed surface of the opened rhizobox when the plants were at 3 weeks post germination. Microdialysis (a novel technology that employ the principle of osmosis) was used to sample nutrient ions intermittently for 44 hours from soils at predetermined positions that are closer or far from maize root types. Samples collected were assayed for NO_3^- and NH_4^+ concentration using colorimetric methods. The net fluxes of NO_3^- and NH_4^+ by root types were also measured using ^{15}N stable isotope tracer. Samples were sent to UC Davis stable isotope facility for analysis.

Results showed that there was difference both between nitrogen sources and root types. For low N-grown plants, the nitrogen (both ammonium and nitrate) depleted quicker adjacent to crown roots compared to adjacent to seminal roots. Under high N conditions, nitrate depleted faster adjacent to seminal roots compared to crown roots but there was minimal difference between root types and ammonium depletion.

Net influx of ^{15}N showed that crown roots preferentially took up ammonium over nitrate in low N treated plants. While the seminal roots appear to capture more N sources at equal rate in high N plants but more ammonium in low N plants. Interestingly plants treated with high N had a higher uptake of stable isotope than those growing on low N for both root types and N-sources.

In summary, these results show that crown roots are better able to capture nitrogen sources in low N background. This suggests that there is more potential for maize crown root selection as a breeding target for improved nitrogen use efficiency.

2.2. Introduction

Nitrate and ammonium are the main forms of N available to roots in arable fields with nitrate being the predominant N source in aerated soils and ammonium as predominant N in anaerobic environment (Hachiya and Sakakibara, 2017; Kiba and Krapp, 2016). Nutrient uptake depends on ion concentration at the root surfaces, root absorption capacity and plant requirements (Fageria, 2014; Fageria. et al., 2011; Gastal. et al., 2015; Shahzad and Amtmann, 2017). Since availability at the root surface is so important (Fageria. et al., 2011), root phenotypes that rapidly move through the soil may be better able to capture nitrogen. Once in the roots, nitrate moves through symplastic pathways, across each cell type and into the xylem for long-distance transport to leaves via the transpiration stream (Hawkesford et al., 2012). Contrastingly, ammonium is usually assimilated into organic compound in the roots and transported through the xylem to other parts of the plant (Hawkesford et al., 2012).

The maize root system comprises of embryonic (primary and seminal) and postembryonic (brace, crown and lateral) roots (Hochholdinger et al., 2004; Hochholdinger et al., 2018; Saengwilai et al., 2014; Yu et al., 2015a, 2019). All maize root types are comprised of distinct cells and tissues with varying specification of gene expression related to their individual roles (Hochholdinger and Tuberosa, 2009). This includes expression of nutrient specific transporters which are present in different root types and affect the efficiency with which roots can take up nitrogen (Dechorgnat et al., 2018). Under low N conditions, the plant will increase transporter expression and increase net nitrogen uptake (Dechorgnat et al., 2018; Garnett et al., 2013), however most studies have focused on seedlings or plants grown in hydroponics. The other gap in understanding maize roots nitrogen use efficiency (NUE) is the relative preference for different nitrogen sources. Analysis of transporter expression can only take this field part of the way; additional techniques are required.

A previous study employed microdialysis and microCT technology to investigate the accumulation and depletion zones of nitrate and ammonium adjacent to maize roots and the background soil (Brackin et al., 2017). They found that nitrates accumulated adjacent to roots while ammonium was greater in the background compared to roots. But how long does it take for applied nitrate and ammonium ions to deplete? And are there differences in the rate of depletion adjacent to each root type? Based on the literature, no nitrogen uptake study reflecting real time spatiotemporal dynamics of applied nitrogen forms around intact roots of maize grown in soil has been carried out. Thus, this study explored the nitrate and ammonium dynamics adjacent to maize crown (post embryonic) and seminal (embryonic) roots as well as the background under low and high nitrogen soil condition using microdialysis and ^{15}N stable isotopes in nitrate and ammonium. Plants were grown on low or high N

soil and then just prior to microdialysis the rhizobox interface was saturated once more with high or low N for monitoring the rates of change.

This was designed to identifying essential root traits that could be optimized for root architecture for incorporation into commercial maize germplasm.

The hypotheses tested were:

Section A: The spatiotemporal dynamics of nitrogen forms.

-Nitrate will more rapidly deplete in soil than ammonium during adequate and limited N supply. This is because nitrate is an anion which can be easily carried by mass flow, unlike ammonium being a cation which will bound to the soil matrix.

- nitrate and ammonium will deplete more faster adjacent to roots of plants grown on low nitrogen background than adjacent to roots of plants grown on high N background. This is due to high plant demand for nitrogen source.

-Supplying high N plants with low N solution and low N plants with high N solution will trigger more rapid depletion of N sources than when high and low N plants were given their usual nitrogen supply especially in plants grown on limited N supply. This will occur because of changes resulting from perceived change in external nutrient availability.

Section B: Net nitrogen uptake by root types.

- Crown roots of plants grown on a low nitrogen background will capture more nitrogen sources than their seminal roots and both root types in plants grown on high nitrogen background. This is due to its larger diameter and larger meta xylem vessels of crown roots than that of the seminal roots.

- Crown roots of all plants irrespective of growth condition will capture more nitrogen sources when the rhizosphere of high and low N plants is sprayed with low and high N respectively, than when they are given their usual nitrogen supply. This will be due to change resulting from a perceived adjustment in external nutrient supply.

2.3. Methods

2.3.1. Experimental design

- *High nitrogen resupply experiment.*

The purpose of the high N resupply experiment was determined how long it would take for one litter of high nitrogen solution to deplete adjacent to the maize crown, seminal roots and the bulk soil which is further from the roots (termed as "background"), in plants grown on low and high nitrogen condition. It also aimed to compare the depletion rates of nitrate and ammonium in relation to the proximity of probes to the roots at 0 hrs (immediately after nutrient spray), 3 hrs (possible short-term induction of nitrate and ammonium transporters), 18, 24 (possible full-term induction of nitrate and ammonium transporters), 27, and 44 hours after high nitrogen resupply.

The experiment was carried out in the glasshouse in a completely randomized design in a 2 x 5 arrangement. The main factors were two levels of nitrogen consisting of adequate N (high N (4 mM)) and sub-optimum N (low N (0.3 mM)) based on Zhan and Lynch, (2015), until the resupply of high nitrogen solution at 21 days post germination during microdialysis. The plants for the experiment (August-September 2018) were planted and treated in 2 batches to enable effective data collection at the same plant age (Figure 2.0.A). During the experiment, 4 high N and 4 low N plants were sprayed with high N solution prior to microdialysis using a hand pump pressure sprayer, while 1 high N and 1 low N plants were left as control. The shoot measurements for long-term longitudinal responses (longitudinal responses in Chapter 3 of the thesis) were taken weekly before the high N resupply, up to 5 weeks after. Table 2.1. summarized the number of plants and concentration of nitrogen resupply per plants for the high nitrogen resupply experiment and the other two microdialysis experiment to be discussed below.

HN	HN	HN	UNSPRAYED	HN
STAGGARED PLANTING FOR SEVEN DAYS				
HN	HN	UNSPRAYED	HN	HN

Figure 2.0.A. Experimental design for the high nitrogen resupply experiment. Cells of light orange colour denotes plants grown on low nitrogen background while the cells of dark green colours are plants grown on high nitrogen background. For the nitrogen resupply, (HN) implies sprayed with high nitrogen solution, and (UNSPRAYED) are the control plants not sprayed with nitrogen solution.

- *Normal nitrogen resupply experiment.*

The purpose of the normal nitrogen resupply experiment was to test whether the supply of low nitrogen to low N plants and high nitrogen to high N plants will influence the depletion rates of nitrogen forms at 0hrs (immediately after nutrient supply) and after 24 hrs of nutrient supply (possible full induction of nitrogen transporters). Also, If the availability of the plants normal nitrogen levels within 24 hours could impact on the uptake rates of nitrogen forms by maize crown or seminal roots due to the anticipated activities of nitrogen transporters. Previous study showed that the influx activities of nitrogen transporters doubled after 24 hrs of nitrogen deprivation (Hole et al., 1990).

The normal nitrogen resupply experiment (November-December 2018) was carried out in the glasshouse in a completely randomized design of 2 x 6 factorial arrangement. The main factors were two levels of nitrogen consisting of adequate N (high N (4 mM)) and sub-optimum N (low N (0.3 mM)) based on Zhan and Lynch, (2015), until resupply of either high or low N (pulses). As with the high nitrogen resupply experiments, the plants for the normal nitrogen resupply were also staggered for seven days during planting time (Figure 2.0.B) to enable data collection from all plants at the same physiological age. During the normal nitrogen resupply experiment, 3 high N plants were sprayed with high N solution while 3 low N plants were sprayed with low N solution (Table 2.1). The remaining 3 high N and 3 low N served as controls for the nitrogen uptake by intact maize crown and seminal roots. The nutrient solution was uniformly sprayed on the entire soil surface of the rhizoboxes using a hand pump pressure sprayer prior to microdialysis after taking shoot measurements. This is to ensure that the plants were not stressed before measuring the uptake of nitrate and ammonium by intact maize crown and seminal roots.

UNSPRAYED	LN	HN
LN	UNSPRAYED	HN
STAGGARED PLANTING FOR SEVEN DAYS		
UNSPRAYED	UNSPRAYED	LN
UNSPRAYED	HN	UNSPRAYED

Figure 2.0.B. Experimental design for the normal nitrogen resupply experiment. Cells of orange colour denotes plants grown on low nitrogen background while the cells of green colours are plants grown on high nitrogen background. For the nitrogen resupply, (HN) implies sprayed with high nitrogen solution, (LN) sprayed with low nitrogen solution, (UNSPRAYED) are the control plants not sprayed with nitrogen solution.

- *Factorial nitrogen resupply experiment:*

The purpose of the factorial nitrogen resupply experiment was to determine whether a difference in nitrate or ammonium depletion rates in relation to the proximity of the microdialysis probes to the roots would occur due to an increase or decrease in the concentration of nitrogen supply in low and high nitrogen plants. Also, if the differences in the depletion rates would be noticed at any sampling time of 0, 3, 6, 18, 24, 27, 33, 44 hours after nutrient resupply. This is in anticipation of the induction of nitrate and ammonium transporters after nutrient pulses were applied. The choice of the sampling times was based on the anticipation of the activities of nitrate and ammonium transporters. Previous study reported double rise in the activities of nitrogen transporters of maize roots after 24 hrs of nitrogen deprivation (Hole et al., 1990). Meanwhile a maize study showed that ZmNRT 2.1 contributed the most to the net nitrate uptake by maize roots during high nitrate supply (Garnett et al., 2013). However, a decrease in the quantity of NRT 2.1 was observed when plants were transferred for at least 3 days on high nitrogen (10mM ammonium nitrate or 5mM nitrate) as compared to plants grown on low nitrogen (0.3mM or 1mM) (Jacquot et al., 2017). Therefore, it is important to understand the depletion rate of nitrogen forms as may be influenced by the concentration of nitrogen applied. The

factorial nitrogen resupply experiment also aimed to determine if the concentration of nitrogen sprayed on plants within 44 hours would cause an increase or decrease in the net effect of nitrate and ammonium uptake by intact maize crown and seminal roots.

The factorial nitrogen resupply experiment (July-August 2019) was carried out in the glasshouse in a completely randomized design of 2 x 12 factorial arrangement. The main factors were two levels of nitrogen consisting of adequate N (high N (4 mM)) and sub-optimum N (low N (0.3 mM)) based on Zhan and Lynch, (2015), until resupply of either high or low N. During the experiment, 4 high N plants were sprayed with high N solution, another 4 high N plants were sprayed with low N solution while the remaining 4 high N plants were left as control. Similarly, 4 low N plants were sprayed with high N solution, another 4 low N plants were sprayed with low N solution while the remaining 4 low N plants were left as control. All controls served as a check on the uptake of N sources by intact maize crown and seminal roots after 44 hours of nitrogen resupply. The nutrients were supplied by homogenously spraying one litter of the required nutrient solution on the entire soil surface using a hand pump pressure sprayer prior to microdialysis. This is after taking shoot measurements for longitudinal responses of the maize shoot to short-term nitrogen availability (longitudinal responses in Chapter 3 of the thesis). Figure 2.0.C. shows the experimental design for the factorial nitrogen resupply experiments and Table 2.1. summarized the number of plants and concentration of nitrogen resupply per plants for the three microdialysis experiments outlined in this Chapter.

LN	LN	UNSPRAYED
HN	UNSPRAYED	HN
STAGGARED PLANTING FOR SEVEN DAYS		
UNSPRAYED	LN	LN
UNSPRAYED	HN	HN
STAGGARED PLANTING FOR SEVEN DAYS		
HN	UNSPRAYED	HN
LN	UNSPRAYED	LN
STAGGARED PLANTING FOR SEVEN DAYS		
HN	HN	UNSPRAYED
LN	UNSPRAYED	LN

Figure 2.0.C. Experimental design for the factorial nitrogen resupply experiment. Cells of orange colour denotes plants grown on low nitrogen background while the cells of green colours are plants grown on high nitrogen background. For the nitrogen resupply, (HN) implies sprayed with high nitrogen solution, (LN) sprayed with low nitrogen solution, (UNSPRAYED) are the control plants not sprayed with nitrogen solution.

Table 2.1. Nutrient resupply regimes during microdialysis. All plants were grown either on high or low nitrogen soil conditions for 3 weeks before the resupply treatments followed by sampling of available dissolved nitrate and ammonium ions using microdialysis in time sequence.

Experiments	*Number of plants*	*Background growth conditions*	*Nutrient resupply*
1. High N resupply	4	High	High N
	4	Low	High N
	1	High	Unsprayed
	1	Low	Unsprayed
	Total =10 plants		
*2. High N background resupplied with high N; Low N background resupplied with low N (*Normal *resupply)*	3	Low	Low
	3	High	High
	3	Low	Unsprayed
	3	High	Unsprayed
	Total =12 plants		
3. Factorial resupply	4	Low	Low
	4	High	Low
	4	Low	High
	4	High	High
	4	Low	Unsprayed
	4	High	Unsprayed
	Total =24 plants		

High= nitrogen concentration of (4mM), Low= nitrogen concentration of (0.3mM) based on Zhan and Lynch, (2015), Unsprayed = plants grown on low or high nitrogen background but were not sprayed for microdialysis. The unsprayed plants serve as control during the measurement of plant physiological response to nitrogen resupply.

2.3.2. Plant materials and growth conditions for all the three microdialysis experiments

Seeds used were from the "KWS experimental maize line" from KWS company limited. The study used openable window rhizoboxes as shown in Figure 2.1. to enable access to intact growing roots non-destructively for live quantitative measurements. The openable window rhizoboxes were placed on trolleys and filled with about 70kg of Klassman silver sand:compost 1:1 soil mix. This resulted in an average bulk density of 0.94g/cm^3 of the soil in each rhizobox. The Klassman tray substrate had pH of 5.8, salt level of 0.5 g/litre, nitrogen: 60 mg/litre, phosphorus (P_2O_5): 70 mg/litre, potassium (K_2O): 120 mg/litre, magnesium (Mg): 100 mg/litre, a mix of frozen black peat and white peat. Soil structure was extra fine 0-5 mm (mixed in University of Nottingham, UK) hereafter referred to as 'soil'). Once filled with soil, the boxes were watered from a hose connected to a tap running at moderate pressure to thoroughly wet the soil and allowed excess water to drain for 24 hrs to achieve field capacity. Field capacity is interchangeably used with the term water holding capacity and represents the amount of soil moisture or water content held in the soil after excess water has drained away from the soil macrospores and the rate of downward movement of water by gravity has significantly decreased (de Oliveira et al.,2015). The soil moisture content at field capacity promotes high water and nutrients absorption by plant (de Oliveira et al.,2015).

The maize seeds were planted directly into the soil at an average depth of 2 cm from soil surface with one seed per rhizobox. The seeds were placed such that the radicle faced the glass side of the rhizobox to enable root visualization and accessibility. Plants were grown on day/night cycle of 14 hrs/10 hrs and day/night average temperature 28°C / 22°C for the experiments to determine the spatiotemporal dynamics of N forms from applied high N solution over time and the factorial nitrogen resupply experiments. The experiment to determine the effects of the 24-hour induction of nitrate and ammonium transporters for N uptake had 12 hrs/12 hrs day/night cycle with average temperatures of 25°C /22°C. The difference in the temperature between experiments is caused by weather conditions the former were experiments carried out in between spring and summer, while the latter (having the lowest temperature was the maximum temperature that can be achieved in the glasshouse) was caried out during the autumn to winter.

Nutrient solution used (excluding nitrogen sources) contained: 5 mM KCl, 5 mM $CaCl_2$, 2 mM $MgSO_4$, 2 mM KH_2PO_4, 0.05 mM FeEDTA. The micronutrients contained: 9 µM $MnCl_2$, 0.2 µM $CuSO_4$, 46 µM H_2BO_3, 0.58 µM Na_2MoO_4, 0.77 µM $ZnSO_4$). For the nitrogen source: High N was 4 mM NH_4NO_3 and low N was 0.3 mM NH_4NO_3 (these nutrients both for high and low N were based on (Zhan and Lynch, 2015)). Plants were fed twice a week with 2 litres of low or high nutrient solution per feeding from 7 days after germination.

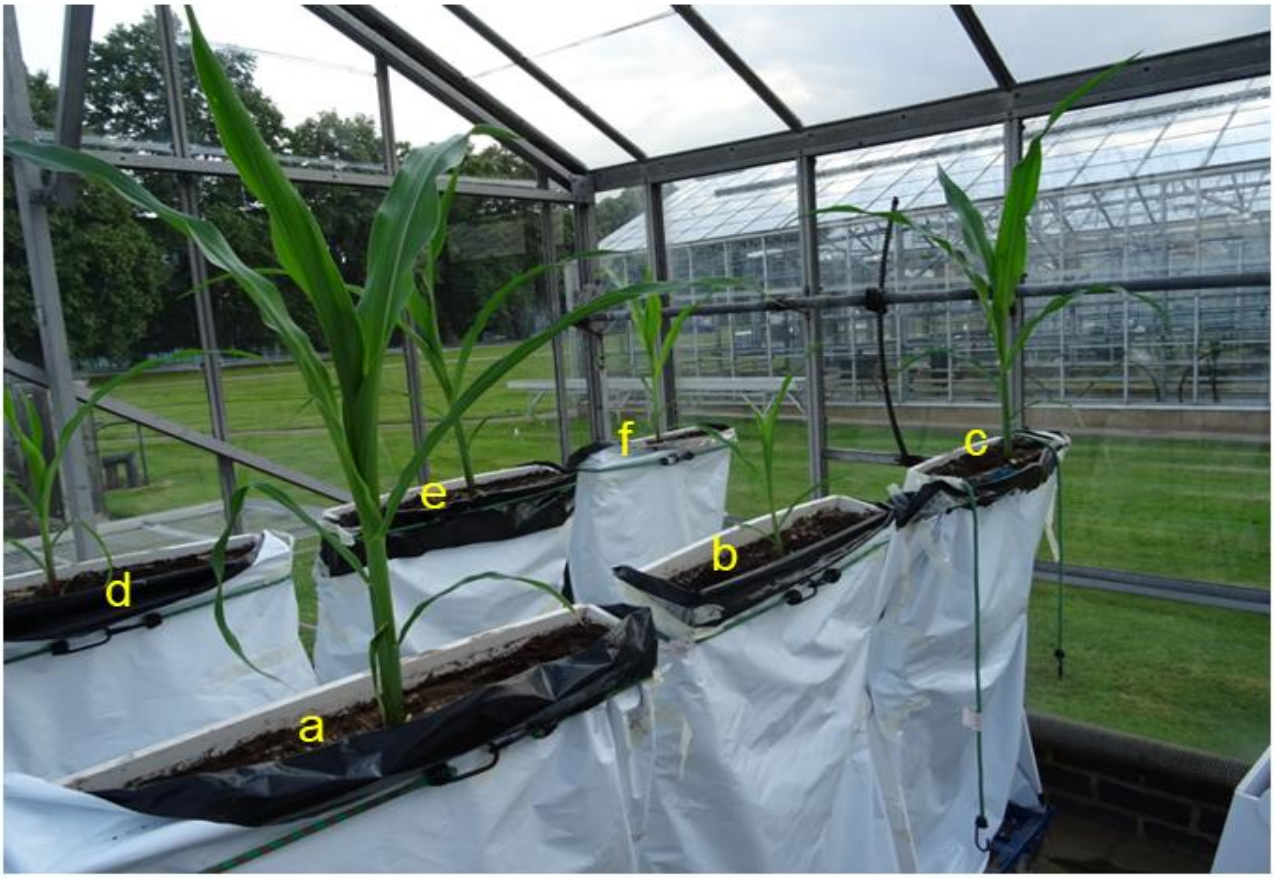


Figure 2.1. Growth conditions: Maize were grown in openable window rhizoboxes as low (b, d, f) or high (a, c, e) nitrogen plants and arranged randomly based on a straight line and almost equal gap from the next line of rhizoboxes to mimic plant spacing to reduce neighbour to neighbour competition. The glass surface of the rhizoboxes was covered with a black and white polyethene sheet to provide darkness for the growing seed and subsequently plant roots. A bungee rope and masking tape were used to tie and stick the polythene sheet to the rhizoboxes. Plants were fed twice a week for 3 weeks before determining the spatio temporal dynamics of nitrogen forms. For all the three microdialysis experiments namely: High nitrogen resupply, Normal nitrogen resupply, and Factorial nitrogen resupply, plantings were staggered for a week to enable data collection from all plants at the same physiological age.

2.3.3. Soil nutrient ion sampling using microdialysis

To sample nitrogen from the soil adjacent to maize crown and seminal roots as well as the background soil, microdialysis was used (Figure 2.2.A). For each plant, two seminal and two crown roots were chosen and a microdialysis probe positioned close to each root. Additionally, 4 or 5 probes were positioned at a greater distance from the roots (>2.5 cm) as an indicator of background nitrogen levels. The microdialysis probes (10 mm long probes with a 20 kDa cut-off-CMA Microdialysis AB, Solna, Sweden) were connected to a microdialysis pump (CMA 4004, CMA Microdialysis AB, Solna Sweden) and deionized water was pumped through the system at the flux rate of 5 µl/min previously proposed (Brackin et al., 2015; Brackin et al., 2017) as shown in Figure 2.2.B. and collected in 1.5 ml Eppendorf tubes. Sampling continued for 1 hour per sampling interval of 0, 3, 18, 24, 27, 44 hours for the high nitrogen resupply experiment, 0 and 24 hrs for the normal nitrogen resupply experiment and 0, 3, 6, 18, 24, 27, 30, 44 hrs for the factorial nitrogen resupply experiment. The samples collected in Eppendorf tubes were frozen at -20°C for subsequent analysis.

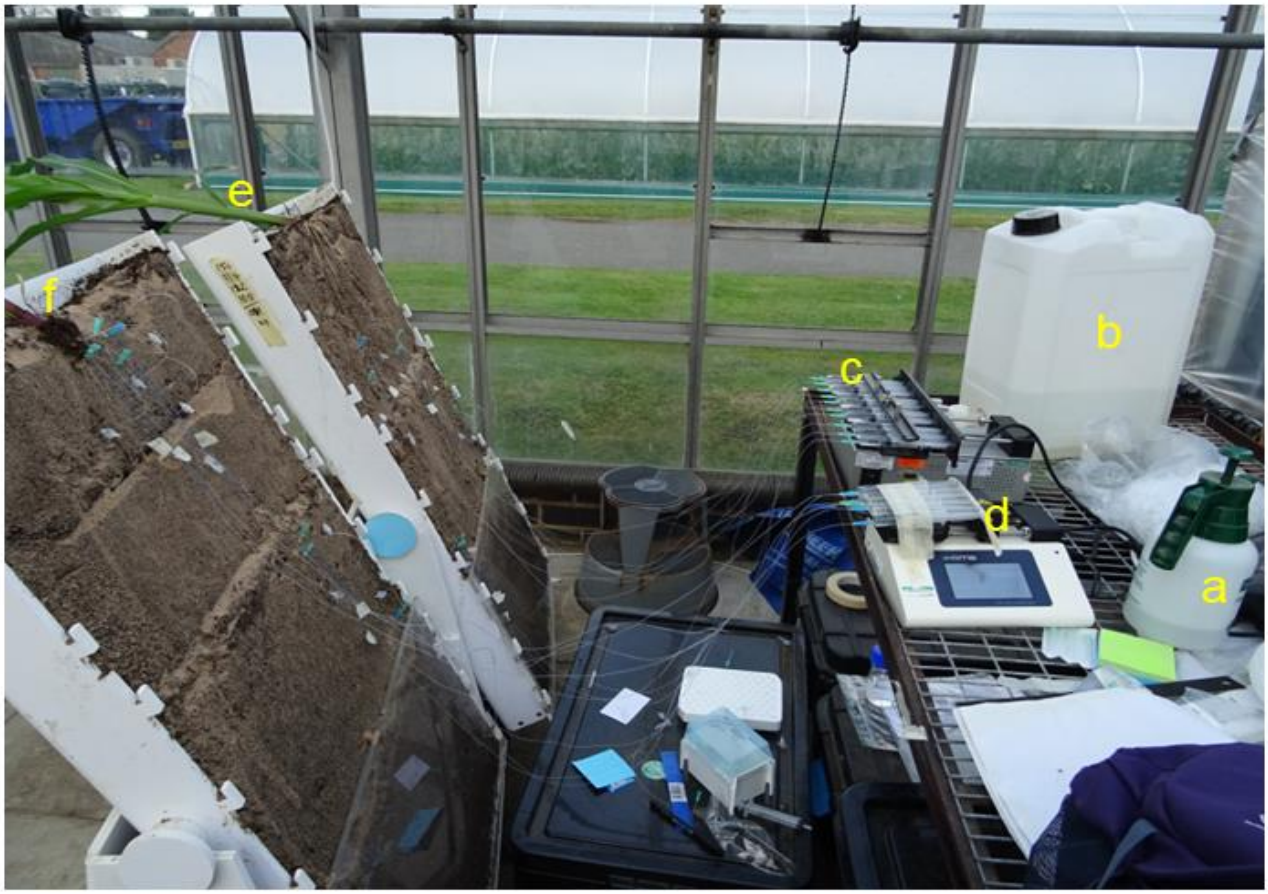


Figure 2.2.A. Microdialysis set-up: . The set-up comprised of (a) pressure hand sprayer, (b) keg of distled water for filling of synringes, (c) and (d) are the microdialysis pump, (e) high N plant and (f) low N plant. For the experiment, a rhizobox of high and low N plants was opened and 1 litre solution of high or low nitrogen in the hand sprayer were sprayed uniformly on the surface soil of the rhizoboxes as follows: High N resupply, high N solution were sprayed on 4 high and 4 low N plants and nutrient ions was sampled at 0, 3, 18, 24, 27, and 44 hours. For the normal nitrogen resupply high N solution were sprayed on 3 high N plants and low N solution on 3 low N plant before sampling of nutrients ions at 0 and 24 hours. For the factorial resupply 4 high and 4 low N plants were sprayed with high N solution, 4 high and 4 low N plants were sprayed with low N solution and nutrient ions were sampled at 0, 3, 6, 18, 24, 27, 33, 44 hours. When the soil surface appeared dry during sampling, clean water was sprayed lightly to allow for mass flow.

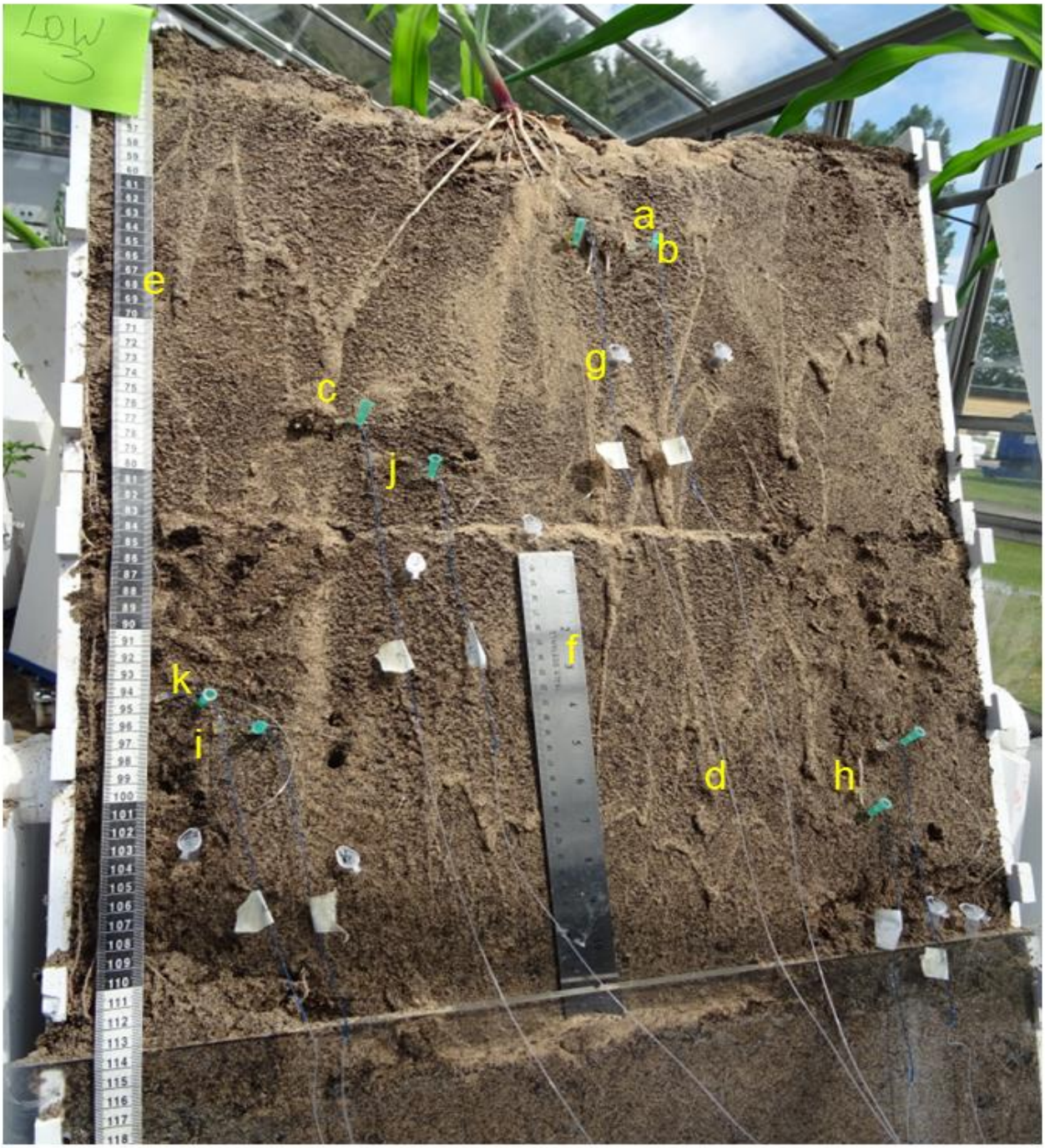


Figure 2.2.B. Probe positioning during microdialysis. All probes adjacent to roots are less than 2.5 cm to the roots (< 2.5 cm), while probes at the background are greater than 3 cm from the roots (> 2.5 cm), but not more than 10 cm from the roots. (a) probe adjacent to crown root (b) injection needle anchoring the probes to the soil with minimal soil disturbance, (c) probe adjacent to crown root, (d) connector dialysate perfusing distilled water at 5 µl during sampling of dissolved soil ions, (e) measuring tape for the determination of distance in cm and conversion of cm measurements to photo pixels unit during image analysis, (f) ruler for determination of distances, (g) Eppendorf tubes for sample collection, (h)visible root out growth away from previous position after 24 hours, (i) and (j) are probes at the background (far from roots), (k), probe closer to seminal root.

2.3.4. Nitrate and ammonium assays

For the analysis, nitrate concentration was measured using vanadium chloride and the Griess reaction (Brackin et al.,2017), while ammonium was measured using the phenol-hypochlorite method (Brackin et al., 2017), adapted for 96 well plate. The colour intensity at 540 nm and 625 nm was used for nitrate and ammonium respectively, measured on a calorimetric plate reader (MRX 11 plate reader, Dynex Technologies) and converted to concentrations using a standard curve of nitrate and ammonium respectively. Numerical values obtained were converted to flux rates (amount of N available per unit surface area of the probe per hour (nmol N-$cm^{-2}h^{-1}$)).

2.3.5. Root nitrogen uptake capacity

Root type nitrogen uptake capacities were measured as net influx measurement. Crown and seminal roots from the treatments described above were selected for nitrogen uptake and preference (Table 2.2). The selection of roots was based on the accessibility without disturbance. Each root was fed a mixture of 2 mM potassium nitrate and 1 mM ammonium sulphate with one compound labelled with ^{15}N in each mixture (making 4 mM of nitrogen in each mixture) (2 mM KNO_3 + 1 mM $(^{15}NH_4)_2SO_4$, or 2 mM $K^{15}NO_3$ + 1 mM $(NH_4)_2SO_4$). Treatments were left for 30 min as shown in Figure 2.3 A & B.

The fed roots were excised, rinsed in 10 mM KCl solution and subsequently in deionized water for removal of surface bound label. The excised washed roots were patted dry with paper towels and photographed for length and diameter and later air dried at room temperature. The dried samples were ground using a mortar and pestle and about 8 mg of each were weighed in tin capsules.

Samples were sent to UC Davis stable isotope lab (USA) for ^{15}N analysis using an elemental analyser interface to a continuous flow isotope ratio mass spectrometer (Sercon Ltd, Cheshire, UK). The net uptake rate of NH_4^+ and NO_3^- per unit weight per hour (umol g^{-1}dw h^{-1}) were calculated from the results of the stable isotope analysis.

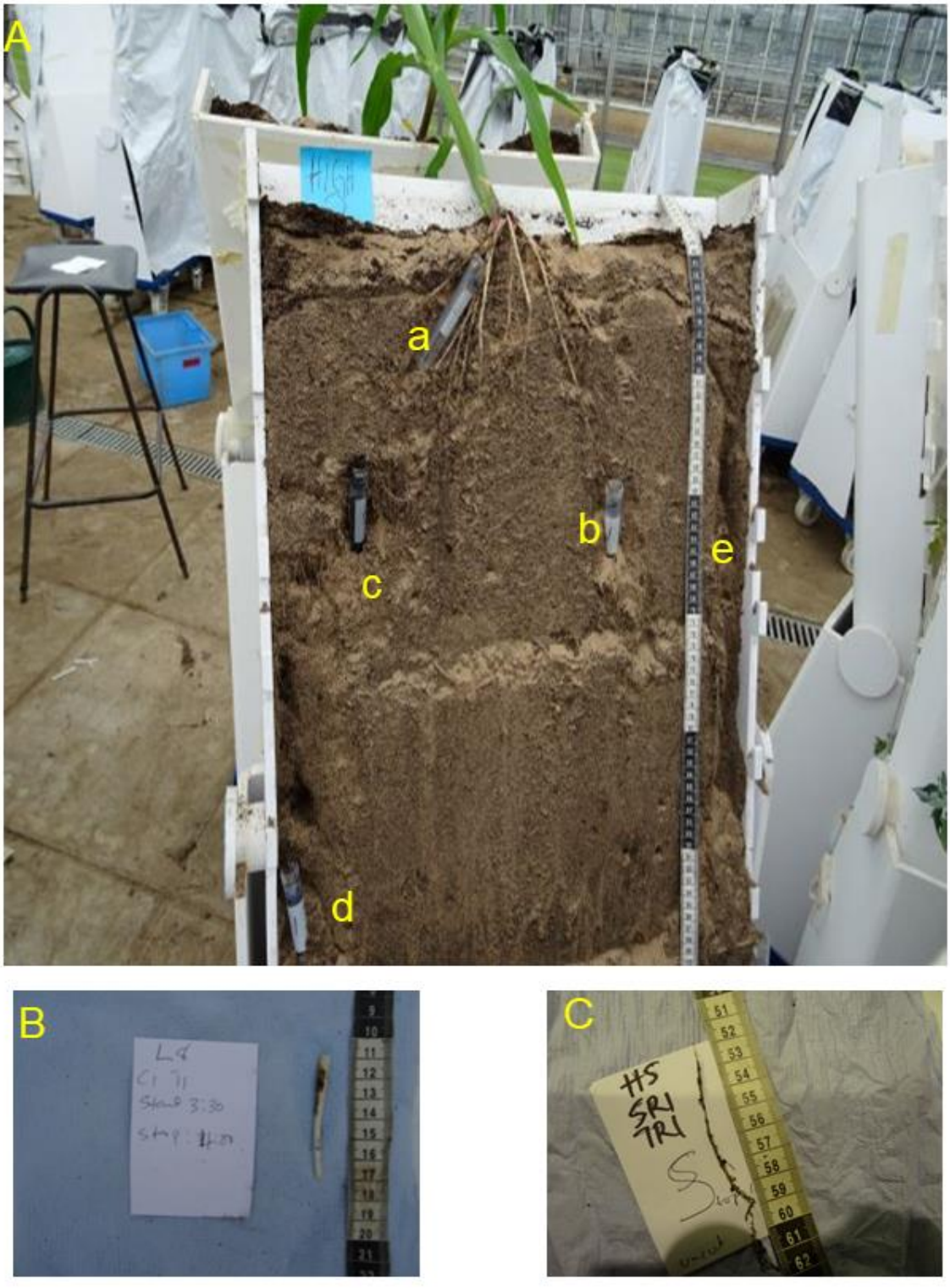


Figure 2.3.A. Stable isotope nitrogen uptake by intact maize roots immediately after the 44 hrs soil nutrient sampling: (A) shows how individual roots were fed with 4mM nitrogen stable isotope. (a) crown root fed with ^{15}N nitrate, (b) crown root fed with ^{15}Nammonium, (c) seminal root fed with ^{15}N ammonium, (d) seminal root fed with ^{15}Nnitrate. (B) and (C) shows the excised crown and seminal root respectively.



Figure 2.3.B. Stable isotope nitrogen uptake by the intact roots of control plants (no microdialysis). (A) shows how individual roots were fed with 4mM nitrogen stable isotope. (a) crown root fed with ^{15}Nammonium, (b) crown root fed with ^{15}N nitrate,(c) seminal root fed with ^{15}N ammonium, (d) seminal root fed with ^{15}Nnitrate.

Table 2.2. Treatment, types, and number of excised roots from each plant after ^{15}N uptake of intact maize root.

Experiment	*Number of Plants*	*Background Nitrogen Treatment*	*Pre -uptake Condition*	*Number of roots fed with $K^{15}NO_3$*	*Number of roots fed with $(N^{15}H_4)_2SO_4$*
Normal nitrogen resupply	3	Low	Induced with low N for 24 hrs	3 CR & 3 SR	3 CR & 3 SR
	3	High	Induced with high N for 24hrs	3 CR & 3 SR	3 CR & 3 SR
	3	Low	Not induced	3 CR & 3 SR	3 CR & 3 SR
	3	High	Not induced	3 CR & 3 SR	3 CR & 3 SR
	Total =12			Total=24 (12 CR & 12 SR)	Total =24 (12 CR & 12 SR)
Factorial nitrogen resupply	4	Low	Induced with low N for 44hrs	4 CR & 4 SR	4 CR & 4 SR
	4	High	Induced with low N for 44hrs	4 CR & 4 SR	4 CR & 4 SR
	4	Low	Induced with high N for 44 hrs	4 CR & 4 SR	4 CR & 4 SR
	4	High	Induced with high N for 44 hrs	4 CR & 4 SR	4 CR & 4 SR
	4	Low	Not induced	4 CR & 4 SR	4 CR & 4 SR
	4	High	Not induced	4 CR & 4 SR	4 CR & 4 SR
	Total =24			Total=48 (12 CR &12 SR)	Total=48 (12 CR &12 SR)

CR=Crown roots, SR= Seminal roots

2.3.1 Data Analysis

To determine whether the mean nitrate and ammonium concentration from each microdialysis and net uptake experiments were statistically significant or not, two-way, and three-way ANOVA using R statistical software were conducted to test for statistical differences between treatment means.

For the high N resupply experiment, the depletion rates of nitrate and ammonium over time (0, 3, 18, 24, 27, 44 hours) were tested as a function of nitrogen background (either high or low N) and distance of the probes to crown roots, seminal roots or background soil using two-way ANOVA. The analysis was followed by Tukey post hoc test to test for significant differences in group means. The choice to analyse the nitrate and ammonium depletion rates at 0, 3, 18, 24, 27, 44 hours sampling time was to determine how long it will take for the high nitrogen supplied to low N plants to deplete adjacent to crown roots or seminal roots and the background soil as compared to the high N plants supplied with high N solution.

For the normal nitrogen resupply experiments, nitrate and ammonium depletion rates were separately tested as a function of distance of the probe to crown roots, seminal roots, or the background soil at 0 and 24 hours. While the net uptake of nitrate and ammonium by maize crown and seminal roots were tested as a function of nitrogen background, condition of whether sprayed with nitrogen before the uptake experiment or not (induction), and root type using two-way ANOVA. The analysis was followed by Tukey post hoc test. The testing of the condition of whether the supply of high nitrogen to low N plants after 24 hrs will cause a higher uptake rate of nitrogen sources by crown roots or seminal roots due to anticipated activities of nitrogen transporters. Previous studies showed that the influx activities of nitrogen transporters doubled after 24 hrs of nitrogen deprivation (Hole et al., 1990).

For the factorial resupply experiment, data from high nitrogen background was separated from that of low nitrogen background and each data were tested for significant difference in nitrate or ammonium depletion rates as may be influenced by the concentration of N resupply, distance of the probes from crown roots, seminal roots or background soil at different sampling times (0, 3, 6, 18, 24, 27, 33, 44 hours) using the three-way ANOVA. For the net uptake experiment, the three-way ANOVA was used to test for the influence of nitrogen background (high or low), the condition of whether sprayed with nitrogen solution before the uptake experiment or not, as well as the concentration of the sprayed nitrogen solution on the net uptake of nitrate or ammonium by maize crown or seminal roots. Each of the ANOVAs was followed by Tukey post hoc test. The Pearson's product moment

correlation coefficient of Microsoft Excel was used to test for the linear relationship between the diameter of crown or seminal roots and net uptake of nitrate or ammonium by maize crown]

2.4. Results

To determine the rate of change in nutrient fluxes adjacent to maize root types and the background soil during adequate and suboptimal N supply, plants were grown in rhizobox soil for 3 weeks on low (0.3 mM) or high (4 mM) nitrogen background. The rhizoboxes were then opened and the soil-window interface of each box was sprayed uniformly with nitrogen solution. The levels of nitrate and ammonium were sampled using microdialysis (2.4.1), and the root uptake capacity determined using ^{15}N isotopes (2.4.2).

2.4.1. The spatio temporal dynamics of nitrogen forms as uncovered by microdialysis:

2.4.1.1. Nitrogen depletion rates at the background of low and high N plants after high N resupply:

Compared to time 0 (first hour of spraying high nitrogen) 3 (possible short-term induction of influx systems), and 24 (possible full-term induction of influx systems), nitrogen fluxes in the background soil of low N plants seemed to have depleted variably at each sampling time, but the depletion rates were not statistically significant (nitrate: Figure 2.3, A-E; ammonium: Figure 2.4, A-E; Table 2.4.). Similarly, the average rates of nitrogen depletion in the background of high N plant at each sampling time as compared to the depletion rates at 0, 3, 24 hours showed no statistical difference (nitrate: Figure 2.3, F-J; ammonium: Figure 2.4, F-J; Table 2.4.). However, the depletion rates of ammonium were faster in the soil of low N plants as compared to that of high N plants ($P=0.04$), while that of nitrates were not statistically significant ($P=0.08$).

2.4.1.2. Nitrogen depletion rates adjacent to crown roots of low N plants after high N resupply:

The rate of nitrate depletion adjacent to crown roots of low N plants were similar at each sampling time as compared to time 0 (first hour of spraying high nitrogen), 3 (possible short-term induction of influx systems), and 24 hours (possible full-term induction of influx systems) (Figure 2.3; Table 2.4). While ammonium depleted significantly faster at 3, 6, 18, 24, 30 and 44 hrs as compared to 0, 3 and 24 hours ($P<0.001$ each; Figure 2.4.; Table 2.4). When the wholistic trend of nitrogen depletion rates adjacent crown roots of low N plants were compared to the depletion rates at the background, it was found that nitrate and ammonium depleted faster at the background than adjacent to crown roots of low N plants (nitrate: $P=0.04$, Figure 2.5.B; ammonium: $P=0.002$ Figure 2.5.D). However, when the depletion rates at each sampling time was considered between crown roots and the background of low N plants, it was found that nitrate depleted at similar rates as the background at each sampling

time, but ammonium depleted fast at the background soil at 0 hrs compared to adjacent to crown roots of low N plants (Figure 2.5; Table 2.5).

2.4.1.3. Nitrogen depletion rates adjacent to seminal roots of low N plants after high N resupply:

The depletion of nitrate adjacent to the seminal roots of low N plants at each sampling time were at similar rates as compared to 0 (first hour of spraying high nitrogen), 3 (possible short-term induction of influx systems), and 24 (possible full-term induction of influx systems) hours (Figure 2.3; Table 2.4), while the rate of ammonium depletion was significantly faster as from 18 hrs after high N resupply as compared to 0, 3, and 24 hours ($P<0.001$; Figure 2.4; Table 2.4). When the wholistic trend of nitrogen depletion rates adjacent seminal roots of low N plants were compared to the depletion rates adjacent to the crown roots of low N plants, it was found that nitrate and ammonium depletion were at similar rates at each sampling time (Table 2.5). Similarly, when the nitrate and ammonium depletion rates adjacent to seminal roots was compared to the depletion rates at the background for each individual sampling time, a faster depletion of nitrate and ammonium occurred at the background at 0 hrs in the rhizosphere of low N plants ($P<0.001$; Figure 2.5; Table 2.5).

2.4.1.4. Nitrogen depletion rates adjacent to crown roots of high N plants after high N resupply:

Nitrate depleted significantly faster adjacent to crown roots of high N plants at 24, 27 and 44 hours ($P<0.001$ each; Figure 2.3; Table 2.4) as compared to sampling time 0 (first hour of spraying high nitrogen) and 3 (possible short-term induction of influx systems). While that of ammonium were at similar rates for each sampling time (Figure 2.4.; Table 2.4). When the depletion trend of nitrate and ammonium adjacent to the crown roots of high N plants at each sampling time was compared to that of the background, it was found that nitrate and ammonium depleted faster at the background than adjacent to crown roots only at 0 hours (nitrate: $P=0.02$, Figure 2.5.A; ammonium: $P=0.002$ Figure 2.5.C; Table 2.5).

2.4.1.5. Nitrogen depletion rates adjacent to seminal roots of high N plants after high N resupply:

Nitrate depleted significantly adjacent to seminal roots from 24 hours sampling time upward ($P=0.02$; Figure 2.3; Table 2.4) as compared to 0 (first hour of spraying high nitrogen) and 3 (possible short-term induction of influx systems). Meanwhile, ammonium depletion adjacent to seminal roots of high N plants were not statistically significant across all sampling time points (Figure 2.4; Table 2.4). When the depletion trend of nitrate and ammonium adjacent to the seminal roots of high N plants was compared to that of the background at ach sampling time, it was found that the depletion rates were not statistically different across each sampling time (Figure 2.5.C; Table 2.5).

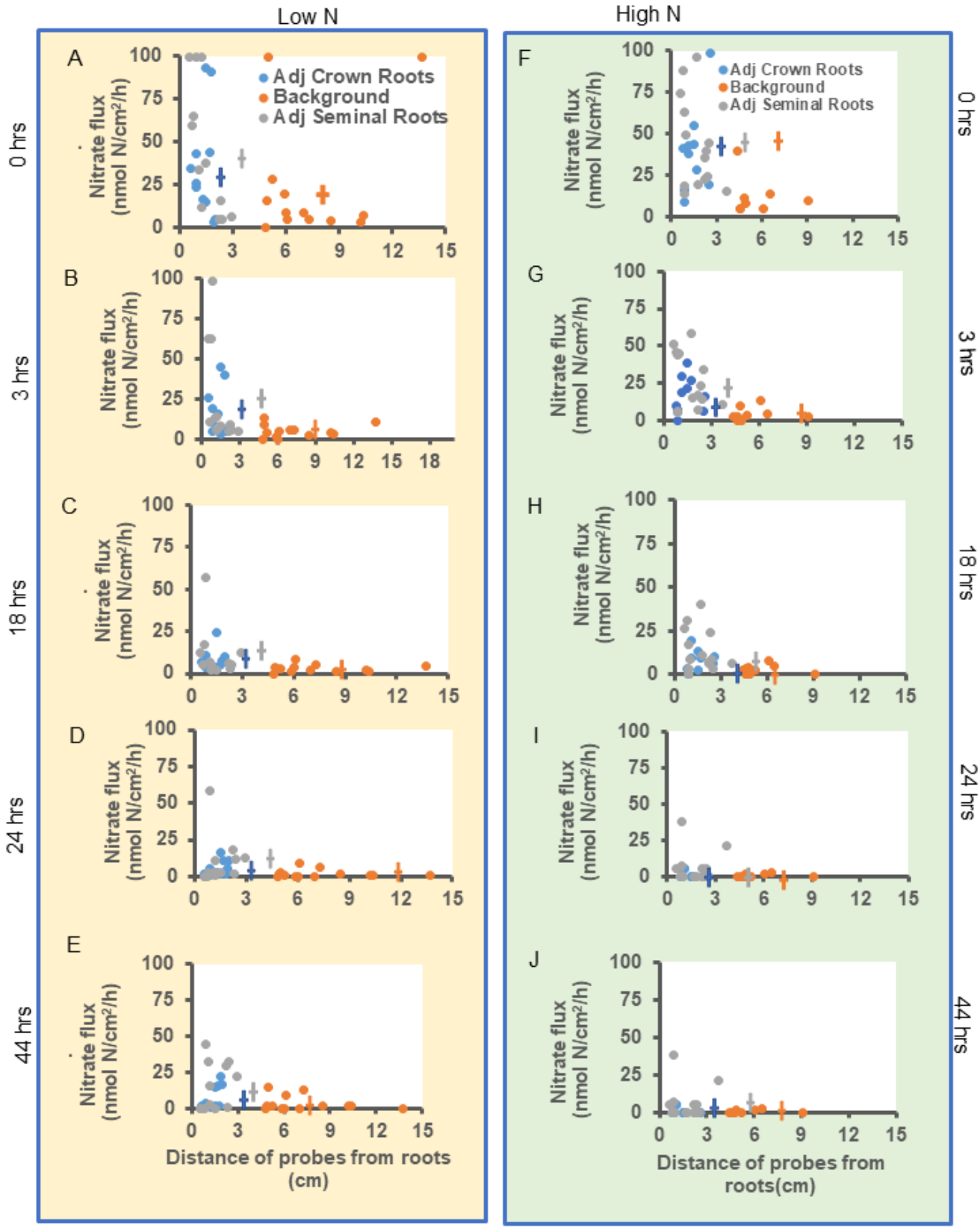


Figure 2.4.Soil nitrate availability rapidly decreases in rhizobox soils. All panels are nitrate fluxes across 1 cm length microdialysis probe, sampled for 1 hour at a flow rate of 5 µl per hour. Orange panels (A-E) are plants grown on low nitrogen and Green panels (F-J) are plants grown on high nitrogen. All plants were sprayed with high N before sampling time 0. Vertically plants were sampled at 0 hrs (first hour; A,F), 3 (B, G), 18 (C, H), 24 (D, I) and 44hrs (E, J). Values are averages (n=9 probes x4 plants each) for all panels. Crosses are averages. (See Table 2.3 and 2.4 for statistical comparison).

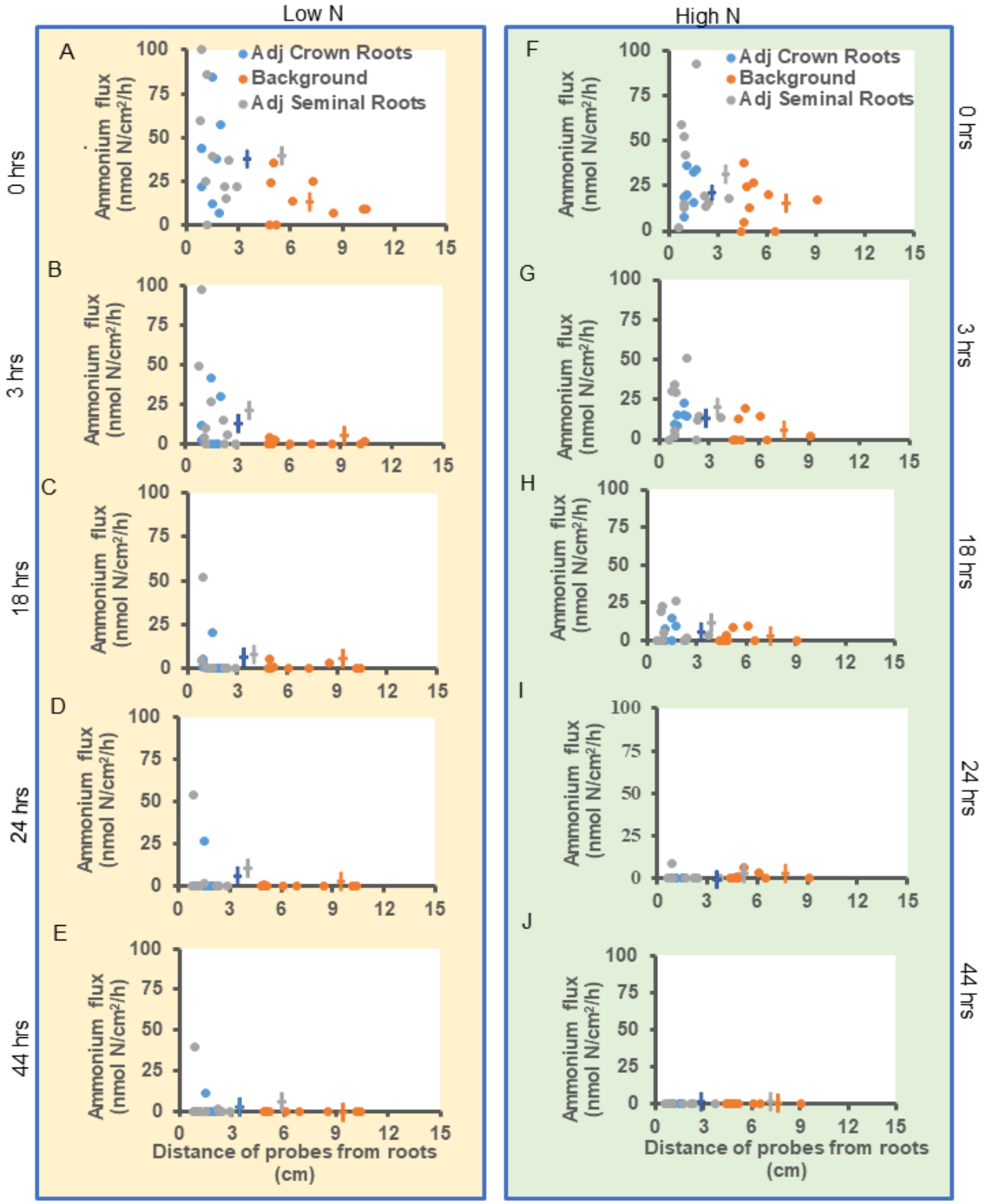


Figure 2.5. Soil Ammonium availability rapidly decreases in rhizobox soils. All panels are ammonium fluxes across 1 cm length microdialysis probe, sampled for 1 hour at a flow rate of 5 µl per hour. Orange panels (A-E) are plants grown on low nitrogen and green panels (F-J) are plants grown on high nitrogen. All plants were sprayed with high N before sampling time 0. Vertically plants were sampled at 0 hrs (first hour; A,F), 3 (B, G), 18 (C, H), 24 (D, I) and 44hrs (E, J). Crosses are mean fluxes while values are averages (n=9 probes x 3 plants each) for all panels. (See Table 2.3 and 2.4 for statistical comparison).

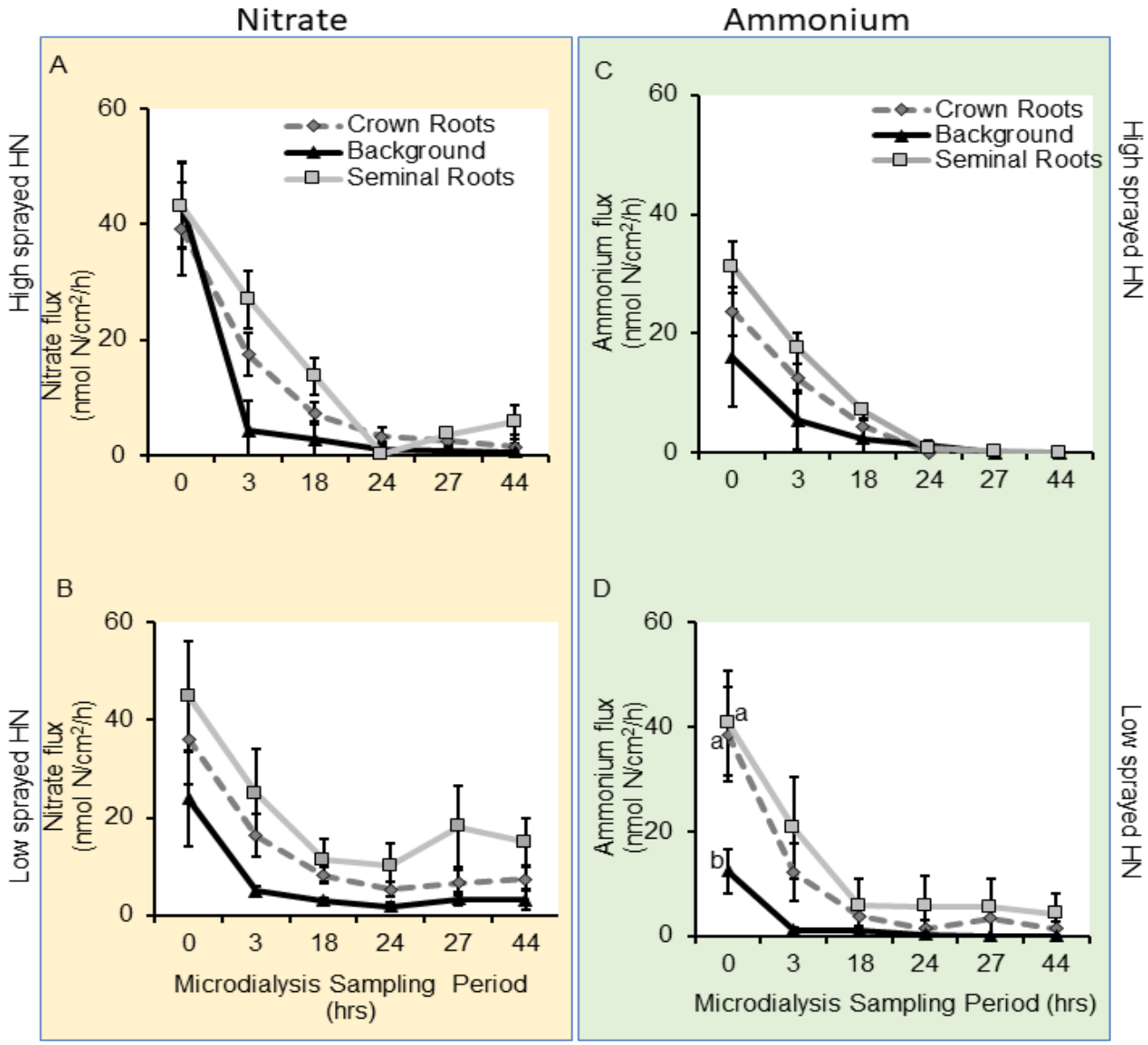


Figure 2.6. Soil nitrate and ammonium availability rapidly decreases in rhizobox soils. This graph is the summary of Figure 2.4. and Figure 2.5. All panels are nitrates or ammonium fluxes across 1 cm length microdialysis probe, sampled for 1 hour at a flow rate of 5 µl per hour. Green panels (A, B) are nitrate fluxes and orange panels (C, D) are ammonium fluxes . All plants were sprayed with high N before sampling time 0. Vertically are plants grown on high nitrogen background (A, C), and plants grown on low nitrogen background (B, D). Values are averages (nitrate: n=9 probes x 4 plants each, ammonium: n=9 probes x 3 plants each) for all panels. (See Table 2.3 and 2.4 for statistical comparison).

Table 2.3. Depletion rates of nitrogen forms at other sampling times compared to 0-, 3-, and 24-hours sampling time after high N resupply (presented in Figure 2.4, 2.5, 2.6).

Nitrogen form	*Nitrogen background of plants*	*Sampling time*	*Adjacent to crown roots.*			*Adjacent to seminal roots.*			*Background (bulk soil).*		
			0	*3*	*24*	*0*	*3*	*24*	*0*	*3*	*24*
Nitrate	Low N plants	0	__	NS	NS	__	NS	NS	__	NS	NS
		3	NS	__	NS	NS	__	NS	NS	__	NS
		18	NS	NS	NS	NS	NS	NS	NS	NS	NS
		24	NS	NS	__	NS	NS	__	NS	NS	__
		27	NS	NS	NS	NS	NS	NS	NS	NS	NS
		44	NS	NS	NS	NS	NS	NS	NS	NS	NS
	High N plants	0	__	NS	***	__	NS	*	__	NS	NS
		3	NS	__	NS	NS	__	NS	NS	__	NS
		18	NS	NS	NS	NS	NS	NS	NS	NS	NS
		24	***	NS	__	*	NS	__	NS	NS	__
		27	***	NS	NS	**	NS	NS	NS	NS	NS
		44	***	NS	NS	**	NS	NS	NS	NS	NS
Ammonium	Low N plants	0	__	***	***	__	NS	***	__	NS	NS
		3	***	__	NS		__	NS	NS	__	NS
		18	***	NS	NS	***	NS	NS	NS	NS	NS
		24	***	NS	__	***	NS	__	NS	NS	__
		27	***	NS	NS	***	NS	NS	NS	NS	NS
		44	***	NS	NS	***	NS	NS	NS	NS	NS
	High N plants	0	__	NS	NS	__	NS	NS	__	NS	NS
		3	NS	__	NS	NS	__	NS	NS	__	NS
		18	NS	NS	NS	NS	NS	NS	NS	NS	NS
		24	NS	NS	__	NS	NS	__	NS	NS	__
		27	NS	NS	NS	NS	NS	NS	NS	NS	NS
		44	NS	NS	NS	NS	NS	NS	NS	NS	NS

NS = not significantly different at 5% level, * = significant at 5% level, ** = significant at 1% level, *** = highly significant ($P < 0.001$).

Table 2.4. Statistical comparison of the depletion rates of nitrate and ammonium between probes adjacent to crown roots and seminal roots, and the background soil at each sampling time after high nitrogen resupply (represented in Figure 2.4, 2.5, and 2.6).

Nitrogen form	*Nitrogen background of plants*	*Sampling time*	*Adjacent to crown roots compared to adjacent seminal roots*	*Adjacent to crown roots compared to the background. (Bulk soil)*	*Adjacent to seminal roots compared to the background. (Bulk soil)*
Nitrate	High N plants	0	NS	NS	NS
		3	NS	NS	NS
		18	NS	NS	NS
		24	NS	NS	NS
		27	NS	NS	NS
		44	NS	NS	NS
	Low N plants	0	NS	NS	***
		3	NS	NS	NS
		18	NS	NS	NS
		24	NS	NS	NS
		27	NS	NS	NS
		44	NS	NS	NS
Ammonium	Low N plants	0	NS	NS	NS
		3	NS	NS	NS
		18	NS	NS	NS
		24	NS	NS	NS
		27	NS	NS	NS
		44	NS	NS	NS
	High N plants	0	NS	***	***
		3	NS	NS	***
		18	NS	NS	NS
		24	NS	NS	NS
		27	NS	NS	NS
		44	NS	NS	NS

NS = not significantly different at 5% level, * = significant at 5% level, ** = significant at 1% level, *** = highly significant ($P < 0.001$).

2.4.1.6. Ammonium depleted as compared to nitrate in both low and high N plants during normal nitrogen resupply:

The depletion rates of ammonium after spraying low N solution on low N plants were rapid within 24 hrs especially adjacent to crown and seminal roots (P= 0.008; Figure 2.7.A). While root-wise concentration of nitrate at 24 hours showed the least concentration adjacent to crown roots as nitrate concentration appeared unchanged adjacent to root types and the background (Figure 2.7.A).

Meanwhile the depletion rates of ammonium in high N plants after spraying high N solution was also rapid adjacent to the roots and the background after 24 hrs (P=0.008; Figure 2.7.B). Nitrate concentration adjacent to crown roots of high N plants was the least followed by the background and then adjacent to seminal roots during the first hour of nutrient sampling. However, at the 24-hour sampling time, nitrate fluxes adjacent to crown roots seemed unchanged while it depleted by 4.3 nmol/N/cm^2/h at the background and increased by 1.75 nmol/N/cm^2/h adjacent to seminal roots (Figure 2.7.B).

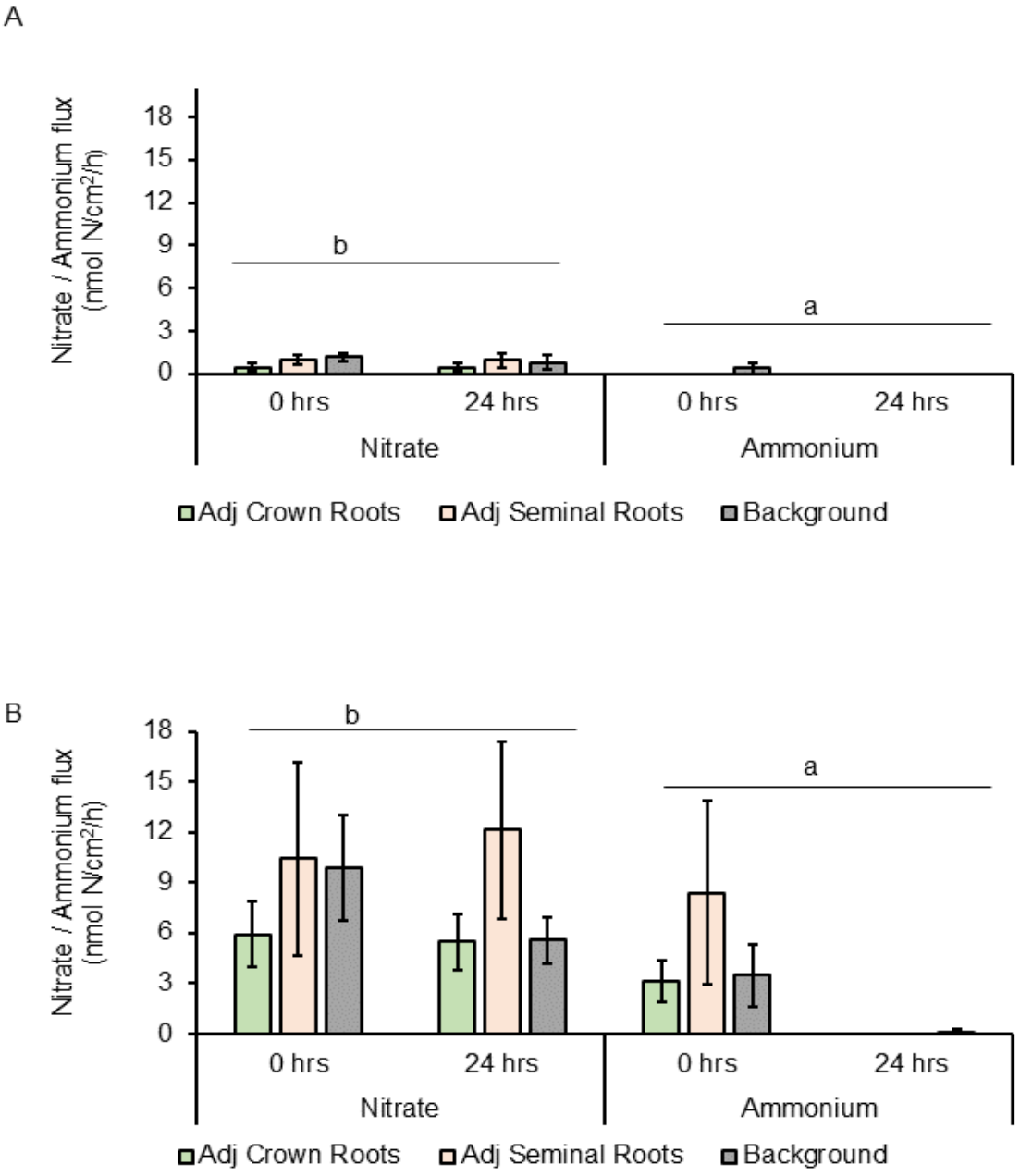


Figure 2.7. Soil ammonium rapidly disappeared from rhizobox surface within one hour of spraying low N solution on the rhizosphere of low N plants (A), and high N solution on high N plants (B). The two graphs are ammonium and nitrate fluxes across 1 cm length microdialysis probe, sampled for 1 hour at a flow rate of 5 µl per hour. Graph (A) is nitrate and ammonium fluxes of plants grown on low nitrogen and given repeat N pulse prior to microdialysis. While graph (B) are nitrate and ammonium fluxes of plants grown on high nitrogen and given repeat N pulse prior to microdialysis. All plants were sampled at 0, and 24 hours. Values represents averages (n=8 probes x3 plants each) for each bar.

The above sections explored how nitrate and ammonium fluxes differed following a pulse of high nitrogen and the normal nitrogen resupply. It then became expedient to explore what happens with different nutrient pulse regimes. Therefore, every combination was made as factorial nitrogen resupply. This meant plants grown on high or low N for 3 weeks after germination were then sprayed with either high or low N at time 0, 3, 6, 18, 24, 27, 30, 44 hrs.

2.4.1.7. Ammonium rapidly disappeared within 6 hours after factorial nitrogen resupply:

The average ammonium recovered from the soil using microdialysis were initially high adjacent to root types and at the background (Figure 2.8.A-D), but rapidly declined ($P<0.001$; Table 2.5) from 3 hrs sampling time to limits beyond detection after six hours, irrespective of nitrogen background or concentration of nitrogen sprayed. However, adjacent to seminal roots and background of high N plants whose rhizosphere was treated with low N solution depleted ammonium faster between 0-3 hrs than adjacent to crown roots ($P=0.03$; Table 2.5.; Figure 2.8.A & B). The low N plants whose rhizosphere were treated with high N solution depleted faster adjacent to roots and the background soil as compared to the low N plants given sprayed with low N ($P=0.002$, Figure 2.8.C & D). Equally important to note was that ammonium depleted faster adjacent to crown roots of all low N plants (irrespective of the concentration of nutrient sprayed) as compared to the background (sprayed high N: $P=0.02$, Figure 2.8.C, sprayed low N: $P=0.004$, Figure 2.8.D) from 0-3 hrs sampling time.

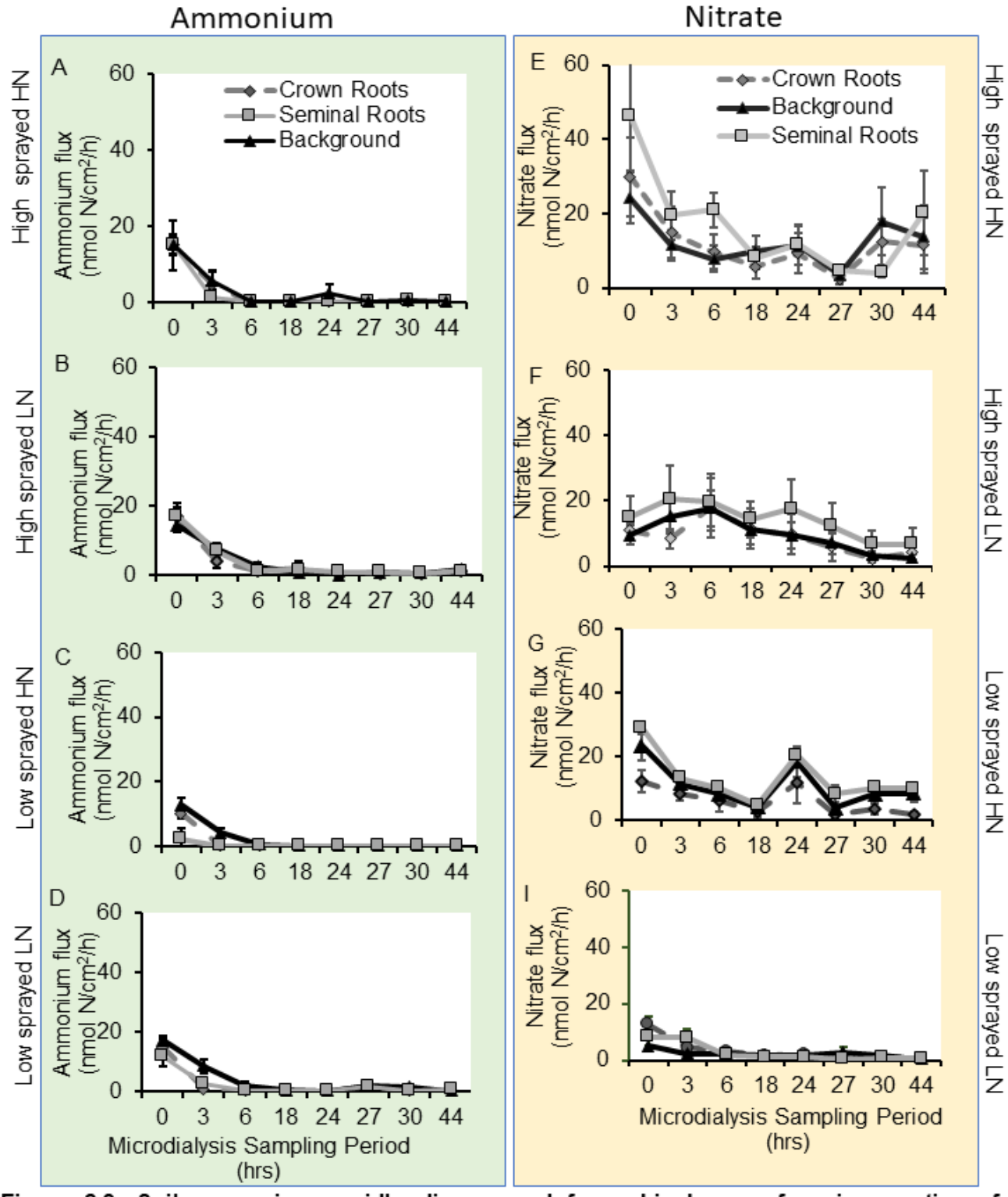


Figure 2.8. Soil ammonium rapidly disappeared from rhizobox surface irrespective of nitrogen background and re-supply while nitrate availability to roots varied with nitrogen background and nitrogen re-supply over time. All panels are ammonium or nitrate fluxes across 1 cm length microdialysis probe, sampled for 1 hour at a flow rate of 5 µl per hour. Green panels (A-D) are ammonium fluxes of plants grown on high (A&B) or low (C&D) nitrogen. While orange panels (E-I) are nitrate fluxes of plants grown on high (E&F) and low (G&I) nitrogen. All plants were sampled at 0,3,6,18,24,27,30,44 hours. Vertically plants were sprayed with either high N solution(A,C and E,G) or low N solution(B,D& F,I) prior to microdialysis. Values represents averages (n=8 probes x4 plants each) for all panels. (See Table 2.5, 2.6 and 2.7 for statistical comparison

Table 2.5. Significant differences in ammonium depletion rates at 0, 3, and 24 hrs each, as compared to other sampling times after factorial nitrogen resupply (represented in Figure 2.8, green panel (A, B, C, D)).

Nitrogen background of plants	*Concentration of nitrogen resupply*	*Sampling time*	*Crown roots.*			*Seminal roots.*			*Background (Bulk soil).*		
			0	*3*	*24*	*0*	*3*	*24*	*0*	*3*	*24*
High N plants	Sprayed high N	0	__	***	***	__	***	**	__	***	***
		3	*	__	NS	***	__	NS	**	__	**
		6	***	NS	NS	***	NS	NS	***	NS	NS
		18	***	NS	NS	***	NS	NS	***	NS	NS
		24	***	NS	__	***	NS	__	***	NS	__
		27	***	NS	NS	***	NS	NS	***	NS	NS
		30	***	NS	NS	***	NS	NS	***	NS	NS
		44	***	NS	NS	***	NS	NS	***	NS	NS
	Sprayed low N	0	__	NS	***	__	*	**	__	*	***
		3	**	__	NS	NS	__	NS	NS	__	NS
		6	***	NS	NS	***	NS	NS	***	*	NS
		18	***	NS	NS	***	NS	NS	***	*	NS
		24	***	NS	__	***	NS	__	***	*	__
		27	***	NS	NS	***	NS	NS	***	*	NS
		30	***	NS	NS	***	NS	NS	***	*	NS
		44	***	NS	NS	***	NS	NS	***	NS	NS
Low N plants	Sprayed high N	0	__	***	***	__	NS	NS	__	***	***
		3	***	__	NS	NS	__	NS	***	__	***
		6	***	NS	NS	NS	NS	NS	***	NS	NS
		18	***	NS	NS	NS	NS	NS	***	***	NS
		24	***	NS	__	NS	NS	__	***	***	__
		27	***	NS	NS	NS	NS	NS	***	***	NS
		30	***	NS	NS	NS	NS	NS	***	**	NS
		44	***	NS	NS	NS	NS	NS	***	*	NS
	Sprayed low N	0	__	***	***	__	***	***	__	***	***
		3	***	__	NS	**	__	NS	***	__	***
		18	***	NS	NS	***	NS	NS	***	**	NS
		24	***	NS	__	***	NS	__	***	***	__
		27	***	NS	NS	***	NS	NS	***	***	NS
		30	***	NS	NS	***	NS	NS	***	***	NS
		44	***	NS	NS	***	NS	NS	***	***	NS

NS = not significantly different at 5% level, * = significant at 5% level, ** = significant at 1% level, *** = highly significant ($P < 0.001$).

2.4.1.8. Nitrate fluxes varies spatially and temporally after spraying either high or low nitrogen solution on the rhizosphere of high and low N plants.

The average nitrate depletion rates at the background soil of high N plants treated with high N solution was appeared rapid from 0-6 hrs, stable between 18-24 hrs, and steadily declined between 24-27 hrs (Figure 2.8.E). However, a gradual increase in nitrate flux rates at the background was notice at 30 hrs which depleted at 44 hrs. Nitrate depletion rates adjacent to seminal roots was significantly rapid at 18, 27 and 30 hrs as compared to 0 hrs ($P= 0.02$ at 18 hrs, $P=0.01$ at 27 hrs and $P<0.001$ at 30 hrs; Figure 2.8.E; Table 2.6). While adjacent to crown roots nitrate showed a significant depletion at 27 hrs as compared to 0 hrs sampling time ($P= 0.05$; Figure 2.8.E; Table 2.6). Root-wise comparisons showed no statistical difference between the root types (Table 2.8).

When high N plants were sprayed with low N solution, the average nitrate fluxes depleted and accumulated intermittently between 0-24 hrs and continue to deplete over time (Figure 2.8.F). No definite sampling time point was statistically significant (Table 2.6), but nitrate fluxes transiently increased at the background between 0-6 hrs, and thereafter, gradually depleted over time. Adjacent to crown and seminal roots also depleted variably with no statistical significance (Table 2.7).

When the rhizosphere of low N plants was sprayed with high N solution, the average nitrate appeared to decline rapidly from 0-6 hrs, gradually from 6-18 hrs, accompanied by a rise in NO^-_3 fluxes between 18 and 24 hrs which rapidly depleted from 24-27 hrs and then steadily afterwards (Figure 2.8.G). Adjacent to crown and seminal roots as well as the background depleted variably with no statistically significant difference (Table 2.7). But the main effect showed that the Spraying of high N and sampling times, independently influenced the nitrate flux rates ($P=0.008$ and $P=0.0002$ respectively).

When the rhizosphere of low N plants was sprayed with low N solution, nitrate variably depleted at the root tips and at the background from 0-6 hrs (Figure 2.8.I). Thus, no statistical significance between root types, the background and the sampling time points (Table 2.6; Table 2.7). However, the main effect of spraying low N and sampling times influenced the depletion rates ($P=0.008$ and $P=0.0002$ for N-pulse and time respectively).

Table 2.6. No significant difference in nitrate depletion rates at 3 hrs and 24 hrs each, as compared to other sampling time across root types and background after factorial nitrogen resupply (represented in Figure 2.8 orange panel (E, F, G, I)).

Nitrogen background of plants	*Concentration of nitrogen resupply*	*Sampling time*	*Crown roots.*			*Seminal roots.*			*Background (Bulk soil).*		
			0	*3*	*24*	*0*	*3*	*24*	*0*	*3*	*24*
High N plants	Sprayed high N	0	__	NS	NS	__	NS	NS	__	NS	NS
		3	NS	__	NS	NS	__	NS	NS	__	NS
		6	NS	NS	NS	NS	NS	NS	NS	NS	NS
		18	NS	NS	NS	*	NS	NS	NS	NS	NS
		24	NS	NS	__	NS	NS	__	NS	NS	__
		27	*	NS	NS	**	NS	NS	NS	NS	NS
		30	NS	NS	NS	***	NS	NS	NS	NS	NS
		44	NS	NS	NS	NS	NS	NS	NS	NS	NS
	Sprayed low N	0	__	NS	NS	__	NS	NS	__	NS	NS
		3	NS	__	NS	NS	__	NS	NS	__	NS
		6	NS	NS	NS	NS	NS	NS	NS	NS	NS
		18	NS	NS	NS	NS	NS	NS	NS	NS	NS
		24	NS	NS	__	NS	NS	__	NS	NS	__
		27	NS	NS	NS	NS	NS	NS	NS	NS	NS
		30	NS	NS	NS	NS	NS	NS	NS	NS	NS
		44	NS	NS	NS	NS	NS	NS	NS	NS	NS
Low N plants	Sprayed high N	0	__	NS	NS	__	NS	NS	__	NS	NS
		3	NS	__	NS	NS	__	NS	NS	__	NS
		6	NS	NS	NS	NS	NS	NS	NS	NS	NS
		18	NS	NS	NS	NS	NS	NS	NS	NS	NS
		24	NS	NS	__	NS	NS	__	NS	NS	__
		27	NS	NS	NS	NS	NS	NS	NS	NS	NS
		30	NS	NS	NS	NS	NS	NS	NS	NS	NS
		44	NS	NS	NS	NS	NS	NS	NS	NS	NS
	Sprayed low N	0	__	NS	NS	__	NS	NS	__	NS	NS
		3	NS	__	NS	NS	__	NS	NS	__	NS
		18	NS	NS	NS	NS	NS	NS	NS	NS	NS
		24	NS	NS	__	NS	NS	__	NS	NS	__
		27	NS	NS	NS	NS	NS	NS	NS	NS	NS
		30	NS	NS	NS	NS	NS	NS	NS	NS	NS
		44	NS	NS	NS	NS	NS	NS	NS	NS	NS

NS = not significantly different at 5% level, * = significant at 5% level, ** = significant at 1% level, *** = highly significant ($P < 0.001$).

Table 2.7. Statistical comparison of nitrate and ammonium depletion rates between probes adjacent to crown roots and seminal roots, and the background soil (far from roots) at each sampling time after factorial nitrogen supply (represented in Figure 2.8).

Nitrogen background of plants	*Concentration of nitrogen resupply*	*Sampling time*	*Crown vs seminal roots.*		*Crown roots vs background.*		*Seminal roots vs background.*	
			NO_3^-	NH_4^+	NO_3^-	NH_4^+	NO_3^-	NH_4^+
High N plants	Sprayed high N	0	NS	NS	NS	NS	NS	NS
		3	NS	NS	NS	NS	NS	NS
		6	NS	NS	NS	NS	NS	NS
		18	NS	NS	NS	NS	NS	NS
		24	NS	NS	NS	NS	NS	NS
		27	NS	NS	NS	NS	NS	NS
		30	NS	NS	NS	NS	NS	NS
		44	NS	NS	NS	NS	NS	NS
	Sprayed low N	0	NS	NS	NS	NS	NS	NS
		3	NS	NS	NS	NS	NS	NS
		6	NS	NS	NS	NS	NS	NS
		18	NS	NS	NS	NS	NS	NS
		24	NS	NS	NS	NS	NS	NS
		27	NS	NS	NS	NS	NS	NS
		30	NS	NS	NS	NS	NS	NS
		44	NS	NS	NS	NS	NS	NS
Low N plants	Sprayed high N	0	NS	NS	NS	NS	NS	***
		3	NS	NS	NS	**	NS	NS
		6	NS	NS	NS	NS	NS	NS
		18	NS	NS	NS	NS	NS	NS
		24	NS	NS	NS	NS	NS	NS
		27	NS	NS	NS	NS	NS	NS
		30	NS	NS	NS	NS	NS	NS
		44	NS	NS	NS	NS	NS	NS
	Sprayed low N	0	NS	NS	NS	NS	NS	NS
		3	NS	NS	NS	**	NS	NS
		18	NS	NS	NS	NS	NS	NS
		24	NS	NS	NS	NS	NS	NS

NS = not significantly different at 5% level, * = significant at 5% level, ** = significant at 1% level, *** = highly significant ($P < 0.001$).

2.4.2. Nitrogen uptake rates by intact maize seminal and crown roots

To understand the uptake capacity of nitrate and ammonium by each root type, when nutrient pulses are applied to plants grown on limited and adequate nitrogen background, Maize plants were grown for 3 weeks in rhizoboxes containing soil in the glasshouse and were fed with either optimum (high: 4 mM) or limited (low: 0.3 mM) nitrogen. For the first uptake trial being the normal nitrogen resupply experiment, half of the high N plants were sprayed with high N solution and half of low N plants with low N solution before sampling for available soil nutrient ions at 0 hrs (immediately after spray) and 24 hrs after. For the second uptake experiment being the third microdialysis experiment, additional treatment regime was included: high N plants sprayed with low N and low N plants sprayed with high N solution and soil nutrient sampling was conducted on the sprayed plants in time sequence (see previous section) for 44 hrs. Then N uptake capacity of intact crown and seminal roots were measured using ^{15}N labelled NO_3^- and NH_4^+ sources to obtain the net effects.

2.4.2.1. Effects of normal nitrogen resupply on the uptake of nitrogen forms by root types after 24 hours.

Repeat N pulse couldn't influence the uptake rate of nitrogen forms, by crown and seminal roots of low and high plants after 24 hours of nutrient spray (Figure 2.9.A). As no difference was seen between the uptake pattern of sprayed plants and the unsprayed. However, the crown roots of low N plants captured 1.3-fold nitrate and 2-fold ammonium than their seminal roots (P=0.05), while the seminal roots of high N plants appeared to capture more ammonium than nitrate at higher rate than their crown roots.

2.4.2.2. Effects of factorial nitrogen resupply on the root type uptake of nitrogen forms after 44 hours of nutrients spray.

Reciprocal N pulse in low and high N plants enabled their crown roots to capture more ammonium and then nitrate than Repeat N pulse (P= 0.01, Figure 2.9.B). Low N plants resupplied with low N appeared to capture more ammonium than nitrate with both seminal and crown roots. While high N resupplied to high N plants tended to enable capture of more? nitrate and ammonium with seminal roots compared to crown roots. Meanwhile the unsprayed plants seem to capture both N forms at similar rates.

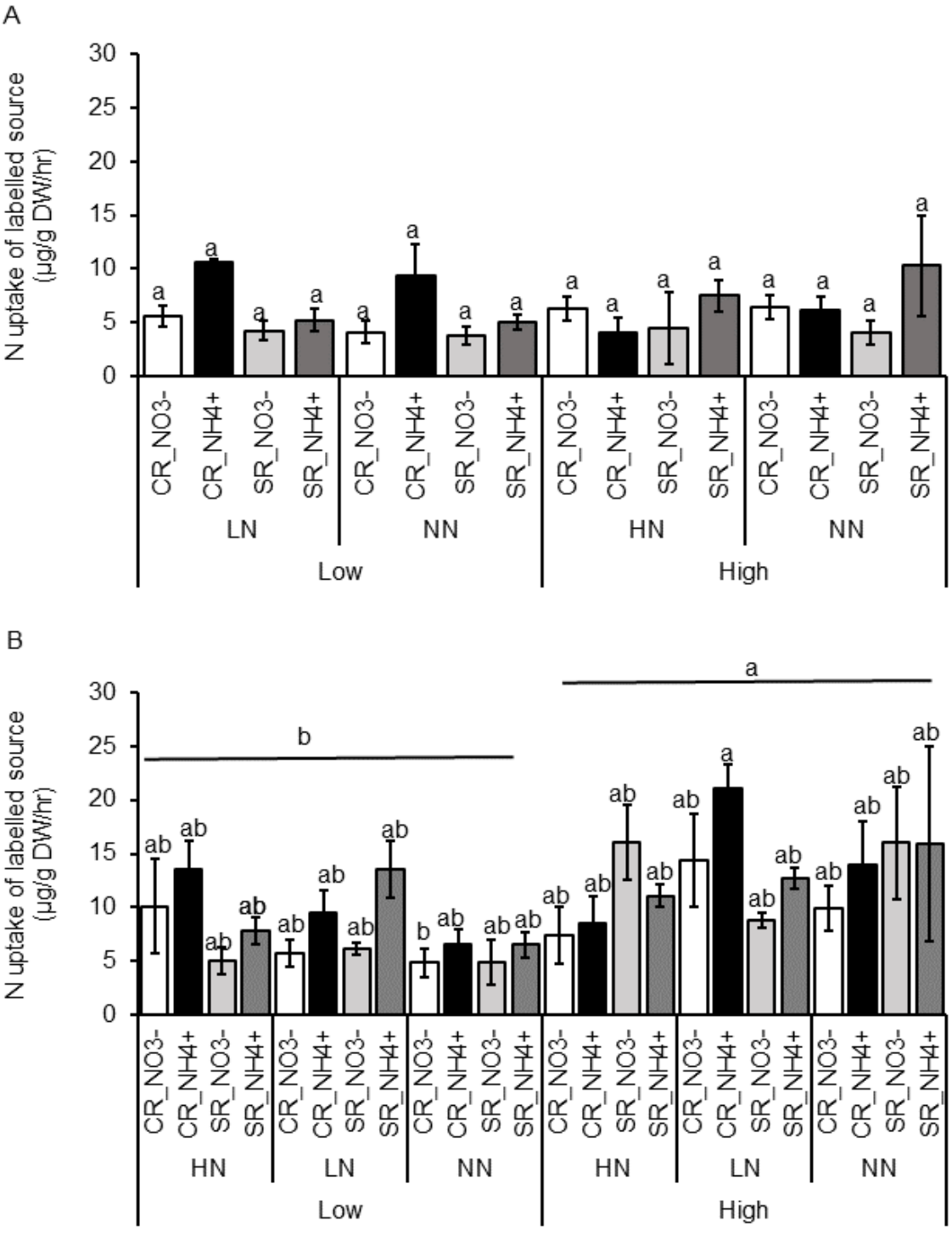


Figure 2.9. Nitrate and ammonium uptake rates were influenced by nitrogen background and concentration of N re-supply after 24hrs (Figure A) and 44hrs (Figure B). All bars are uptake of nitrate or ammonium from intact maize crown (CR) and seminal (SR) roots, sampled for 30 minutes after spraying with high (HN) or low (LN) nitrogen solution, or not sprayed (NN) from maize grown on limited (Low) and optimum (High) nitrogen background in two experiments. Bars are averages (n=1 root sample x 3 plants each (Figure A, normal nitrogen resupply experiment) and n=1 root sample x 4 plants each (Figure B, factorial nitrogen resupply experiment)).Error bars are standard error of the means. Different letters denotes statistical difference at 5%.

2.4.2.3. Nitrogen background influenced the quantity of captured N sources:

From the first uptake experiment being the second microdialysis experiment (12 plants: 6 high & 6 low), the mean ammonium uptake rate was 1.7-fold higher than nitrate in low N plants (P=0.05, Figure 2.10.A), while there was no significant difference in the uptake of ammonium and nitrate in high N plants.

Meanwhile from the second experiment being the third microdialysis experiment (24 plants: 12 high & 12 low of which for each, 4 plants were sprayed with high N, 4 with low N and 4 unsprayed), roots of low and high N plants individually captured ammonium and nitrate at similar rates (Figure 2.10.C). However, ammonium appeared to be the preferred nitrogen source in all plants. Whilst nitrate uptake by roots of high N plants was more than that of the low N plants (P= 0.01, Figure 2.10.C). Notwithstanding, the mean nitrogen uptake rates were higher in high N grown plants than the low N plants (P<0.001, Figure 2.9.B).

2.4.2.4. *Growth condition influenced the uptake rates of nitrogen sources by crown and seminal roots:*

From the first experiment, the crown roots of low N plants captured twice as much ammonium (2.0-fold) as the crown roots of high N plants (P= 0.05, Figure 2.10.B). While the seminal roots captured ammonium at similar rates. During this condition, both the crown and seminal roots of low and high plants captured nitrate at similar rates.

Meanwhile from the second experiment, the crown roots of low and high N plants individually captured ammonium and nitrate at similar rates (Figure 2.10.D). Their seminal roots also captured ammonium at similar rate. But the seminal roots of high N plants capture more nitrate than that of the low N plants (P=0.04, Figure 2.10.D).

Wholistic N uptake comparison between low and high N plants shows that nitrate and ammonium uptake in high N plants were significantly higher than in low N plants (nitrate: P=0.01, ammonium: P=0.002), but no significant difference between the mean ammonium uptake of high N and low N plants (P=0.09).

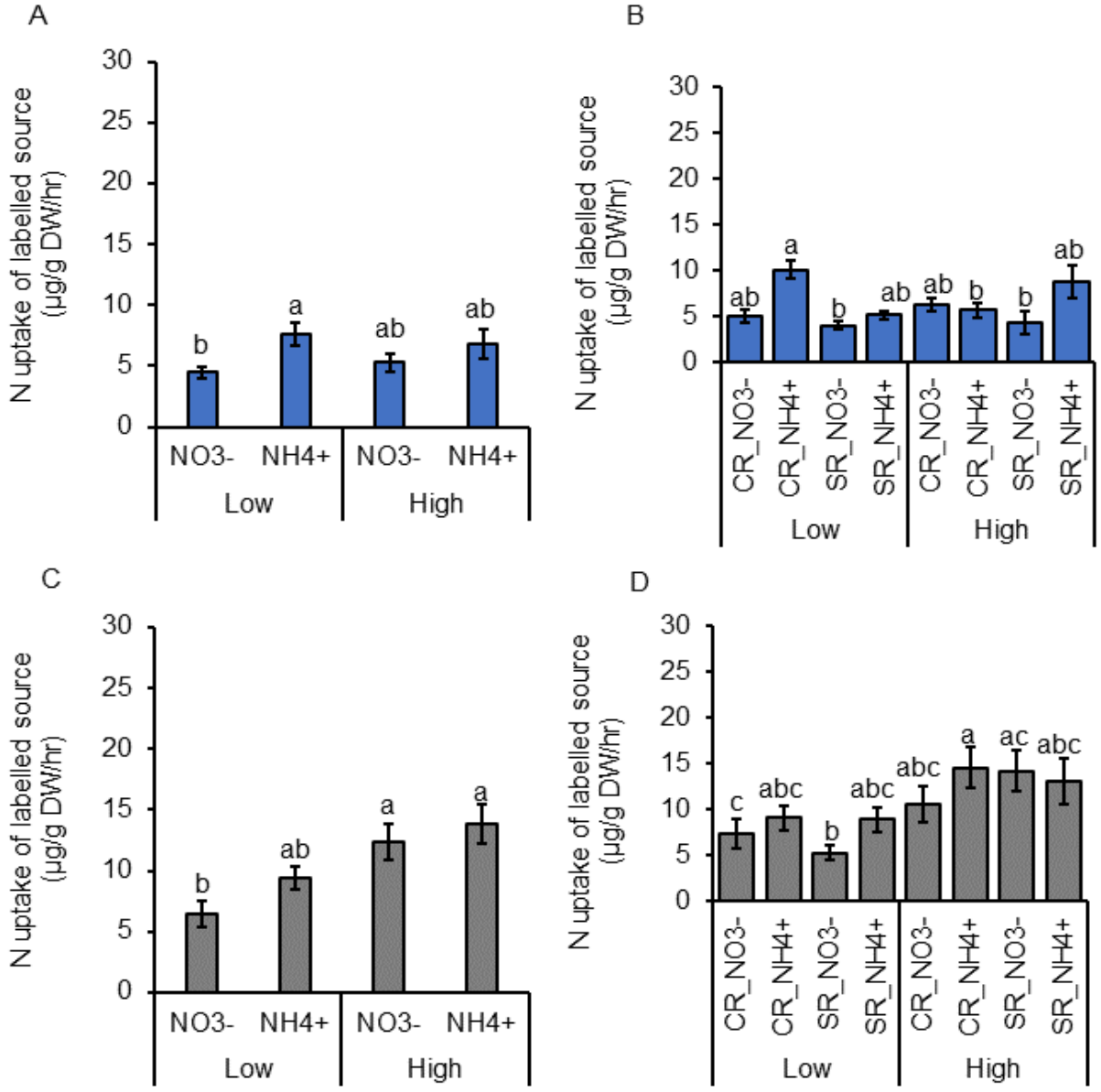


Figure 2.10. Nitrate and ammonium uptake rate by maize crown and seminal roots were influenced by nitrogen background. The effect of nitrogen background on the uptake of nitrate and ammonium (A,C) while (B,D) represent the effect of nitrogen background on nitrate and ammonium uptake by crown roots (CR) and seminal roots (SR) from both normal nitrogen resupply experiment (blue) and factorial nitrogen resupply experiment (brown). All bars are averages of (n=1 root type x 6 plants and n=1 root type x 12 plants each for the first second experiment respectively) different lower script letters denote statistical difference at 5%. Error bars are standard error of the mean.

2.4.2.5. The nitrogen uptake capacity of maize roots depends on the concentration of available N sources but not the root diameter.

The relationship between nitrogen uptake by crown and seminal roots of high and low N plants show no individual correlation with their corresponding root diameter (Figure 2.11.), using the Pearson's product-moment correlation coefficient test (r= 0.01). However, there appeared to be a weak positive relationship between nitrogen uptake and root diameter when high N plants were sprayed with low N solution (r= 0.6, Figure 2.11.B) and when low N plants were sprayed with high N (r= 0.5 Figure 2.11 D), and a weak negative correlation between the root diameter of the unsprayed high N plants and uptake of N sources (r= -0.6, Figure 2.11. C).

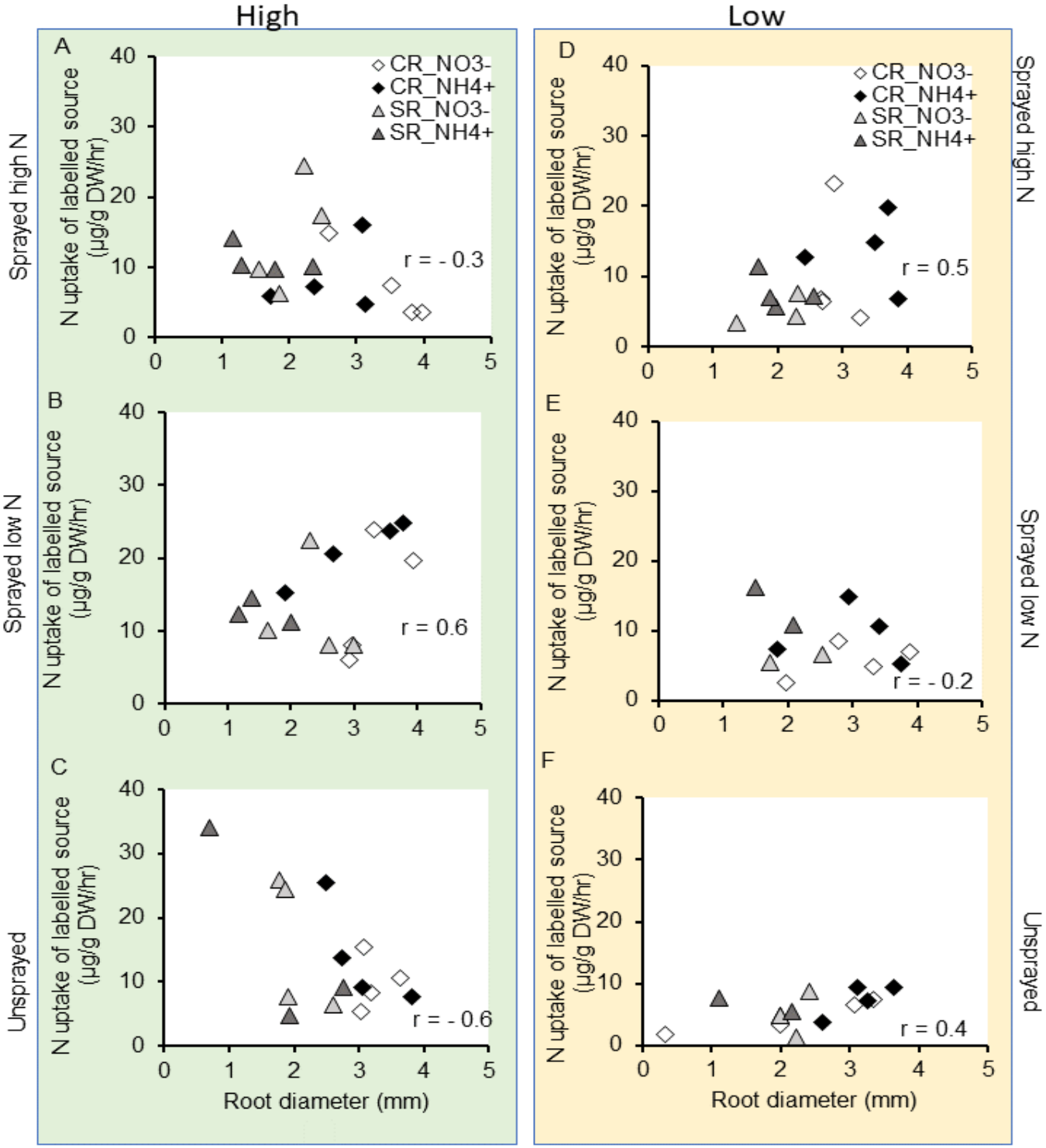


Figure 2.11. Nitrate and ammonium uptake by maize crown and seminal roots showed a weak tendency to correlate with their root diameter after a switched nitrogen re-supply. All data points are nitrate or ammonium fluxes by intact maize crown and seminal roots, sampled for 30 minutes after spraying with high (A,D) or low (B,E) nitrogen solution, or unsprayed (C,F) maize grown on limited and optimum nitrogen background. Green panel (A-C)are plants grown on high N background while the orange panels (D-F) are plants grown on low N background. markers are averages(n=1 root sample x 4 plants each. Data are from the factorial nitrogen resupply experiment.

2.4. Discussion

While nitrogen fertilizer is used in growing maize for improved yield, the real-time rates at which these nutrient ions deplete from the soil is poorly understood. This Chapter hypothesized that nitrate ions would deplete in the soil faster than ammonium ions when maize plants were grown on adequate or limited nitrogen. This is because the negative charge ion of nitrate will be repelled by the negative charge on the surface of soil particles and so nitrate will be mobile and can be carried easily through mass flow. Ammonium on the other hand is a cation and so is bound tightly to the soil matrix and so may not be readily carried through mass flow (Brackin et al., 2017; Fageria, 2014). The microdialysis technique was used to sample for nitrate and ammonium ion available for uptake by plant roots at positions adjacent to crown roots, seminal roots, and the background soil across time sequences. This was done after spraying high or low nitrogen solution on the entire surface of the rhixoboxes, containing maize plants growing on low and/or high nitrogen background. To extend this knowledge of depletion via uptake, it was expedient to measure the uptake abilities of crown and seminal roots when induced for uptake at 24 or 44 hours, to help understand the differences in the root type uptake abilities. This would offer the opportunity to identify root traits that can be optimised for improved nitrogen uptake from the soil. Here, the Chapter aim to discuss the real-time spatio-temporal dynamics of nitrate and ammonium at the background soil and adjacent to maize root types and to compare with the root type nitrogen uptake ability.

Data suggests that ammonium more rapidly depleted from the soil surface compared to nitrate. This occurred earlier in soils of low nitrogen background and when nitrogen resupply is of lower concentration. With this, ammonium appeared to be the preferred nitrogen form as compared to nitrate during low nitrogen condition. However, the lack of distinct uniform pattern of depletion in the three microdialysis experiments carried out means that environmental factors were important influencers of this sudden disappearance of ammonium from the soil surface. But root type uptake study also showed that the maize crown root captured more ammonium during perceived low external nitrogen by plants growing on both low and high nitrogen background soils. While the maize seminal roots capture more nitrate during adequate nitrogen supply. These variation in uptake by intact maize roots were not associated with the differences in size of their root diameter but appeared to depend on the concentration of nutrient resupply. Equally important to note, is that the net nitrogen uptake was lower in plants grown on low nitrogen background as compared to those grown on adequate nitrogen irrespective of the concentration of the nitrogen resupplied. And these will now be discussed below.

2.5.1. The spatiotemporal dynamics of nitrogen forms in relation to soil background and root types

2.5.1.1. The depletion rates of nitrogen forms at the soil background (far from roots):

The depletion rate of nitrate at the background was not rapid but was gradual at most of the times regardless of the nitrogen background or the concentration of nitrogen applied. This means that the nitrate ions were loose on the soil surface and were carried away from the background through mass flow. A situation that can lead to environmental pollution through leaching in agricultural lands (Brackin et al., 2017; Fageria, 2014; Fageria. et al., 2011; Hawkesford and Whalley, 2021). The real time depletion rate of nitrate at the background in this study was not linear and an increase in nitrate concentration was observed in the background of low and high N plants after 18 hrs and 27 hrs respectively, following high nitrogen pulse and these patterns were observed in two experiments. This may mean a form of nitrogen transformation such as mineralization by the soil microbes domiciled in the background soil. This finding is partly in line with Brackin et al., (2017), that found the soil background as the depletion zone for nitrate. Although their study did not apply any nutrient when they examined the accumulation and depletion of the nitrogen forms in the soil.

For ammonium, the rate of depletion at the background soil was rapid within 3 hrs and by 6 hrs no ion was detected. This occurred earlier in soils of low nitrogen background. The rapid depletion cannot be directly attributed to plant uptake, but a possible loss of ammonium through microbial activities and possible volatilisation aided by atmospheric factors. Earlier research using isotope dilution pools and modelling approaches to determine short-term (24 hours) soil nitrogen dynamics in annual grassland ecosystem at two different seasons ((Jackson et al., 2008; Jackson et al., 1989), showed that the turnover of microbial pools was rapid and that microbes took more ammonium and nitrate than plants, with a higher percentage of ammonium captured by microbes and plants than nitrate (Jackson et al., 1989). Considering these, This Chapter hypothesized that the rapid depletion of ammonium compared to nitrate could be orchestrated mainly by microbial activities of heterotrophs followed by plant uptake. This is because the soil used was Klassman silver sand:compost 1:1 soil mix, of low nitrogen implying high carbon to nitrogen ratio. The soil physical and chemical properties may exert changes in the activities of the microbial community that might multiply by the influence of growth condition. However, the microbial diversity of the annual grassland may not directly relate with the experimental set-up in the glasshouse. But may have certain common elements due to the physicochemical soil properties, compost in the soil, and growth condition.

Microbial soil nitrogen transformation processes include nitrification, denitrification and immobilization of inorganic nitrogen sources (Fageria, 2014; Fageria. et al., 2011; Robertson and Groffman, 2015; Tatsumi et al., 2019). The type and degree of each transformation process that takes

place depends on the availability of nitrogen, the carbon and nitrogen ratio of soil organic matter, the dominant groups of soil microbes, soil temperature and moisture content (Fageria, 2014; Nevin et al., 2020; Robertson and Groffman, 2015) as well as the physicochemical properties of the soil (Tatsumi et al., 2019). Given the condition of the experiment, it is presumed that nitrification by soil heterotrophs and other nitrifying bacteria and archaea might have contributed to the rapid ammonium loss. An overview of the abiotic factors that might have interacted during the experiments could help to identify the cause of rapid ammonium loss and understand how to minimise their activities.

The growth condition (increased temperature, aeration and moisture) could be favourable for the activities of the ammonium oxidising microbes especially when nitrogen solution was applied. As some scholars (Fageria, 2014; Nevin et al., 2020; Robertson and Groffman, 2015) explained that the most important factor controlling nitrification is ammonium supply followed by increased aeration and temperature. Moreso, the ratio of carbon to nitrogen in the soil used is higher (since the soil used is a low N soil), a situation that can encourage scavenging of inorganic nitrogen by soil microbes for their survival (Chen et al., 2015; Fageria, 2014; Martikainen, 2022; Nevin et al., 2020; Robertson and Groffman, 2015), thus leading to either immobilization or nitrification. The fact that ammonium depleted rapidly without subsequent recovery in the soil background with earlier occurrence in low N soil implies the activities of chemolithotroph and heterotrophic nitrifiers. Chemolithotrophic nitrifiers are suspected because a group of bacteria comammox *Nitrospira* are widely found on most soil types (Nevin et al., 2020; Robertson and Groffman, 2015). While heterotrophs are highly suspected because of the high carbon to nitrogen ratio in the soil used and rhizosphere of plants could serve as substrate for nitrification (Chen et al., 2015; Martikainen, 2022). It is difficult to distinguish between the two *in situ* because both processes are predominant in arable lands within a wide pH range. But one difference between the duo is that heterotrophs also oxidise organic matter which can be easily detected by laboratory analysis after soil incubation at room temperature. Also, they can tolerate higher concentration of acetylene. Whereas just a little concentration of acetylene can inhibit the enzymatic process of oxidising ammonium (Robertson and Groffman, 2015).

In the light of the above, further studies is required to quantify the net and gross uptake of ammonium by this possible microbial community, since the goal of this study is to understand the rate of nitrogen depletion after application. Identifying the composition of the soil microbes and quantifying the uptake rate will help with the understanding of soil-plants nutrients dynamics in maize. Also, to understand if other channel of losses such as volatilization were involved and if the effect was substantial. This will help to make inform decision on ways to manipulate experimental set-up to

mitigate ammonium loss. Also, the suitability of the rhizobox as a tool for root-nutrients interaction studies need to be investigated for modification.

2.5.1.2. The spatiotemporal dynamics of nitrogen forms adjacent to root types:

The rapid depletion rates of nitrate in the rhizosphere of low N plants can be connected to uptake by a possible up regulation of the high affinity transport system, ZmNRT2.1, ZmNRT2.2 and other nitrate inducible transporters responding to plants nitrogen demand during nitrogen provision, commonly known as the primary nitrate response (Dechorgnat et al., 2018; Garnett et al., 2013; Noguero and Lacombe, 2016; Plett et al., 2018; Vega et al., 2019; Vidal et al., 2020). The pattern of nitrate depletion after low N plants were sprayed with high N (linear-wise response) could be orchestrated by ZmNRT1.1 following continuous high N availability. This portrayed over-dependence on the nitrate low affinity transport system (York et al., 2016). However, the increase in nitrate level between 18-24 hours in low N plants given high N pulse may be due to mineralization of nutrients by the microbes in the rhizosphere. Since plants on low nitrogen soil could out-smart competitors by enriching the rhizosphere with microbial communities that enhance nutrient capture and utilization (Garcia and Kao-Kniffin, 2018; Jacoby et al., 2017). As the rhizosphere of low N plants given low N pulse was lacking nitrogen source, a situation that depicts competition between plant roots and soil microbe since no accumulation of nitrate was seen afterwards.

The nutrient-induced variation in depletion pattern in the rhizosphere of high N plants could be associated with differences in transcriptional regulation of uptake. The density of transporters per unit length of roots, and the activities of internal transporters as regulated by plants demand processes influence the plants uptake abilities (Bouranis and Chorianopoulou, 2021; Garnett et al., 2013; Lemaire and Ciampitti, 2020; Plett et al., 2018; White, 2012). The low affinity transporters ZmNRT1.1a and ZmNRT1.1b were demonstrated to capture soil nitrate at high concentration (Dechorgnat et al., 2018). But the high affinity transport system (HATs) ZmNRT2.1 and ZmNRT2.2 was also reported to respond to plants demand and contribute to total uptake at high external N concentration (Garnett et al., 2013; Plett et al., 2018). The expression levels of ZmNRT2.1 and ZmNRT2.2 was said to be 1000-fold higher than ZmNRT1.1a, ZmNRT1.1b and ZmNRT1.2 even when plants were grown on adequate N condition (Garnett et al., 2013; Yu et al., 2014)). Although a previous study showed that NRT1.1 was involved in activating NRT2.1 upon nitrate provision, as knockout mutations of NRT1.1 was unable to increase the expression of NRT2.1 in Arabidopsis (Nacry et al., 2013). Further research into real-time kinetics of transcriptional regulation is required for better understanding.

One outstanding issue is that the fluxes of nitrate adjacent to roots decreased to lowest level at 27 hrs and gradually increased afterwards following high nitrogen supply to high N plants. This could mean

mineralization by microbes. Remineralization of nitrogen sources was said to occur faster in soils with live roots than soil without plants (Burger and Jackson, 2004), due to great diversity of microbes that are free living or intricately linked to the plant host (Garcia and Kao-Kniffin, 2018). While the high N plants sprayed with low N solution decrease continually without increase in nitrate afterwards. This pattern of nitrate depletion (almost linear-wise) could be attributed to the characteristics of the main nitrate transporter enabling uptake in the high N plant. For maize growing on high N background, the low affinity transporter ZmNRT1.1 (K_m~4 mM) is assumed to be the main transporter for nitrate uptake. But both low and high affinity transporters (LATs and HATs) were said to coexist in different proportion in the plants root epidermis and be post-translationally regulated for kinetics (York et al., 2016).

For the rapid depletion rate of ammonium adjacent to roots of low and high N plants, the lower energy cost for ammonium assimilation might have enhanced its preferential uptake as compared to nitrate. Because plants expend a quarter fold of their total energy in shoots and roots to assimilate nitrate (Bloom, 2015). So, NH_4^+ is preferentially taken up by many plants when equimolar level of nitrate and ammonium are supplied during low N condition (Hawkesford et al., 2012). In maize, *ZmAMT 1.1a* and *ZmAMT 1.3* were implicated for substrate inducible high affinity transport (HATs) for ammonium uptake, with Km affinities of 48 and 33 µM respectively (Dechorgnat et al., 2019; Gu et al., 2013; Kiba and Krapp, 2016). The transcript abundances of ZmAMT1.1a, ZmAMT1.1b and ZmAMT1.3 rapidly upregulated within 3 hrs of root exposing maize roots to 0.5mM NH_4NO_3 solution (Hao et al., 2020). While ZmAMF1.1 and ZmAMF1.2 were the low affinity ammonium transporters expressed in similar proportion in all maize organs irrespective of ontogenetic phase but are not functionally characterized(Dechorgnat et al., 2019). In this study, the nitrogen supply might have triggered the up regulation of the ZmAMTs at the plasma membrane of root types within the first 3 hours for uptake. However microbial nitrifiers are suspected of competing with the roots due to the rapidity of depletion and the lack of recovery of ammonium even after 44 hrs adjacent to roots or background.

2.5.2. The Net effect of nitrate and ammonium uptake by intact maize roots

2.5.2.1. *The effect of nitrogen background on the amount of ammonium and nitrate captured:*

Ammonium was the preferred N source with higher net uptake as compared to nitrate. This result is consistent with the findings from the microdialysis experiment where ammonium depletion was faster than nitrate and even faster in low N plants. This could be due to the reduced energy cost required to synthesize organic N compounds during ammonium acquisition and assimilation as compared to nitrate (Bloom, 2015; Hachiya and Sakakibara, 2017; Liu and von Wirén, 2017). Gu et al., (2013),

demonstrated the preferential uptake of ammonium over nitrate within 5 minutes of exposing maize roots to equimolar concentration of nitrate and ammonium, using ^{15}N tracer.

2.5.2.2. *Growth condition influenced the uptake rates of nitrogen sources by crown and seminal roots*:

The crown roots captured more ammonium than nitrate during low nitrogen condition in both low and high N plants. while the seminal root captured more nitrate during N satiate condition. This support previous study that the crown root contributed to most of the net N uptake across the HATs and LATs +HATs (HATs= high affinity transport system, LATs= low affinity transport system) ranges, with the seminal roots being the least active for N uptake (Dechorgnat et al., 2018). These could explain why the crown roots in my study appeared to take up more N source when there was increase in plants demand in relation to external nitrogen supply.

2.5.2.3. Root type uptake of nitrogen forms did not significantly differ following 24 hours of nutrient spray:

The lack of significant difference between root types of induced and uninduced plants was compared with the corresponding microdialysis. I found higher depletion of ammonium adjacent to both root type within one hour in low N plants, and within 24 hours of treatments in high N plants, while nitrates were readily available in both low and high plants. This implies that the nitrate transporters were readily induced since the roots were already in contact with N source prior to uptake measurement, but the activities of the ZmNRTs might have been suppressed by ammonium uptake activities in both low and high N plants. Previous reports suggest production of one proton per molecule following ammonium uptake by roots. These protons are secreted into the external medium to maintain cellular pH and electron neutrality that favours ammonium uptake as compared to nitrate (Hawkesford et al., 2012). The proton generated by ammonium assimilation could be utilized for nitrate reduction, making it easier for plants to control intracellular pH when both N forms are applied (Hawkesford et al., 2012).

2.5.2.4. Factorial N pulse influenced the root type uptake of nitrogen forms following 44 hours of nutrients spray:

Like above, the influx rates at 4 mM were also chosen but in a factorial manner. The expectation was that uptake rate by root types for N sources when nutrient concentration was interchanged. This expectation was met as crown roots preferentially captured ammonium and then nitrates when there was need to maximise uptake. While seminal roots appeared to capture more N sources during usual concentration. Induction enabled the uptake of N sources in low N plants. Mapping this to that of the microdialysis at 44 hours showed a probable sustained induction of transporter genes.

A study on gene expression pattern by maize root types in maize inbred line B73 revealed that the stress related genes and transcription factors including (LATERAL ORGAN BOUNDARY DOMAIN (LBD), GOLDEN$_2$ (G$_2$LIKE), BASIC/HELIX-LOOP-HELIX (bHLH,) ETYLENE RESPONSE FACTOR (ERF), DNA binding with one finger (DOF) and WRKY) were over expressed in seminal roots relative to crown and primary roots (Tai et al., 2016). But that the genes implicated for growth and development, cell division and response to environmental stimuli (ARFs and HD-ZIP) were over expressed in crown and primary roots relative to seminal roots (Tai et al., 2016). These findings could relate to my studies because the recombinant inbreed line of KWS maize cultivar which I used had preference for ammonium uptake than nitrate of which B73 maize cultivar was reported to have higher preference for ammonium over nitrate (Dechorgnat et al., 2018). Therefore, relating this transcriptional variation between crown and seminal roots to my studies could account for why the crown roots capture more N source when there was need to maximise uptake, since the genes responding to environmental stimuli were said to be predominantly expressed in crown roots. Also, the seminal roots of low N plants seemingly took up more amount of ammonium then nitrate as compared to the crown roots after 44 hrs of usual N supply. This could mean an enhanced activity of the stress response genes in the seminal roots resulting from a short period of N starvation.

Both the crown and seminal roots of uninduced low N plants capture the N sources at similar rates but was also the least amount captured compared to the induced low N plants. This implies that the activation of transcription factors takes time following roots contacts with nitrogen. The uninduced plants were said to possess low-capacity constitutive nitrate transporters and that the constitutive nitrates transporters do not saturate as the external nitrate concentration increases (Hole et al., 1990; White, 2012), but that 20 minute of roots contact with nitrates was required for the induction of the high affinity nitrate influx system (White, 2012). Also, Carbohydrate shortage might be the reason why the net uptake of nitrogen sources by all low N plants were significantly lower than that of high N Plants (Jackson and Volks, 1992).

2.5.2.5. No relationship between the nitrogen uptake capacity of root types and their diameter:

Crown roots were said to contribute more of the nutrient uptake throughout the maize lifecycle (Yu et al., 2015b), and that their larger diameters were thought to be the reason for the enhanced water uptake than the seminal roots (Schneider et al., 2020). While the size of the metaxylem vessels of roots were said to influence plant capacity to transport water and nutrients to the shoots (Yu et al., 2015b), as crown roots were said to have an increased average root diameter, total number and area of metaxylem vessels (Tai et al., 2016; Yu et al., 2015b). My data suggest no correlation between uptake rate and root diameter of crown and seminal roots. Therefore, relating the previous reports

with my study, I inferred that the plants nutrients status and external environmental condition predominately influenced the selective and uptake rates of nutrients by maize root types more than anatomical traits.

2.5.3. Extent of genetic variation in maize population for crown root traits:

The maize genome has extensive structural, genetic, and transcriptional variation, of which each mutagenesis study is restricted to the differences in selected genotypes (Bray and Topp, 2018). A search for natural variation in crown root traits may present new insight for root development (Wang et al., 2021). The search could be achieved through the identification of quantitative trait locus (QTL), which can offer some understanding into the improvement of crop root functions, adapted for biotic and abiotic challenges (Burton et al., 2018). QTLs are used in marker-assisted selection for profiling of genotypes endowed with desirable traits (Burton et al., 2018). The marker assisted selection in conjunction with genome wide association study (GWAS) provides useful information for improving complex traits including crown root number, crown root angle and crown root diameter (Wang et al., 2021). This understanding led to several QTL mapping studies for crown root number, angle, and diameter (Li et al., 2020; Matthew and Shimelis, 2022; Wang et al., 2021). One of the QTL analyses in single and multiple environments revealed that 46 QTL were found in a single environment and 25 QTL were identified in multiple environments, with most of the trait loci as minor effect QTL for crown roots traits (Wang et al., 2021).

Environmental factors were reported to have some level of influence on phenotypic variation for crown root traits (Li et al., 2020). But the root traits could be constitutive (consistent across many growing environment) or adaptive (influenced by the specificity of the environment) (Mathew and Shimelis, 2022). Previous study showed that nitrogen stress increased root diameter in some maize phenotypes as an adaptive means to respond to nitrogen limitation (Wang et al., 2021). This view supports the findings by Dechorgnant et al., (2018), which showed that the B73 inbred maize line originating from Iowa (mollisols-soil, mainly deep surface soils with high organic and nutrient content) exhibited higher number of crown roots that are relatively shallow in contrast to the F44 inbred maize line originating from Florida (spodosols-soil and ultisols-soil, mainly acidic soils with poor nutrient status resulting from leaching), which had lower crown root number that were longer with increased surface area and volumes, when both B73 and F44 lines were grown in PVC pipes in a controlled growth chamber under moderate nitrogen condition (Dechognant et al., 2018). But it is unclear if there would be a notable variation in crown roots number, diameter and angle between plants grown in the glasshouse (pots or rhizoboxes) and plants grown in the field. More comparative study of crown root

traits of the same genotype in the field and glasshouse (pots and rhizoboxes) would expand our understanding on the extend of variation in crown roots traits as caused by different environment.

Crown roots number and diameter are more sensitive to node position than crown root angle, but the extend of sensitivity depends on ontogeny (all developmental events that occurs during the existence of plants) and soil nitrogen level (Li et al., 2020). Wang et al., (2021), reported that fewer crown root number improve nitrogen acquisition in low nitrogen soils. Interestingly, several QTLs controlling crown root number were found to correspond with flowering-times genes, when root traits from tropical teosinte were compared with the temperate maize inbred line W22 (Zhang et al., 2018; Bray and Topp, 2018). Flowering time directly regulate root traits in terms of size (lengths, surface areas, volumes, and biomass) and numbers (axile and primary branches), while the principal flowering time locus *vgt,* was found to be associated with a seminal root QTL number on chromosome 8. (Bray and Topp, 2018). Additionally, a sholvelomics-based study followed by genome wide analyses study of 316 inbred maize lines at maturity in 3 fields trials, revealed that a total of 12, 10 and 16 QTLs were dictated for crown root angle, crown root diameter and crown root number respectively (Wang et al., 2021). However, Li et al., (2020), reported that crown root angle did not vary by developmental stages but by genetic variation between genotypes. A study found that nearly one third of the genetic variation in crown root traits was attributed to flowering time and plant architecture (Sha et al., 2023). From the available literature, not much is known about phenotypic variation of crown roots traits prior to plants establishment.

Crown root traits are complex quantitative traits controlled by multiple genes (Sha et al., 2023). Studies showed that some polygenes with minor effects regulate the fundamental structures of crown root traits and traits response to soil condition (Lynch et al., 2019; Prince et al., 2019; Mathew and Shimelis, 2022). For instance, Wang and colleagues (2021) identified an Aux/IAA protein encoding genes GRM2M2G141205 which were associated with crown root number, and GRM2M2G175910 encoded a cytokinin-O-glucosyltransferase involved in the determination of crown root number (Wang et al., 2021). Out of a total of 38 QTLs that were found by a single locus and 3 multi-locus GWAS, 3 genes including GRM2M2141205 (IAA), GRM2M2G138511 (HSP), and GRM2M2G175910 (cytokinin-O-glucosyltransferase) were chosen as potential candidate genes in connection with the crown root development (Wang et al., 2021). Interestingly, GRM2M2G141205 encoding (Aux/IAA) was found to be associated with the regulation of crown root number in different environment (Wang et al., 2021). Another gene found to regulate crown root trait during drought stress is ZmDRO1 (homologous gene of OsDRO1) (Sha et al., 2023). ZmDRO1 facilitate maize tolerance to drought stress by altering the crown root angle in the field (Sha et al., 2023; Feng et al., 2022). ZmCIPK15 was also found to be strongly associated with crown root angle (Sha et al., 2023).

2.6. Conclusion

This study provides evidence that the maize rhizosphere were the nitrate accumulation zones while the background remained the zones of depletion as previously reported (Brackin et al., 2017), and that ammonium depleted fastest than nitrate in the background soil (contrary to expectation) and root zone. The rapid ammonium depletion rates were partly due to uptake as demonstrated in the three microdialysis and the two ^{15}N uptake studies where roots were seen to have preferential uptake of ammonium than nitrate in most cases. However, the rapid rate at which ammonium depleted at the background soil remain to be explored for better elucidation. A change in nutrient concentration enhances N uptake as compared to usual nutrient concentration, suggesting that growing maize on low nitrogen background and supplying at least one or two shots of high/adequate dose of N fertiliser intermittently, can enhance nitrogen use efficiency for optimum productivity with minimal environmental pollution. This will be substantiated further in the next chapter that explored the short- and long-term response of maize shoots to these N pulses. Furthermore, mixed ammonium and nitrate nutrition could facilitate efficient nitrogen capture especially when plants are grown on low nitrogen condition as uptake experiments showed enhanced uptake of ammonium and nitrate during reciprocal N pulse. Although the study did not investigate the uptake pattern if plants were grown on sole nitrate and/or sole ammonium.

This study also demonstrated that the maize crown roots captured more nitrogen source during low N condition as compared to seminal roots, and that reciprocal N pulse enhanced the ability of the crown roots to capture more N sources presumably due to higher expression of genes encoding environmental signalling as compared to the seminal roots. This implies that the crown roots possess the root traits required to improve uptake of soil resources which has been the subjects of research in the past 2 decades. Breeders can explore the gene pools and breed for maize plants with crown roots that are properly optimized for resource capture.

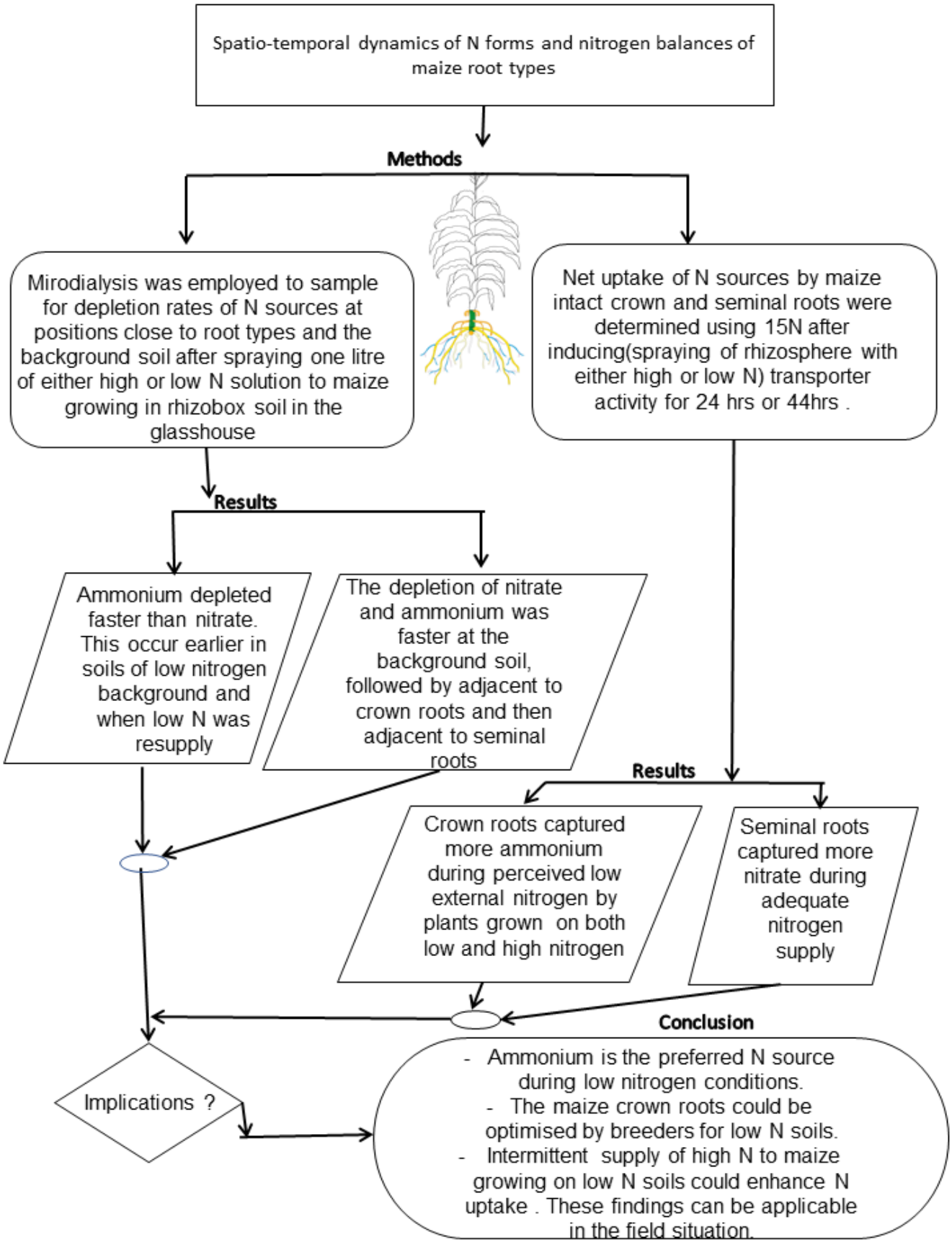


Figure 2.12. Summary of methods and findings in Chapter 2

2.7. References

Bloom, A. J. (2015). The increasing importance of distinguishing among plant nitrogen sources. *Current opinion in plant biology* **25**, 10-16.

Bouranis, D. L., and Chorianopoulou, S. N. (2021). Adavances in understandimg plants roots anatomy and nutrients acquisition. *In* "Understanding and improving crop roots function" (P. Gregory, ed.). Burleigh Dodd Science Publishing, United Kingdom.

Brackin, R., Atkinson, B. S., Sturrock, C. J., and Rasmussen, A. (2017). Roots-eye view: Using microdialysis and microCT to non-destructively map root nutrient depletion and accumulation zones. *Plant, cell and environment* **40**, 3135-3142.

Burger, M., and Jackson, L. E. (2004). Plant and microbial nitrogen use and turnover: Rapid conversion of nitrate to ammonium in soil with roots. *Plant and soil* **266**, 289-301.

Chen, Z., Ding, W., Xu, Y., Müller, C., Rütting, T., Yu, H., Fan, J., Zhang, J., and Zhu, T. (2015). Importance of heterotrophic nitrification and dissimilatory nitrate reduction to ammonium in a cropland soil: Evidences from a 15N tracing study to literature synthesis. *Soil biology & biochemistry* **91**, 65-75.

Dechorgnat, J., Francis, K., Dhugga, K. S., Rafalski, J. A., Tyerman, S. D., and Kaiser, B. N. (2018). Root Ideotype Influences Nitrogen Transport and Assimilation in Maize. *Frontiers in Plant Science* **9**, undefined-undefined.

Dechorgnat, J., Francis, K. L., Dhugga, K. S., Rafalski, J. A., Tyerman, S. D., and Kaiser, B. N. (2019). Tissue and nitrogen-linked expression profiles of ammonium and nitrate transporters in maize. *BMC plant biology* **19**, 206-206.

Fageria, N. K. (2014). "Nitrogen Management in Crop Production," First edition/Ed. CRC Press, Boca Raton.

Fageria., N. K., Baligar., V. C., and Jones., C. A. (2011). "Growth and Mineral Nutrition of Fields Crops," Third Edition/Ed. CRC Press, Boca Raton.

Garcia, J., and Kao-Kniffin, J. (2018). Microbial Group Dynamics in Plant Rhizospheres and Their Implications on Nutrient Cycling. *Frontiers in microbiology* **9**, 1516-1516.

Garnett, T., Conn, V., Plett, D., Conn, S., Zanghellini, J., Mackenzie, N., Enju, A., Francis, K., Holtham, L., Roessner, U., Boughton, B., Bacic, A., Shirley, N., Rafalski, A., Dhugga, K., Tester, M., and Kaiser, B. N. (2013). The response of the maize nitrate transport system to nitrogen demand and supply across the lifecycle. *New Phytologist* **198**, 82-94.

Gastal., F., Lemaire., G., Durand., J.-L., and Louarn., G. (2015). Quantifying crop responses to nitrogen and avenues to improve nitrogen-use efficiency. *In* "Crop Physiology

Applications for Genetic Improvement and Agronomy" (V. O. S. a. D. F. Calderini, ed.), pp. 161-206. Academic Press, United Kingdom.

Gu, R., Duan, F., An, X., Zhang, F., Von Wirén, N., and Yuan, L. (2013). Characterization of AMT-mediated high-affinity ammonium uptake in roots of maize (Zea mays L.). *Plant and cell physiology* **54**, 1515-1524.

Hachiya, T., and Sakakibara, H. (2017). Interactions between nitrate and ammonium in their uptake, allocation, assimilation, and signaling in plants. *Journal of experimental botany* **68**, 2501-2512.

Hao, D.-L., Zhou, J.-Y., Yang, S.-Y., Qi, W., Yang, K.-J., and Su, Y.-H. (2020). Function and regulation of ammonium transporters in plants. *International journal of molecular sciences* **21**, 3557.

Hawkesford, M., Horst, W., Kichey, T., Lambers, H., Schjoerring, J., Moller I.S., and White, P. (2012). Functions of Micronutrients. (P. e. Marschiner, ed.), Vol. 3, pp. 135-189. Academic Press, Mineral Nutrition of Higher Plants.

Hawkesford, M. J., and Whalley, W. R. (2021). Advances in the understanding of nitrogen uptake by plant roots. *In* "Understanding and improving crop root function" (P. Gregory, ed.), Vol. 1, pp. Pages 303-320. Burleigh dodds, United Kingdom.

Hochholdinger, F., Park, W. J., Sauer, M., and Woll, K. (2004). From weeds to crops: genetic analysis of root development in cereals. *Trends in plant science* **9**, 42-48.

Hochholdinger, F., and Tuberosa, R. (2009). Genetic and genomic dissection of maize root development and architecture: Genome studies and molecular genetics. *Current opinion in plant biology* **12**, 172-177.

Hochholdinger, F., Yu, P., and Marcon, C. (2018). Genetic Control of Root System Development in Maize. *Trends in Plant Science* **23**, 79-88.

Hole, D. J., Emran, A. M., Fares, Y., and Drew, M. C. (1990). Induction of nitrate transport in maize roots, and kinetics of influx, measured with nitrogen-13. *Plant physiology (Bethesda)* **93**, 642-647.

Jackson, L. E., Burger, M., and Cavagnaro, T. R. (2008). Roots, Nitrogen Transformations, and Ecosystem Services. *Annual review of plant biology* **59**, 341-363.

Jackson, L. E., Schimel, J. P., and Firestone, M. K. (1989). Short-term partitioning of ammonium and nitrate between plants and microbes in an annual grassland. *Soil biology & biochemistry* **21**, 409-415.

Jacoby, R., Peukert, M., Succurro, A., Koprivova, A., and Kopriva, S. (2017). The Role of Soil Microorganisms in Plant Mineral Nutrition-Current Knowledge and Future Directions. *Frontiers in plant science* **8**, 1617-1617.

Jacquot, A., Li, Z., Gojon, A., Schulze, W., and Lejay, L. (2017). Post-translational regulation of nitrogen transporters in plants and microorganisms. *Journal of experimental botany* **68**, 2567-2580.

Kiba, T., and Krapp, A. (2016). Plant Nitrogen Acquisition Under Low Availability: Regulation of Uptake and Root Architecture. *Plant and Cell Physiology* **57**, 707-714.

Lemaire, G., and Ciampitti, I. (2020). Crop Mass and N Status as Prerequisite Covariables for Unraveling Nitrogen Use Efficiency across Genotype-by-Environment-by-Management Scenarios: A Review. *Plants (Basel)* **9**, 1309.

Liu, Y., and von Wirén, N. (2017). Ammonium as a signal for physiological and morphological responses in plants. *Journal of experimental botany* **68**, 2581-2592.

Martikainen, P. J. (2022). Heterotrophic nitrification – An eternal mystery in the nitrogen cycle. *Soil biology & biochemistry* **168**, 108611.

Nacry, P., Bouguyon, E., and Gojon, A. (2013). Nitrogen acquisition by roots: physiological and developmental mechanisms ensuring plant adaptation to a fluctuating resource. *Plant and Soil* **370**, 1-29.

Nevin, C. J., Strauss, S. L., and Inglett, P. (2020). An overview of key soil nitrogen cycling transformation. *In* "SL471 University of Florida", Florida.

Noguero, M., and Lacombe, B. (2016). Transporters Involved in Root Nitrate Uptake and Sensing by Arabidopsis. *Frontiers in plant science* **7**, 1391-1391.

Plett, D. C., Holtham, L. R., Okamoto, M., and Garnett, T. P. (2018). Nitrate uptake and its regulation in relation to improving nitrogen use efficiency in cereals. *Seminars in Cell and Developmental Biology* **74**, 97-104.

Robertson, G. P., and Groffman, P. M. (2015). Nitrogen Transformations. *In* "Soil Microbiology,Ecolgy and Biochemistry" (E. A. Paul, ed.), pp. Pages 421-446. Academic Press, Burlington Massachuset, USA.

Saengwilai, P., Tian, X., and Lynch, J. P. (2014). Low Crown Root Number Enhances Nitrogen Acquisition from Low-Nitrogen Soils in Maize. *Plant physiology (Bethesda)* **166**, 581-589.

Schneider, H. M., Postma, J. A., Kochs, J., Pflugfelder, D., Lynch, J. P., and van Dusschoten, D. (2020). Spatio-Temporal Variation in Water Uptake in Seminal and Nodal Root Systems of Barley Plants Grown in Soil. *Frontiers in plant science* **11**, 1247-1247.

Shahzad, Z., and Amtmann, A. (2017). Food for thought: how nutrients regulate root system architecture. *Current Opinion in Plant Biology* **39**, 80-87.

Tai, H., Lu, X., Opitz, N., Marcon, C., Paschold, A., Lithio, A., Nettleton, D., and Hochholdinger, F. (2016). Transcriptomic and anatomical complexity of primary, seminal, and crown roots highlight root

type-specific functional diversity in maize (Zea mays L.). *Journal of experimental botany* **67**, 1123-1135.

Tatsumi, C., Taniguchi, T., Du, S., Yamanaka, N., and Tateno, R. (2019). The steps in the soil nitrogen transformation process vary along an aridity gradient via changes in the microbial community. *Biogeochemistry* **144**, 15-29.

Vega, A., O'Brien, J. A., and Gutiérrez, R. A. (2019). Nitrate and hormonal signaling crosstalk for plant growth and development. *Current opinion in plant biology* **52**, 155-163.

Vidal, E. A., Alvarez, J. M., Araus, V., Riveras, E., Brooks, M. D., Krouk, G., Ruffel, S., Lejay, L., Crawford, N. M., Coruzzi, G. M., and Gutiérrez, R. A. (2020). Nitrate in 2020: Thirty years from transport to signaling networks. *The Plant cell* **32**, 2094-2119.

White, P. J. (2012). Ion Uptake Mechanisms of Individual Cells and Roots: Short-distance Transport. *In* "Marschner's Mineral Nutrition for Higher Plants" (P. Marschner, ed.). Academic Press, United States of America.

York, L. M., Silberbush, M., and Lynch, J. P. (2016). Spatiotemporal variation of nitrate uptake kinetics within the maize (Zea mays L.) root system is associated with greater nitrate uptake and interactions with architectural phenes. *Journal of experimental botany* **67**, 3763-3775.

Yu, P., Hochholdinger, F., and Li, C. (2015a). Root-type-specific plasticity in response to localized high nitrate supply in maize (Zea mays). *Annals of Botany* **116**, 751-762.

Yu, P., Hochholdinger, F., and Li, C. (2019). Plasticity of Lateral Root Branching in Maize. *Frontiers in Plant Science* **10**.

Yu, P., Li, X., White, P. J., and Li, C. (2015b). A large and deep root system underlies high nitrogen-use efficiency in maize production. *PloS one* **10**, e0126293-e0126293.

Yu, P., White, P., Hochholdinger, F., and Li, C. (2014). Phenotypic plasticity of the maize root system in response to heterogeneous nitrogen availability. *An International Journal of Plant Biology* **240**, 667-678.

Chapter 3

Maize shoot longitudinal response to nitrogen pulses

3.1. Abstract

It is not fully understood what the short- and long-term response of maize is to additional supply of nitrogen in plants growing on low nitrogen background soils.

To address this gap in knowledge, maize plants from the microdialysis experiment (Chapter 2) were monitored for physiological changes in the shoot when either low or high nitrogen solution was applied to the rhizosphere during the nutrient depletion rates experiments. The PhotosynQ (Multispec v 2.0) was used to measure the chlorophyll level of leaves, the leaf temperature differential (proxy means of accessing transpiration), energy partitioning within the photosystem including ϕPSII (efficiency of the photosystem II), ϕNPQ (quenching of excess light as a safety mechanism), ϕNO (potential harmful energy to the photosystems), ϕNPQt (thermal quenching), linear electron flow and FV'/FM' (Quantum yield of the photosystem) during the short-term response to nitrogen pulse in a concentration dependent manner. The (Dualex (Force-A, Paris, France) were used to determine the nitrogen balance index (NBI), chlorophyll levels and flavonoids in all the experiment. While the SPAD was to measure the chlorophyll levels of leaves during the first experiments the plants of the first experiment were left to reach reproductive stage and plants heights, leaf length and leaf width, as well as leaf number were determined weekly. The plant height was used to obtain the longitudinal response such as the relative growth rate and estimated net assimilation rate. The leaf length and width were used to determine the leaf area duration of all visible leaves on the plants during the first experiment.

Findings from the short-term response showed that additional supply of high N solution to low N plants elevated the chlorophyll levels of low N plants within the three days of root nitrogen interaction. Increased efficiency of the photosystem two (ϕPSII) but dissipated energy (ϕNO) and quenched some as safety mechanism (ϕNPQ) as also evident with a temporal elevation of flavonoid shortly after nutrient application. The quantum yield of the photosystem II and the rate of electron transport between photosystem I and photosystem II were not affected by growth condition or concentration of nutrients resupplied. In terms of long-term responses, the relative growth rates were highest at 21 days after germination in all plants regardless of growth condition or additional supply of nitrogen, the estimated net assimilation rate was higher during the vegetative stage as compared to reproductive stage in low N plants and leaf area duration was higher in the during the onset of reproductive stage as compared to the vegetative stage.

In summary, these results demonstrated that regardless of the nitrogen condition of maize plants, the plants continued to photosynthesize efficiently, and that growing maize on low nitrogen background

and intermittently giving it a boost of high dose of nitrogen fertiliser may prove economical and minimise environmental degradation from excessive use of nitrogen fertilizer.

3.2. Introduction

Plant nitrogen use depends on processes including nitrogen uptake and assimilation, translocation, and recycling as well as remobilization. These processes depend on the internal and environmental cues surrounding the plants. For instance, the rate of nitrogen uptake from the soil depends on the internal nitrogen concentration of the plant (Fageria, 2014; Garnett et al., 2015; Gastal. et al., 2015; Muratore et al., 2021; White, 2012). The critical plant nitrogen concentration is the minimum nitrogen level needed by plants to reach maximum plant mass based on their growth rate potential (Gastal. et al., 2015). While, the association between critical crop nitrogen uptake and growth rate is independent of external variables (Gastal. et al., 2015), crop nitrogen uptake is directly related to the level of nitrogen within the rooting medium and feedback controlled by crop growth rate (Gastal. et al., 2015). The nutritional status of plant shoots mediate adaptive responses to nutrient starvation through mobilization of nutrients from intractable compounds and biochemical processes (White, 2012). This shows that the whole plant (root and shoot) responds to internal and the external nitrogen availability.

It is important to understand the link between root and shoot nutrient status and signalling before focusing on shoot responses (the focus of this chapter). Two opposite regulatory processes (positive and negative) are associated with the uptake of nitrogen sources from the soil. One example includes a positive regulation emanating from a carbon signal relating to photosynthetic assimilate transported by the phloem from leaves to roots, and a negative signal emanating from organic nitrogen recirculated from the shoot to the roots (Gastal. et al., 2015). The negative signal acts as a nitrogen satiety signal suppressing nitrogen uptake of plants when its capacity to store organic nitrogen compound within new growing organs is overwhelmed (Gastal. et al., 2015). The immediate effect of nutrient influx is due to posttranslational changes in influx systems while the long-term effect of nutrient influx is mediated by transcriptional responses (Plett et al., 2018; White, 2012; York et al., 2016). The assimilation of nitrate in the roots and shoots involves the reduction of nitrate to nitrite by the cytosolic enzyme, nitrate reductase (NR) and then further reduction to ammonium by nitrite reductase (NiR) in the plastid (Dechorgnat et al., 2018; Hawkesford et al., 2012). The ammonium obtained from root uptake and nitrate reduction are assimilated in the plasmid through the glutamine synthetase and glutamate synthase pathways (Dechorgnat et al., 2018), for growth and developmental processes.

The plant nitrogen economy involves partitioning of nitrogen between the metabolic and the structural protein pools (Gastal. et al., 2015; Urban et al., 2021). The metabolic pools comprise

proteins associated with photosynthesis and meristematic activities, while the structural protein pools are associated with plants architecture, all affecting plant mass (Gastal. et al., 2015). The photosynthetic process is a complex network of mechanisms including biochemical capacity (e.g carboxylation and electron transport), anatomical traits (e.g stomata conductance and mesophyll conductance), and the capacity of the plants to interact with changing environments (McAusland et al., 2019). Nitrogen is the central element in chlorophyll, meaning that leaf nitrogen content influences photosynthesis and that chlorophyll content can be used as a proxy for leaf nitrogen content (Gastal et al., Engels et al., 2012).

An important parameter that shows changes in the photosynthetic process as a function of growth environment is chlorophyll fluorescence (Garcia-Parra et al., 2020; Mu et al., 2017; Murchie and Lawson, 2013). Chlorophyll fluorescence is a non-invasive measurement of photosystem II activities (Murchie and Lawson, 2013). Chlorophyll fluorescence provides information regarding the proportion of absorbed light energy channelled for photochemistry, the proportion loss as thermal dissipation and the proportion loss as light re-emittance for the prevention of damage to the photosynthetic apparatus (Murchie and Lawson, 2013). During photosynthesis, the thylakoid membrane nitrogen is shared between proteins associated with bioenergetics (Cyt b/f and Cf_1/Cf_0 involved in photosynthetic electron transport and photophosphorylation) and light harvesting proteins such as photosystem I, photosystem II, and light harvesting complex II (Evans and Clarke, 2019; Mu et al., 2016, 2017; Urban et al., 2021). The leaf nitrogen content relates to the light saturated photosynthesis (Engels. et al., 2012; Urban et al., 2021) and while photosynthesis can be increased by increasing the photosynthetic activities of the leaves it can also be increased by having a larger photosynthetic area (Engels. et al., 2012). Finally, an increase in plant growth rate is concurrent with increased leaf area and photosynthesis (Gastal. et al., 2015).

Previous studies found the shoot nitrogen concentration to be significantly lower in low N maize plants across the lifecycle despite a one-time switch from low nitrogen, to medium, to high nitrogen hydroponic solutions (Garnett et al., 2013). Other works showed that leaf area expansion during low nitrogen supply must lead to a decrease in leaf nitrogen concentration per unit leaf area in maize (Gastal. et al., 2015). However, the integration of root and shoot responses to nitrogen starvation in maize in soil beyond seedling stage has been neglected. Moreso, Liebigs law of the minimum says growth is determined not by the total resource available but by the scarcest resource or limiting factor (although some scientists argue with his theory (Gorban et al., 2011; Paris, 1992)). This chapter aimed to examine the above-ground shoot response to the nitrogen regimes reported for below ground responses in Chapter 2 of this thesis. To achieve the aims and objectives, chlorophyll concentration,

chlorophyll fluorescence and other photosynthetic parameters were measured. The leaf area and plant height were determined and incorporated into plant growth indices.

Hypotheses:

To determine the response of maize shoot (above the ground section) to nitrogen background and nitrogen re-supply, the hypotheses evaluated were:

-Additional supply of nitrogen to maize grown on limited nitrogen will trigger dynamic changes in shoot photosynthetic parameters and growth rates. These changes will be greater with increase in concentration of nitrogen re-supplied.

-The growth rates of shoot functional organs will be greater during the vegetative stage as compared to the reproductive stage because of increased cell division during the vegetative stage. This increase will be greater in low nitrogen plants when high nitrogen pulse is applied at the beginning of the vegetative stage.

3.3. Methods

For plant growth conditions see "Methods" section in Chapter 2.

3.3.1. Above ground measurements.

Leaf number was counted, and leaf length (cm), leaf width (cm), and plant height (cm) were obtained weekly using a measuring tape, from 28 days after germination until the end of each experiment.

The leaf area (LA) was calculated as:

LA = leaf length x leaf width x K Equation 1

K represents the coefficient of variation of the wider and narrower part of the maize leaf when calculating leaf area = 0.75 (Wang et al., 2019).

The Plant height was used to obtain the relative growth rate (RGR) also known as the efficiency index, was calculated by:

$r = (\log_e H_2 - \log_e H_1) / (t_2 - t_1)$ $(m.m^{-1}\ day^{-1})$ Equation 2

(Where r is the relative growth rate/efficiency index, H_1 and H_2 are plants heights at time t_1 and t_2 respectively; Hoffmann and Poorter, 2002, Rees et al., 2010, Pandey et al.,2017).

Most plant growth analyses between different plant species use change in plant dry weight to calculate RGR or efficiency index to account for ontogenetic drift (Pandey. et al., 2017; Price. and Munns., 1999; Rees et al., 2010). Ontogenetic drift in this context refers to the pattern of decreasing RGR with increasing size. However, the RGR here, was obtained from plant height (cm) since the data was on the same plant species and with limited replication.

The net gain in height/biomass is the outcome of CO_2 assimilation by leaves minus respiratory loss by the entire plant. Leaf area can therefore be seen as a driving variable and height increase by unit time can be divided by the leaf area to yield the net assimilation rate.

$NAR = (H_2-H_1/t_2-t_1) * (\log_e (LA_2) - \log_e (LA_1) / (LA_2-LA_1)$ Equation 3

$(m.\ m^2.day^{-1})$

Where H_2 and H_1 are plant heights at time t_2 and t_1, $\log_e LA_2$ and $\log_e LA_1$ are the natural log of leaf area LA_2 and LA_1 at times t_2 and t_1

3.3.2. Measurements of chlorophyll and other photosynthetic parameters.

Three devices were used to measure the photosynthetic parameters: PhotosynQ Multispec v 2.0 (PhotosynQ LLC, East Lansing, Missouri, USA), Dualex (Force-A, Paris, France); and Spad (SPAD-502 Minolta corporation, Ltd., Osaka, Japan).

For the high N resupply experiment, the PhotosynQ, Dualex and Spad were all used. The PhotosynQ was initially used but was substituted by Dualex following temporary synchronisation issues. The Dualex (as an older device) developed light leak and had to be sent for service during the experiment. So, the Spad was used to complete the experiment. The measurements were taken from three positions (leaf tip, mid leaf, and leaf base) on all fully expanded leaves of each plant before opening the rhizoboxes, after opening the rhizoboxes, after spraying nutrients solution, and during each our hour of microdialysis. The mean values of the measurements from the three positions on leaves were presented for each measured parameter. The chlorophyll measurement continued once a week for 5 more weeks after microdialysis.

For the normal nitrogen resupply experiment, only the photosynQ was used and measurements were conducted just before microdialysis and after 24hrs (second microdialysis sampling).

For the factorial nitrogen resupply experiment, the measured parameters with PhotosynQ include the relative chlorophyll; leaf temperature differential (the difference between the leaf temperature and the ambient temperature); linear electron flow (the rate of electron transport from photosystem I to photosystem II); Non-Photochemical Quenching potential (ϕNPQt); the photosynthetic efficiency of

Photosystem II (ϕPSII), ϕNPQ is the proportion being quenched in the leaf for protection when insolation is in excess and ϕNO is the proportion released as free energy that can damage the photosystems – these last three all add up to 1 as they are proportions of the total energy. The dualex was also used to measure chlorophyll, nitrogen balance index (NBI) and flavonoid immediately after the initial measurements with PhotosynQ.

3.3.3. *Comparison of Dualex and PhotosynQ readings for photosynthetic apparatus.*

To harmonise the photosynQ and Dualex chlorophyll measurements in the first experiment, a correlation graph (scatter plot) was plotted to obtain a linear equation with the PhotosynQ and Dualex chlorophyll values obtained from the factorial nitrogen resupply experiment. This was used to predict the chlorophyll values from each device reading by solving for the unknown using the equation Y= mx + c as presented in Figure 3.0.

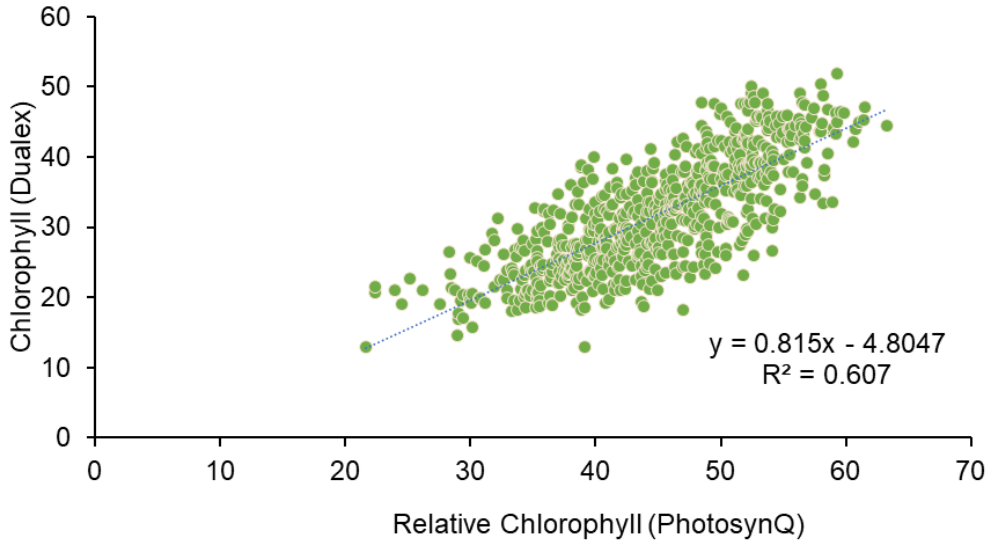


Figure 3.0. The correlation chart used to predict the values of chlorophyll measured with Dualex from the photosynQ readings and vice versa. The straight-line equation from the graph was used to calculate the predicted values.

Summarily, to understand the short and long-term effects of nitrogen background and resupply, plant shoots were measured to determine the changes in photosynthetic parameters and growth rates as influenced by the interaction of nitrogen background and concentration of N resupply. Plants from the factorial nitrogen resupply experiment were monitored for short-term effects. These plants were given either a high or low N pulse then were monitored for 44 hours. Plants from the high nitrogen resupply experiment were allowed to continue to grow up to the beginning of reproductive phase (56

DAG) for monitoring of long-term effects. These plants (both high and low N) were given only High N-pulses at 21 days post germination.

3.3.4. Data Analysis

Before analysis, data from the photosynQ were downloaded from the cloud while other data from Dualex, plant height and leaf measurement were imported into Microsoft Excel. All data were analysed using two-way ANOVA with R statistical software (R version 4.2.0 (2022-04-22 ucrt)).

3.4. Results

3.4.1. Short-term effect of nitrogen on maize shoot:

3.4.1.1. Chlorophyll and Nitrogen Balance Index were influenced by nitrogen background and slightly by the concentration of additional nitrogen resupplied.

Addition of nitrogen (irrespective of concentration) to high N plants did not significantly increase their chlorophyll level even after 72 hours of root interaction with soil nitrogen (P=0.9, Figure 3.1.A). However, the low N plants sprayed with high nitrogen had increased chlorophyll contents, as the high plants sprayed with high N and the unsprayed high plants on their first and second day of root interaction with soil nitrogen (P=0.02, Figure 3.1.C).

Meanwhile the nitrogen balance index of all high plants was 2-fold higher than that of the low plants (P<0.001). However, the low plants treated with high N solution on the first day of nutrient application had an increased nitrogen balance index above that of the unsprayed on the second and third day after nutrient supply (P=0.002, Figure 3.1.D).

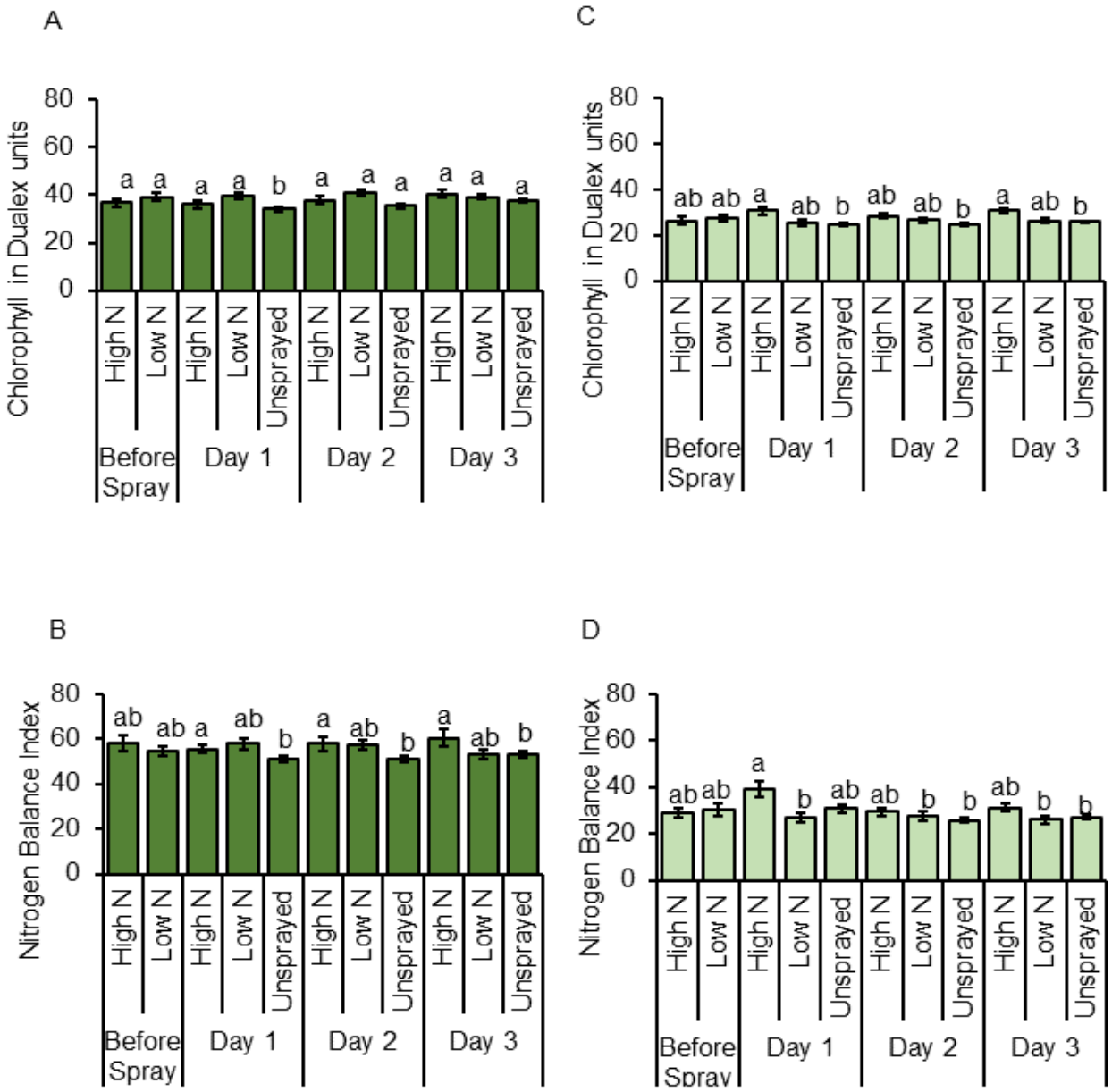


Figure 3.1. Chlorophyll related parameters were greatly influenced by nitrogen background and high nitrogen re-supply. Plants were grown on adequate/high (A&B, dark green bars) and limited/low (C&D, light green bars) nitrogen background and were either sprayed with high (High N) or low (Low N) nitrogen solution, or not sprayed (Unsprayed) on the first day of microdialysis. All bars are averages (n=5 leaves x 4 plants each). Different lower script letters denote statistical difference at 5%. Error bars are standard error of the mean.

.

3.4.1.2. High soil nitrogen level caused cooler canopy as compared to low soil nitrogen.

It was observed that the leaf temperature differential (difference between ambient and leaf temperatures) became more negative for each measurement time following the opening of rhizobox windows ($P<0.001$, Figure 3.2.A). This started to recover by 1 hour later. Spraying low plants with high N solution resulted in a decrease in average leaf temperature differential from -3 prior to opening rhizobox, to -7.8 after nutrient spray ($P<0.001$, Figure 3.2.A). When this decrease in leaf temperature differential was compared to similar change in other plants, it was observed that the percentage decrease got higher by 77% as compared to high plants given high nitrogen ($P=0.03$).

For measurements up to 72 hours of N resupply, the Day 1 measurements were taken immediately after the boxes were opened and sprayed, and the average leaf temperature differential of all sprayed plants increased by more than 50% within 48 hours as compared to that of the first day of N-pulse ($P<0.001$ each, Figure 3.2.B). The increase in leaf temperature differential was highest (83%) in low plants given low N-pulse, followed by high plants given low N-pulse (64%), then low plants given high N-pulse (62%) and finally high plants given high N-pulse (54%). The unsprayed plants recorded a raise in leaf temperature within 48 hours by 19% and 11% in high and low plants respectively (high: $P=0.02$, low: $P=0.9$, Figure 3.2.B). By the third day (72 hours) of nutrient sampling from the root-soil interface, the average leaf temperature differential of the sprayed plants decreased by 31, 30, 12 and 10% in low plants given repeat N-pulse, high plants given reciprocal N-pulse, high plants given repeat N-pulse and low plants given reciprocal N-pulse respectively as compared to their leaf temperature within 48 hours (second day). The percentage change in leaf temperature of the unsprayed high and low plants within 72 hours (third day) as compared to the second day were 1% and 7% respectively (Figure 3.2.B).

Interestingly the unsprayed plants (NN in the figure) showed less changes in leaf temperature differential as compared to those for the sprayed boxes. For the leaf temperature differential between the sprayed and the unsprayed plants on each of the three days of roots-soil nutrient interplay, no significant difference was observed between the high plants given high N-pulse and the unsprayed high plants within the three days (Day 1: $P=0.9$, Day 2: $P=0.6$, Day 3: $P=0.2$, Figure 3.2.B). Meanwhile, the average leaf temperature differential of high plants given low N-pulse as compared with that of the unsprayed high plants was significant higher (less negative) on the second and third day (Day 2: $P<0.001$, Day 3: $P=0.01$, Figure 3.2.B) but not the first day of treatment ($P=0.9$, Figure 3.2.B). Similarly, the average leaf temperature differential of low plants given high N-pulse compared with the unsprayed low plants were statistically higher on the second and third day ($P<0.001$ each, Figure 3.2.B) but not the first day of roots interaction with soil nitrogen ($P=0.9$, Figure 3.2.B). Accordingly, the average leaf temperature of the low plants given repeat N-pulse compared to that of the unsprayed

were significant higher on the second and third day ($P<0.001$ each, Figure 3.2.B) but not the first day of root nitrogen interaction ($P=0.6$, Figure 3.2.B).

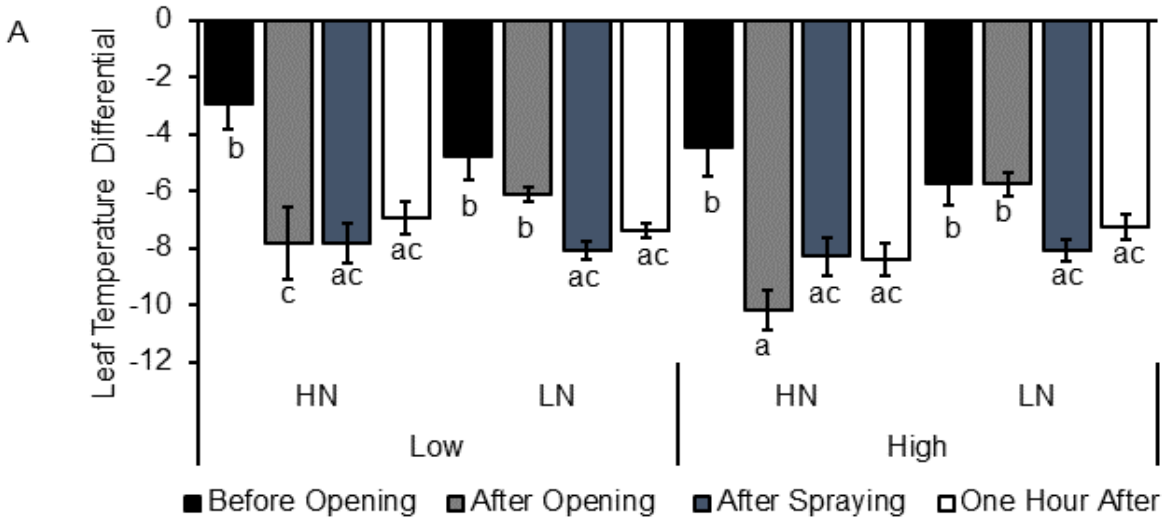


Figure 3.2.A. Opening of rhizobox increased the maize leaf temperature differential. Plants were either grown on adequate nitrogen (High) or limited nitrogen (Low). Measurements were taken as follows: Before opening the rhizoboxes, (black bars) After opening the rhizobox windows (gray bars), After spraying with either high nitrogen(HN) or with low(LN) (blue bars) and During the first hour of root nutrient interaction (white bars). Bars are averages (n= 4 leaves x 4 plants each). Lower case letters denotes statistical difference from 5% level of significance. Error bars are standard error of the mean.

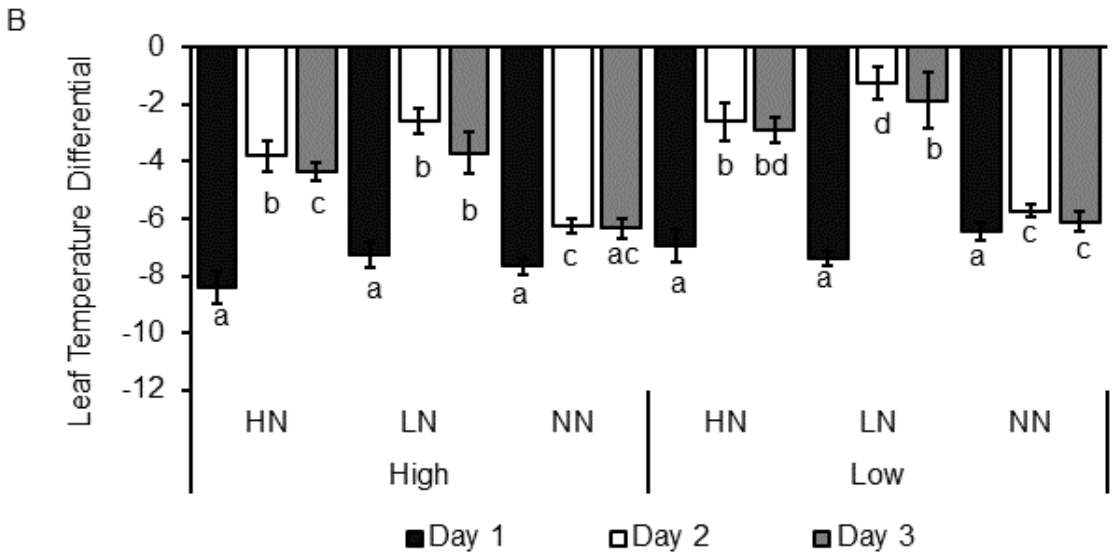


Figure 3.2.B. Nitrogen background and the concentration of nitrogen resupply influenced the maize leaf temperature differential. Plants were either grown on adequate nitrogen (High) or limited nitrogen(Low). They were either sprayed with high nitrogen(HN) or with low(LN) or unsprayed (NN). Day 1 implies the first day of nitrogen resupply while day 2 and Day 3 are the respective subsequent days after nutrients resupply. Bars are averages (n= 4 leaves x 4 plants each). Lower case letters denotes statistical difference from 5% level of significance.Error bars are standard error of the mean.

3.4.1.3. Changes in ϕNPQt *(heat dissipation of chlorophyll excitation energy) was noticed 24 hrs after root interaction with soil nitrogen.*

The average ϕNPQt in both high and low plants within 24 hours were not statistically significant (P=0.7, Figure 3.3.A). However, after 24 hrs (day 2- 3), the average ϕNPQt of low N plants were significantly higher than that of the high plants irrespective of the concentration of nitrogen pulses received (main effect: P=0.02, Figure 3.3.B). The interaction between nitrogen background, concentration of N re-supplied, and days were not statistically significant (P=0.5), but average ϕNPQt on the second and third day after nutrient pulse application were statistically significant (P=0.008 and P<0.001 respectively, Figure 3.3.B) in both high and low plants.

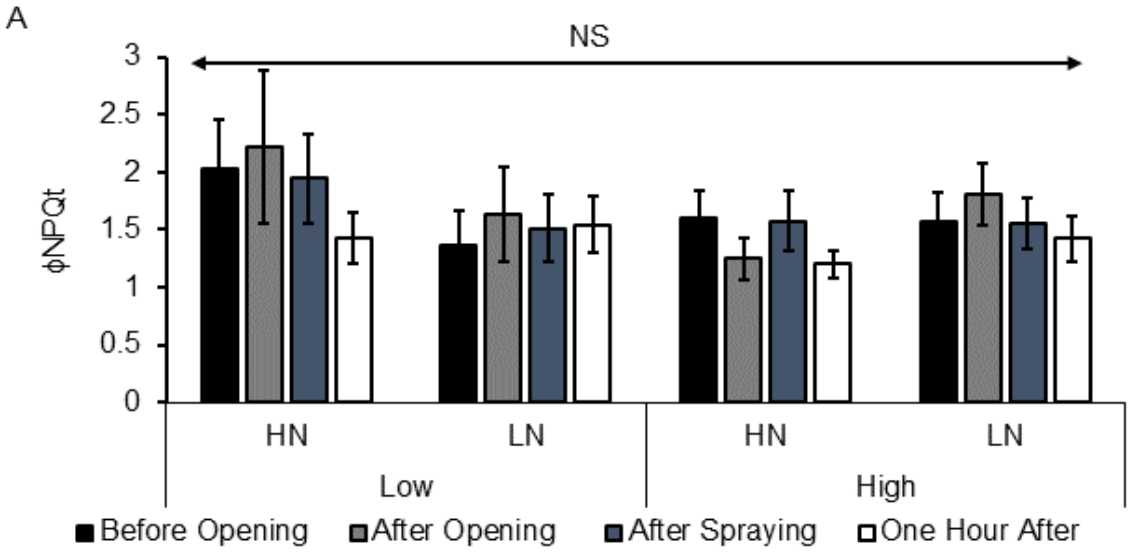


Figure 3.3.A. The opening of rhizobox and spraying with N solution did not cause immediate change in ϕNPQt. Plants were either grown on adequate nitrogen (High) or limited nitrogen (Low). Measurements were taken: Before opening rhizobox (black bars), After opening rhizobox (grey bars), After spraying with either high nitrogen (HN) or with low (LN) solution (blue bars), During the first hour of root interaction with soil nitrogen (white bars). Bars are averages (n= 4 leaves x 4 plants each). NS means not statistically significant. Error bars are the standard error of the mean.

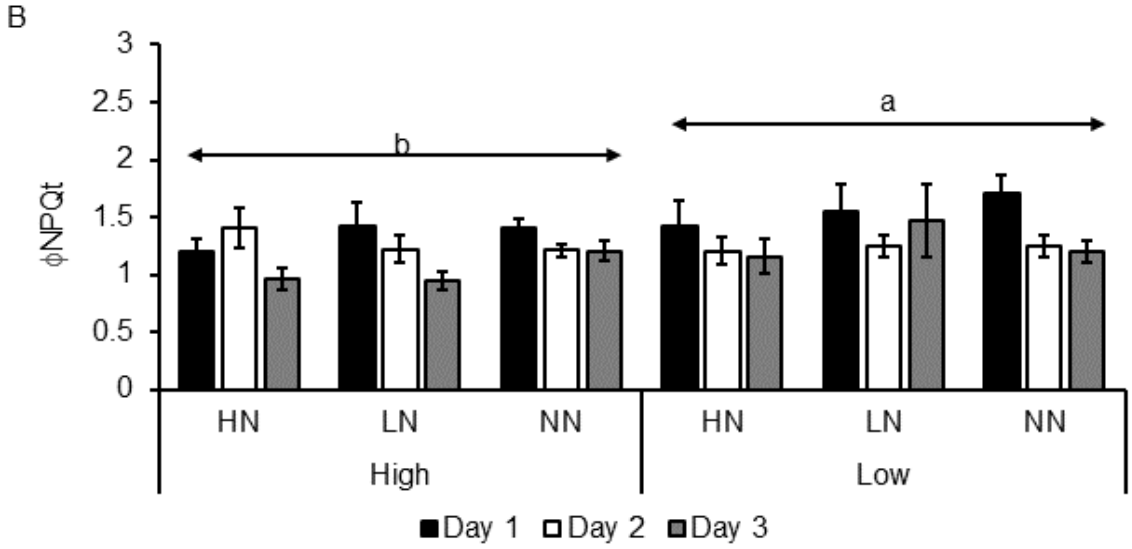


Figure 3.3.B. Nitrogen background influenced the rate of ϕ NPQt in 3 days. The NPQt increased in low nitrogen plants within 3 days irrespective of the concentration of nitrogen applied. Plants were either grown on adequate nitrogen (High) or limited nitrogen(Low). They were either sprayed with high nitrogen (HN) or with low (LN) or unsprayed (NN). Bars are averages (n= 4 leaves x 4 plants each). Lower case letters denotes statistical difference from 5% level of significance. Error bars are standard error of the mean.

3.4.1.4. The relative allocation of energy was influenced by growth condition within hours of root-zone spraying.

It was observed that the average ϕPSII (the photosynthetic efficiency of Photosystem II) and ϕNPQ (proportion of energy being quenched in the leaf for protection when insolation is in excess) in both high and low plants were not affected by nitrogen pulses within 24 hours of root nutrient interaction (ϕPSII: P=0.2, ϕNPQ: P=0.4, Figure 3.4.A). But the average ϕNO (the proportion released as free energy that can damage the photosystems) of plants increased shortly after high nitrogen spray than those sprayed with low nitrogen irrespective of nitrogen background (P=0.01, Figure 3.4.A). Meanwhile the average ϕPSII increased in high plants that received high N pulse than low plants given low N pulse within 72 hours (3 days) of nutrient application (P=0.03, Figure 3.4.B). Furthermore, the average ϕNPQ of low N plants increased in the first day of nutrients pulse application as compared to high plants on the third day, whether given additional N treatment or not (P<0.001, Figure 3.4.B). Application of high N pulse to low plants had increased ϕNO on the second day after treatment in relation to its proportion on the first day (P=0.02) and as compared to the unsprayed high N plants at both first and third day of nutrient pulse application (P=0.03 each, Figure 3.4.B).

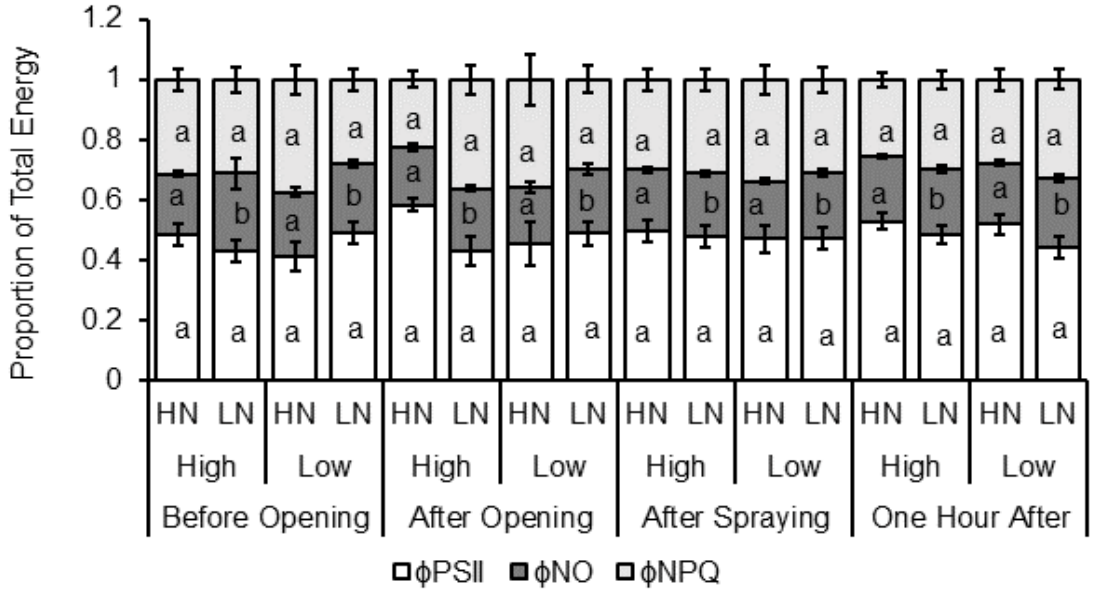


Figure 3.4.A. High nitrogen condition increased the thermal dissipating mechanism of leaves (ϕNO) regardless of nitrogen background. Plants were either grown on adequate nitrogen (High) or limited nitrogen(Low). They were either sprayed with high nitrogen (HN) or with low (LN) or left unsprayed (NN). All bars are averages (n=5 leaves x 4 plants each from the factorial nitrogen resupply experiment, different lower script letters denote significant difference at 5% level. Error bars are the standard error of the mean.

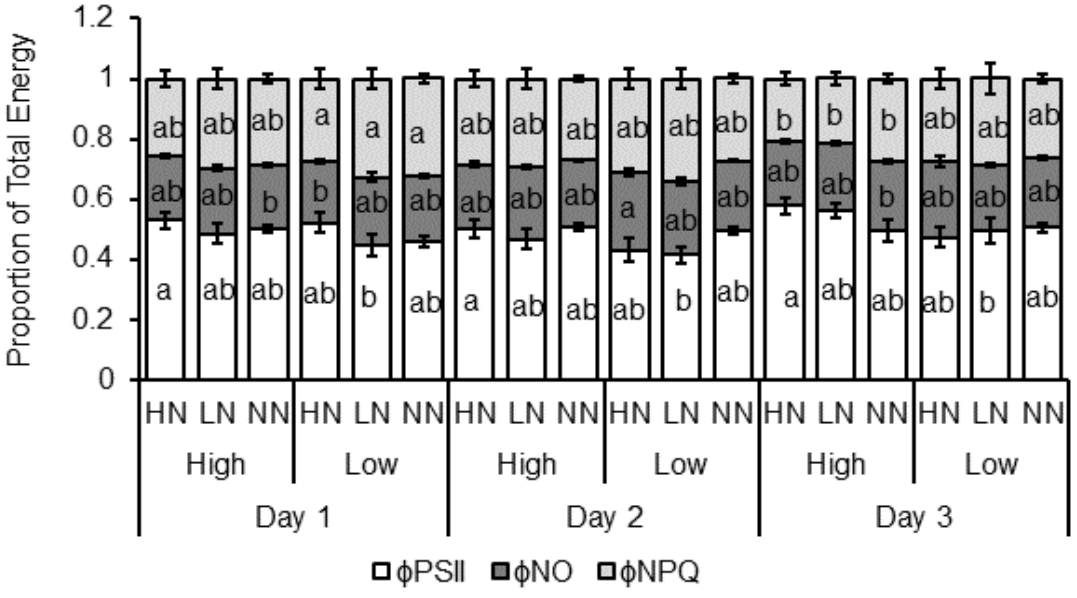


Figure 3.4.B. Nitrogen background and re-supply slightly influenced the partitioning of energy within three days of root-soil nitrogen interaction. Plants were either grown on adequate nitrogen (High) or limited nitrogen (Low). They were either sprayed in Day 1 with high nitrogen (HN) or with low (LN) or left unsprayed (NN). All bars are averages (n=5 leaves x 4 plants each from the factorial nitrogen resupply experiment, different lower script letters denote significant difference at 5% level. Error bars are the standard error of the mean.

3.4.1.5. The linear electron flux was not influenced by soil nitrogen level or nitrogen resupply.

It was observed that the average linear electron flux (the number of molecules of electron per seconds moving from the reaction centre chlorophyll (P680) to the primary quinone acceptor of PSII (Q_A) for sugar production) was not significantly different during the process of nutrient pulse application and an hour after nutrient spray (P=0.4, Figure 3.5.A). There was no statistically significant difference between low and high plants (P=0.6) as well as the concentration of nitrogen resupplied (P=0.9). The only statistically significant difference was the high plants given repeat N-pulse on the second day after nutrient application as compared to the high unsprayed plants on the third day (P=0.03, Figure 3.5.B).

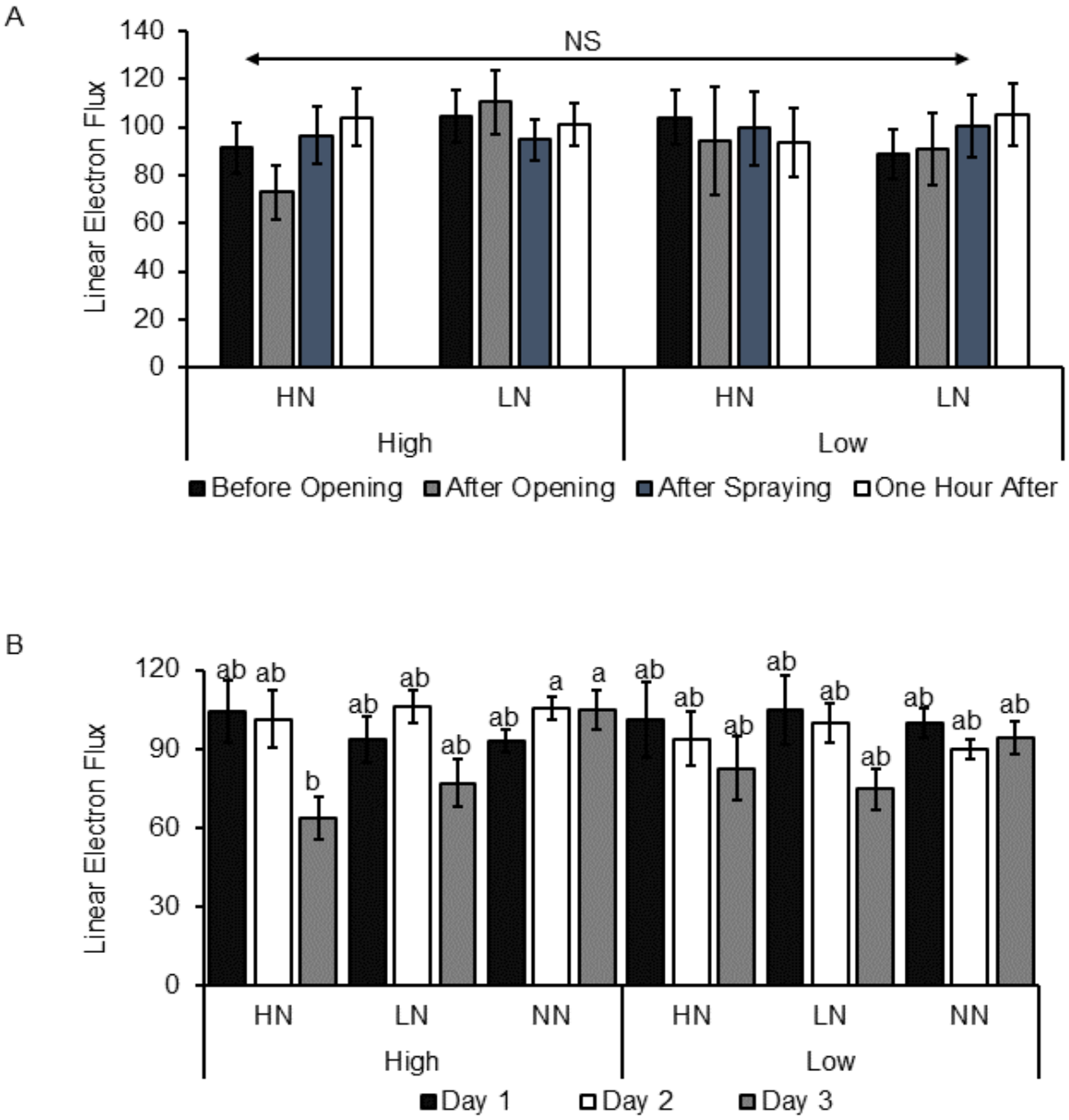


Figure 3.5. The linear electron flux was not affected by growth condition but slightly affected by the concentration of nutrients resupplied. The instantaneous average linear electron flux (A) was not influenced by growth condition or nutrient pulse. The average linear electron flux within 3 days of nutrient spray (B) was affected. Plants were either grown on adequate nitrogen (High) or limited nitrogen (Low). They were either sprayed with high nitrogen (HN) or with low (LN) or unsprayed (NN). Bars are averages (n= 5 leaves x 4 plants each from the factorial resupply (third experiment)). Lower case letters denotes statistical difference from 5% level of significance. Error bars are the standard error of the mean.

3.4.1.6. Photosystem II efficiency was not affected by growth condition, or the concentration of nitrogen pulse administered.

Fv'/Fm' is used to describe the maximum quantum yield of chlorophyll fluorescence as the ratio between the minimal amount of fluorescence from the leaf to the maximum fluorescence in light conditions and is an indication of plant stress (Maxwell and Johnson, 2000; Sheng et al., 2008). The Fv'/Fm' was similar in all treatments shortly after nutrient resupply, irrespective of nitrogen background and concentration of nitrogen sprayed (P= 0.06, Figure 3.6.A). The lack of increase or decrease in the Fv'/Fm' continued throughout the 3 days of measurements (P=0.7, Figure 3.6.B).

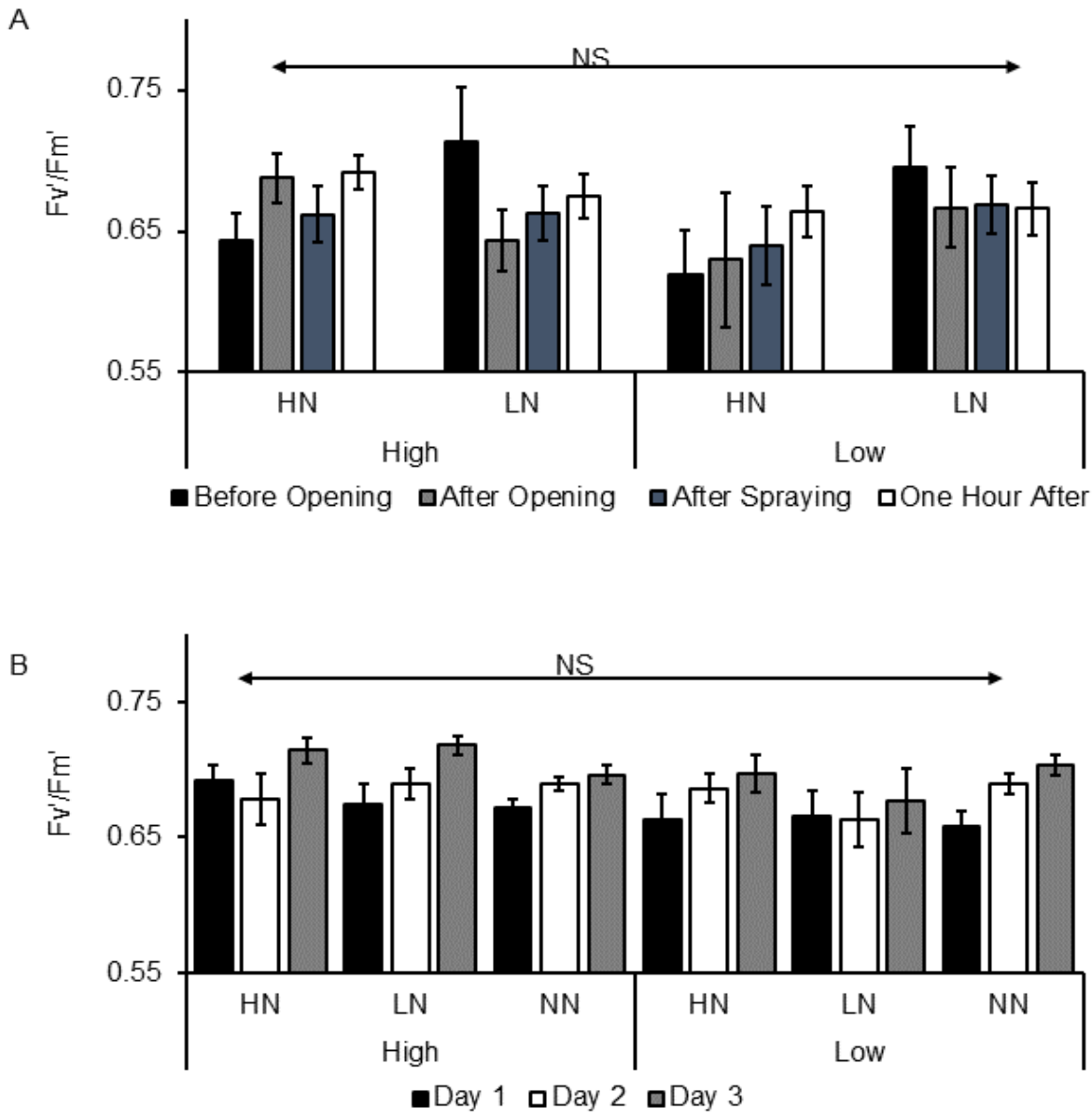


Figure 3.6. Nitrogen background and re-supply did not affect the efficiency of the photosystem II shortly after root soil nutrient interaction (A) or within the 3 days of root-soil nutrient sampling. Plants were either grown on adequate nitrogen (High) or limited nitrogen (Low). They were either sprayed with high nitrogen (HN) or with low (LN) or unsprayed (NN). All bars are averages (n=5 leaves x 4 plants each from the factorial resupply (third experiment)), "NS" means not significant at 5% level. Error bars are the standard error of the mean.

3.4.1.7. Low N plants showed an elevated flavonoid content in leaves and more so with immediate supply of high nitrogen solution.

It was observed that the average flavonoid contents of leaves grown on high nitrogen background were 70% lower than those grown on low nitrogen background ($P<0.001$, Figure 3.7.A), with no significant change after nitrogen resupply ($P=0.9$). Addition of high N to low N plants lead to increased flavonoids by 60% as compared to initial levels prior to nutrient resupply ($P=0.2$, Figure 3.7.A), which decreased by 45% one hour later ($P=0.05$, Figure 3.7.A). This decrease was short-lived as by day 2 and 3 the flavonoid levels of all low N plants were similar irrespective of whether sprayed with additional N solution or not (Figure 3.7.B).

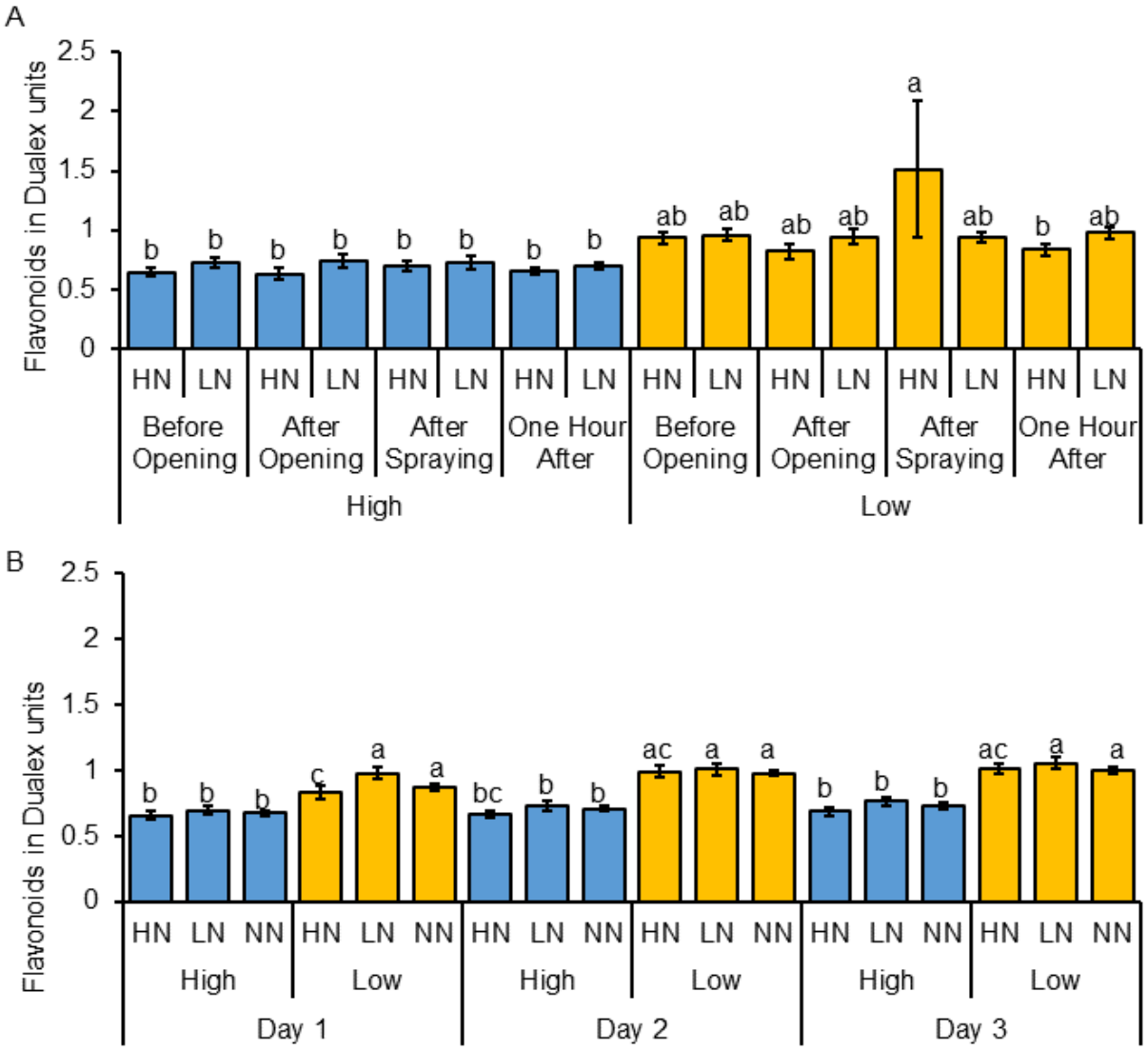


Figure 3.7. Nitrogen stress increased the flavonoid contents of maize leaves. Plants were sprayed with either high N (HN), low N (LN), or unsprayed (NN). Day 1, 2, & 3 represents measurements taken after root interaction with soil nitrogen on the first, second and third day respectively. All bars are averages (n= 5 leaves x 4 plants each from the third experiment). Different lower script letters denote significant difference at 1% level. Error bars are the standard errors of the mean.

3.4.2. Long term effects of Nitrogen:

3.4.2.1. The relative growth rate was influenced by time and growth conditions.

The relative growth rate, also known as the efficiency index, provides information on the rate of increase in growth per unit of biomass already present over time and here, was obtained from plant height. This parameter was influenced by time ($P<0.001$), with all plants having increased relative growth rates at 21 days post germination compared to other time points irrespective of nitrogen background and nutrient pulse applied (Figure 3.8. A, Table 3.1). When the relative growth rate of all the plants were compared with that of the low sprayed plants at 21 days post germination, it was seen that the relative growth rate reduced in all plants at 28 days post germination, but only the change in the relative growth rate of the sprayed low and high plants were statistically significant ($P<0.001$, Table 3.1). The relative growth rate of the unsprayed low plants was significantly reduced only from 35 days post germination upward as compared to the low sprayed plants where it was 21 days post germination (Figure 3.8. A, Table 3.1).

Accordingly, the relative growth rate at two physiological stages of growth (vegetative and reproductive) were also evaluated. During the vegetative stage (up to 42 days post germination) there was a higher relative growth rate ($P=0.04$) as compared to the reproductive stage (49-56 days post germination) as in (Figure 3.9.A). Spray of high N solution on low N pants led to an increased relative growth rate at the vegetative stage as compared to the growth rate of the low unsprayed plant at same age ($P=0.03$, Figure 3.9.A).

3.4.2.2. The net assimilation rate was influenced by time and nutrient pulse.

The net assimilation rate represents the measure of the quantity of photosynthetic products going into plant material or the estimate of net photosynthetic carbon assimilated by photosynthesis minus the carbon lost by respiration. It was observed that nutrient pulse at 21 days post germination led to increased net assimilation rate in the low and high plants by 19% and 38% respectively, within 2 weeks of treatment ($P=0.04$, Figure 3.8.B). The unsprayed plants had a decreased net assimilation rate by 78% and 25% respectively. When the net assimilation rates of all plants were compared with that of the low sprayed plants at 21 days post germination, it was discovered the nutrient pulse led to increased net assimilation rates of the low and high plants for 2 and 3 weeks respectively ($P<0.001$, Table 3.1, Figure 3.8.B). The changes in net assimilation rate of the unsprayed plants from 35-56 days post germination were not statistically significant when compared to that of the sprayed low plants at 21 days post germination.

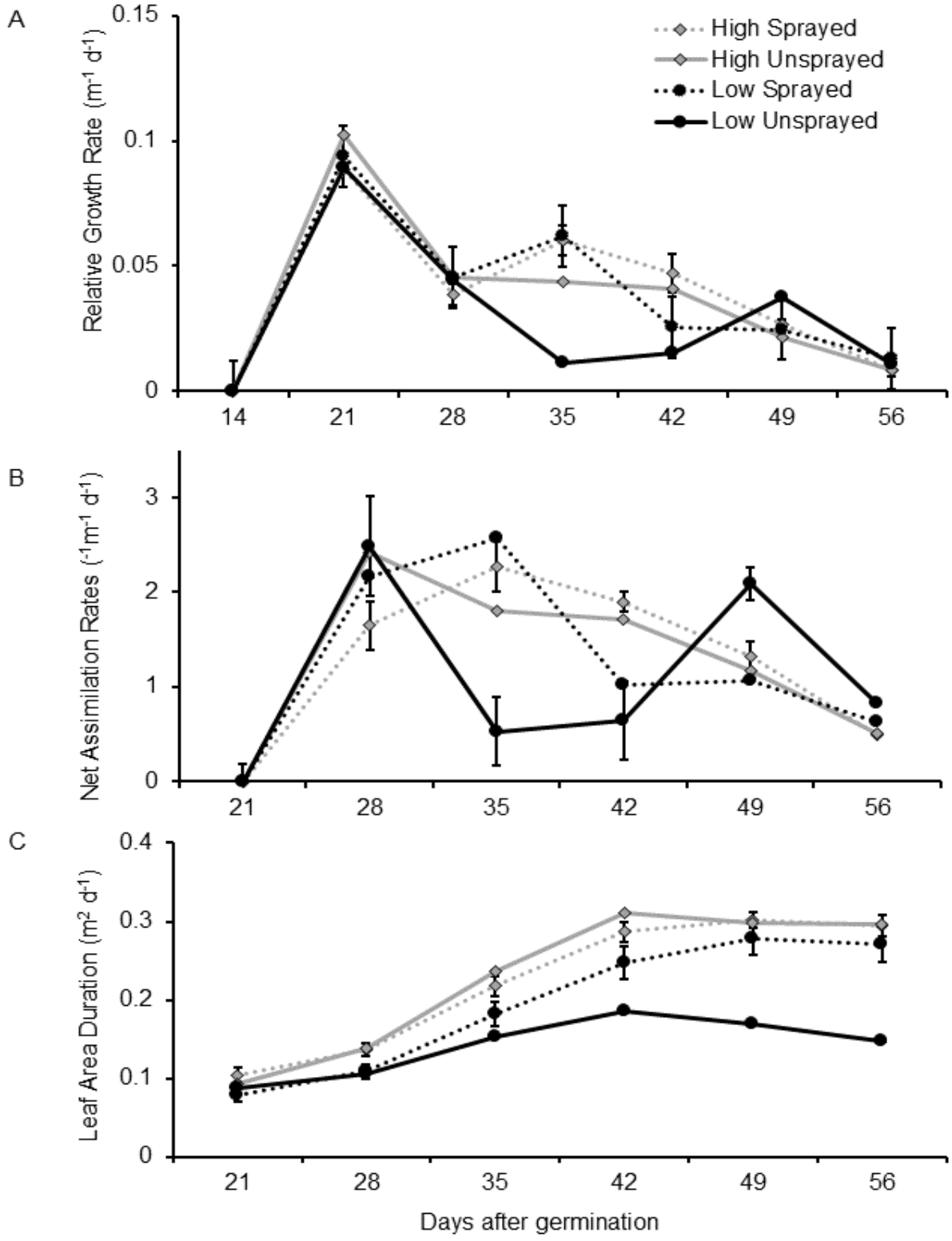


Figure 3.8. The influence of nitrogen background and nitrogen pulse on major shoot growth parameters over time. Maize plants were grown in adequate (High) or limited (Low) nitrogen background. Plants were either treated with high nitrogen(Sprayed) during microdialysis or not treated (Unsprayed). Data points are averages (sprayed:n=1x4plants each, unsprayed:n=1x1plants each from the first experiment (high N resupply)). Error bars are standard error of the means Refer to **Table 3.1 for statistical differences.**

Table 3.1. The significant differences in relative growth rates, net assimilation rates and leaf area duration over time, as influenced by nitrogen background and nitrogen resupply at 21 days post germination (represented in Figure 3.8).

		Low		***High***	
	Time (Days)	**Sprayed**	**Unsprayed**	**Sprayed**	**Unsprayed**
Relative Growth Rates ($m^{-1}d^{-1}$)	14	***	***	***	**
	21	__	NS	NS	NS
	28	***	NS	***	NS
	35	NS	***	*	NS
	42	**	***	***	*
	49	**	*	***	***
	56	***	***	**	***
Net Assimilation Rates ($^{-1}m^{-1}d^{-1}$)	21	__	NS	NS	NS
	28	***	***	*	*
	35	***	NS	***	NS
	42	NS	NS	***	NS
	49	NS	NS	NS	NS
	56	NS	NS	NS	NS
Leaf Area Duration (m^2d^{-1})	21	__	NS	NS	NS
	28	NS	NS	NS	NS
	35	**	NS	***	**
	42	***	NS	***	***
	49	***	NS	***	***
	56	***	NS	***	***

All parameters were compared with values of the sprayed low N plants at 21 days post germination (denoted:_) as reference point. Thus, NS= not significant at 5% level. * = significant at 5% level, ** = significant at 1% level, *** = highly significant ($P < 0.001$).

Furthermore, the net assimilation rates of the plants were compared at the vegetative (up to 42 days after germination) and the reproductive stages (49-56 days post germination), and net assimilation rate was found to be 2-fold higher in the vegetative stages than the reproductive stages of all high plants and the sprayed low plants ($P<0.001$, Figure 3.9.B). Whereas there was no significant difference between the net assimilation rate of the vegetative and reproductive stage of low unsprayed plant ($P=0.9$, Figure 3.9.B).

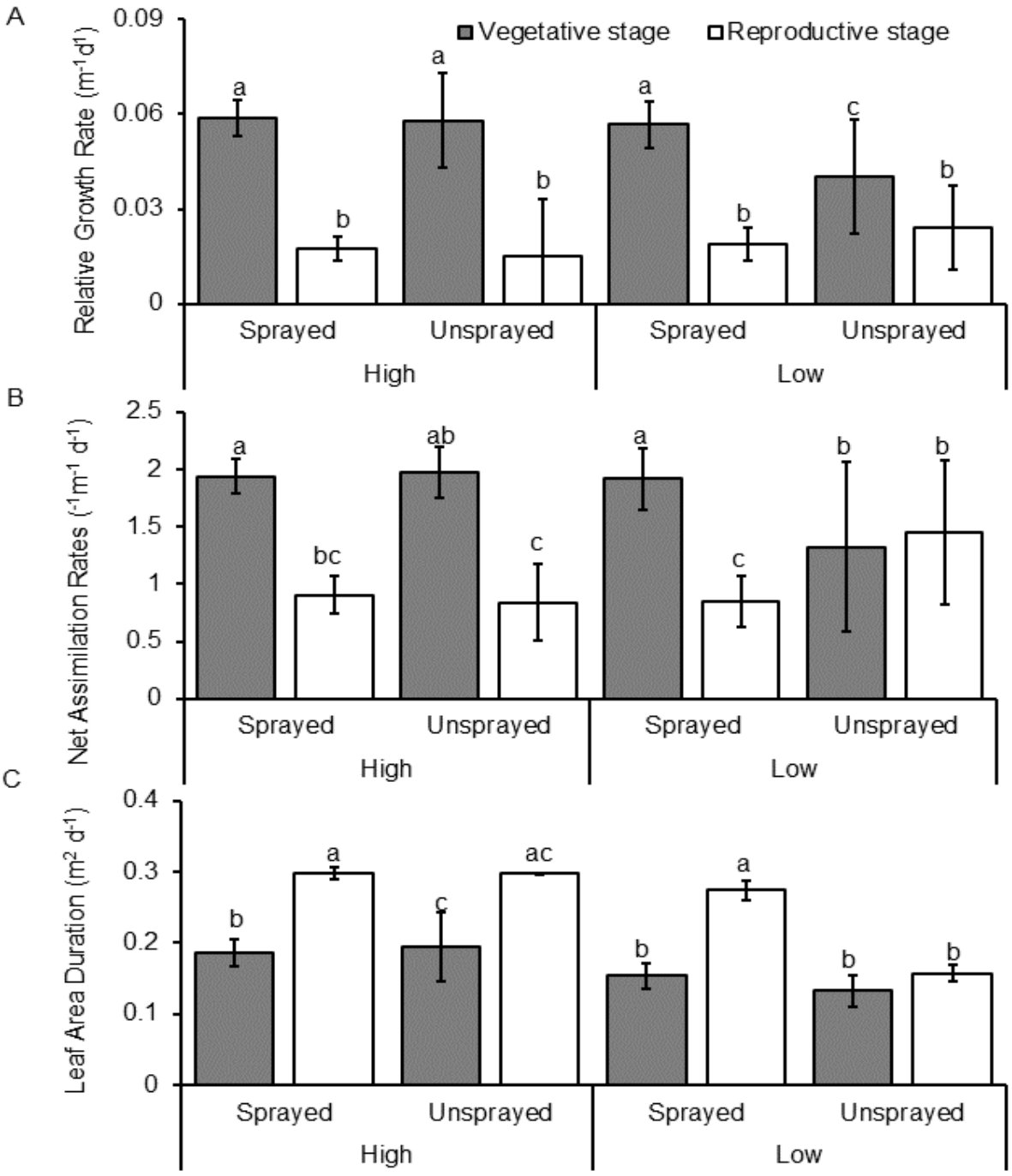


Figure 3.9. The effects of nitrogen background and nitrogen pulse on major shoot growth parameters during the vegetative and onset of reproductive stage. Plants were either grown on adequate (High) or limited (Low) nitrogen background. They were either treated with high nitrogen (Sprayed) during the first experiment (high N resupply) or not treated (Unsprayed). Bars are averages (Sprayed:n=1x4 plants each; Unsprayed:n=1x1 plant each). Gray bars represents vegetative stage (21-42) days post germination, while the white bars represents the onset of reproductive stage (49-56) days after germination. Different lowercase letters denote statistically significant difference at 5% level. Error bars are the standard error of the mean.

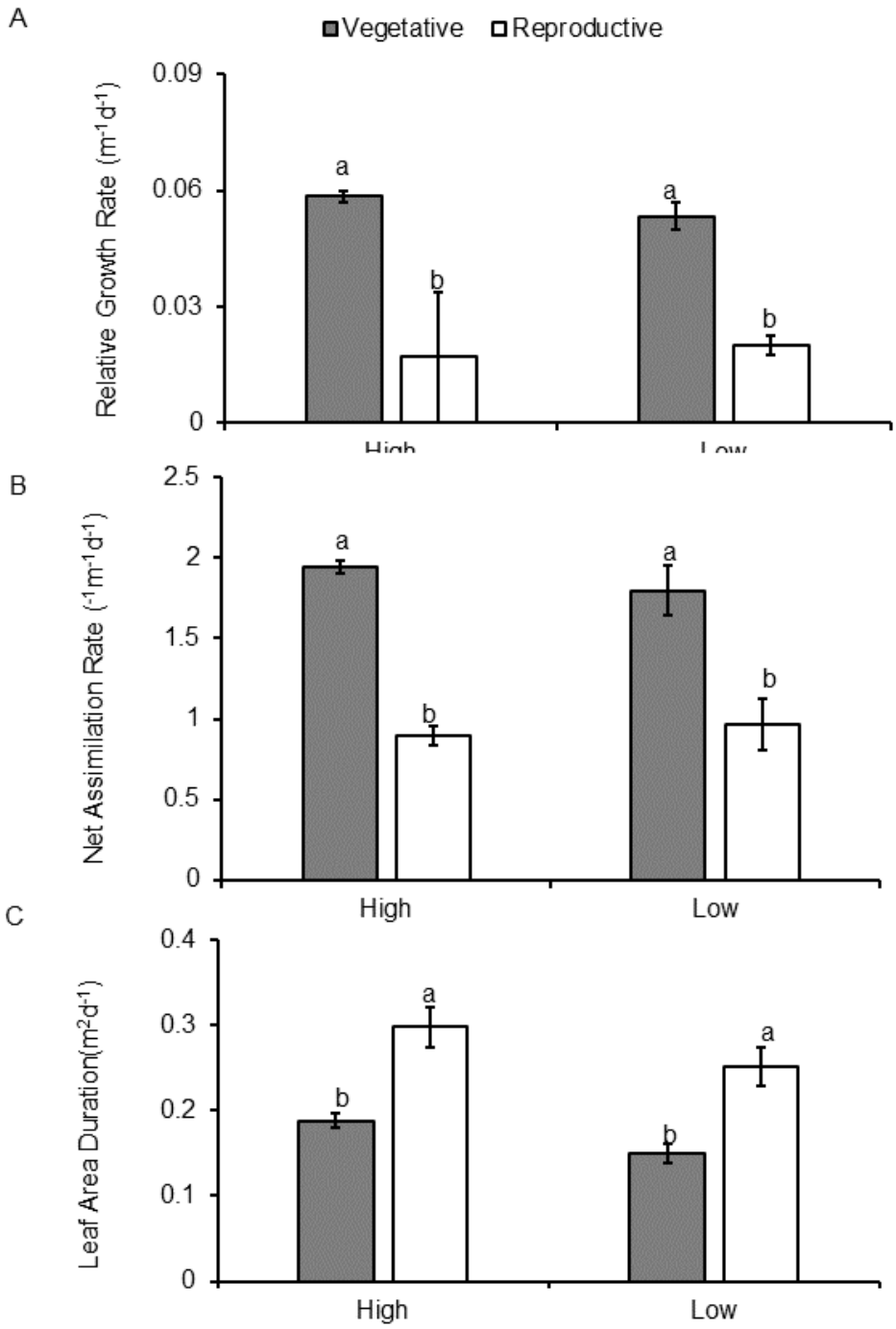


Figure 3.10. The effects of nitrogen background and nitrogen pulse on major shoot growth parameters during the vegetative and onset of reproductive stage. Plants were either grown on adequate (High) or limited (Low) nitrogen background. Bars are averages (n=1x5 plants each). Gray bars represents the vegetative stage (21-42) days post germination, while the white bars represents the onset of reproductive stage (49-56) days after germination. Different lowercase letters denote statistically significant difference from 5% level. Error bars are the standard error of the mean.

3.4.2.3. The leaf area duration was strongly influenced by nitrogen background and nitrogen pulse.

The leaf area duration represents the integrated leaf area over time. The leaf area duration was first used in crop science research when a strong correlation was observed between leaf area duration dry weight at each successive measurement of the effects of experimental variables (Power et al., 1967). Thus, it became a proxy way to determine changes in dry mater content of plants non-destructively. This is an important measure especially in glasshouse experiment where replication might be limited as compared to field plants (Power et al., 1967). In this case, it was observed that addition of high N to low N plants increased leaf area duration more than that of the sprayed high plants by 7, 21, 38, 63, 61% (P=0.007) and the unsprayed low plant by 18, 56, 103, 161 and 176% (P=0.002) at 28,35 ,42,49, and 56 days post germination respectively (Figure 3.8.C). There was a significant interaction between nitrogen background and nitrogen pulse on leaf expansion (P=0.003). When these leaf area durations were compared to those of the sprayed low N plants at 21 days post germination, all the high N plants (sprayed and unsprayed) and the sprayed low plants were significantly larger from 35-56 days post germination whereas the unsprayed low plants remained like the sprayed low plants at 21 days post germination (Table 3.1).

3.4.2.4. Ontogenetic phase changes influenced leaf area duration.

During the vegetative stage (up to 42 days after germination), there were six visible leaves (5 fully expanded) for both high and low N plants at 21 days post germination before the high N-pulses were administered. A week after, the sprayed low and high plants developed eight visible leaves with six fully expanded each, but the unsprayed high plant had twelve visible leaves with ten fully expanded while the sprayed high plants had nine leaves with seven fully expanded leaves and emerging tiller buds. By 35 days post germination, the sprayed high and low plants had an average of 12 and 11 visible leaves while the unsprayed high and low plants had 13 and 9 visible leaves, respectively. By 42 days post germination, the sprayed high plants had thirteen visible and ten fully expanded leaves with tiller buds comprising of the average of six leaves. The reproductive stage started when the bottom-most branch of tassels emerged (between 42- 49 days post germination, although all high plants reached reproductive stage about 7 and 14 days earlier than sprayed low and unsprayed low plants respectively). The 11^{th}-14^{th} leaves of all plants emerged during the vegetative stage and continued to grow in the reproductive stage.

The rate of leaf expansion was higher at the onset of reproduction than that of the vegetative stage in sprayed high and low plants (High: P=0.003, Low: P=0.002, Figure 3.9.C and Figure 3.10.C). The leaf area durations were similar during the vegetative and the onset of reproductive stages of the unsprayed high and low plants (High: P=0.3, Low: P=0.9, Figure 3.9.C and Figure 3.10.C).

Additionally, the leaf area duration during the vegetative and the onset of reproduction were evaluated at the individual leaf level, and it was found that during the vegetative phase, addition of high N to low N plants enabled the expansion of their 4^{th}, 5^{th}, 6^{th}, $7^{th}$and 8^{th} leaves to a similar rate as the sprayed and unsprayed high N plants (P=0.02, Figure 3.11). The reproductive phase of all plants had a faster expansion of the 7^{th}-n^{th} leaves than their 4^{th}-6^{th} leaves in vegetative phase (P<0.001, Figure 3.11).

Within the reproductive phase, low N plants had 1 to 3-fold decrease in the expansion of their 9^{th}, 10^{th}, 11^{th}, $12^{th}$and 13^{th} leaves as compared to high N plants (P<0.001) with the low N unsprayed plants having the raising decrease in leaf growth over time. The low N plants sprayed with high N recorded a higher individual leaf area duration than the unsprayed low N plants during their reproductive stage (P<0.001), while there was no significant statistical difference between the leaf area duration of the sprayed and the unsprayed high plants (P=0.9, Figure 3.11).

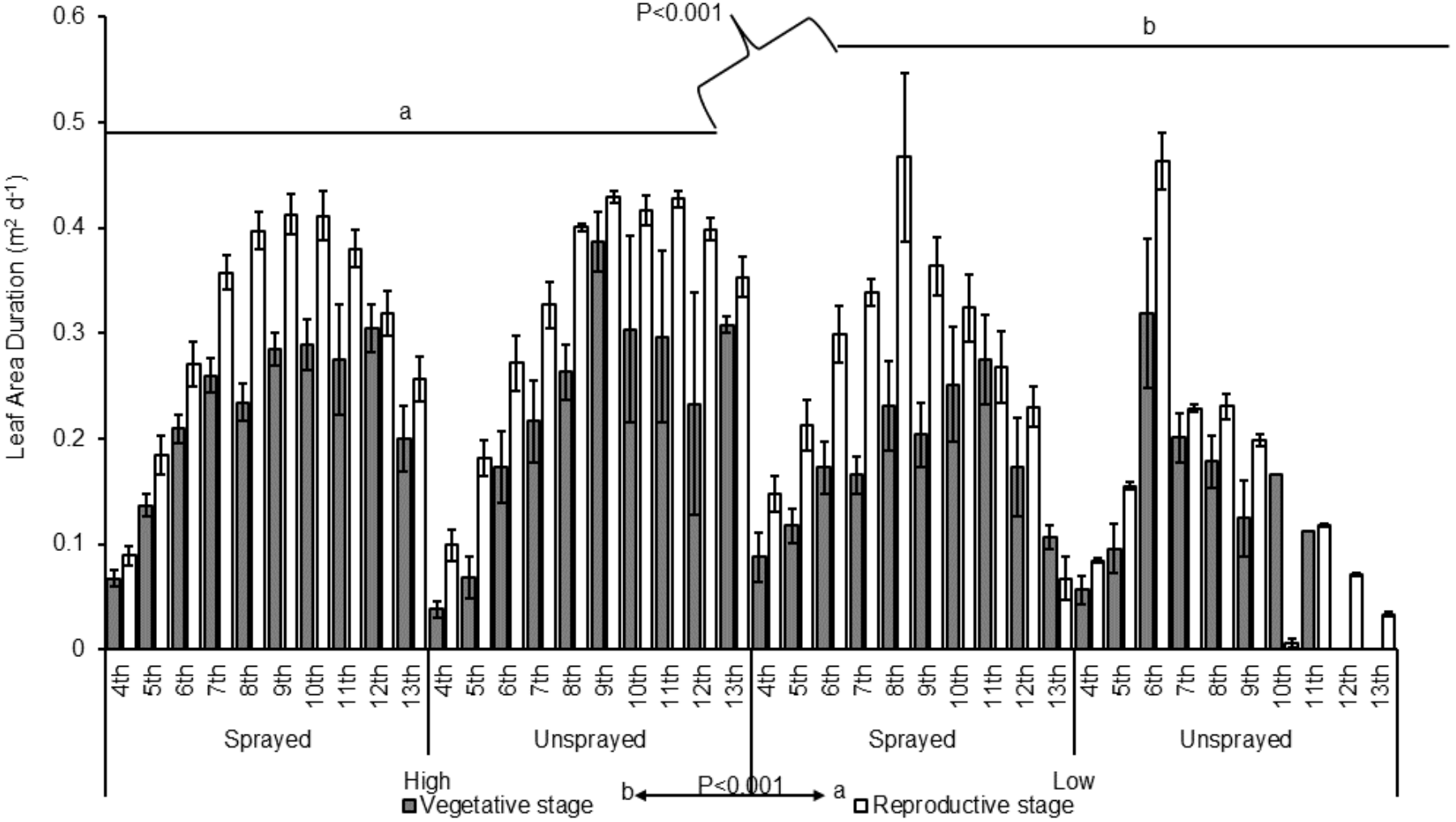


Figure 3.11.The rate of increase in leaf area of all measurable leaves at vegetative and reproductive stages, following nutrient pulse application. Plants were either grown on adequate (High) or limited (Low) nitrogen background. They were either given high nitrogen pulse (Sprayed) during microdialysis or not given (Unsprayed). Gray bars represents the leaf area duration during vegetative stage (21-42) days post germination, while the white bars represents the leaf area duration at the onset of reproductive stage (49-56) days post germination. Bars are averages (Sprayed:n=1x4 plants each; Unsprayed:n=1x1plant each). Different lowercase letters denotes statistical difference from 5% level, (Table 3.2 for statistical differences). Error bars are standard error of the mean.

3.4.2.5. High nitrogen availability triggered expansion of middle and then upper canopy leaves.

All the measurable leaves were categorized into three positions on the maize canopy (Figure 3.12): lower (4th, 5^{th,} and 6th leaves), middle (7th, 8th, and 9th leaves) and upper (10th, 11th,12^{th,} and 13th leaves). The rate of leaf expansion was faster in the middle leaves irrespective of nitrogen background ($P<0.001$, Figure 3.12) followed by the upper leaves in high plants ($P=0.002$, Figure 3.12.A) compared to the lower leaves.

Addition of high N to low N plants led to the expansion of their middle leaves at very similar rates to both sprayed and unsprayed high plants during the onset of reproduction ($P=1$, Figure 3.12.B). When these leaf expansion rates were compared between leaf positions and the two physiological stages of growth, it was seen that the middle leaves during reproductive stage of low plants treated with high N were expanding to a greater size than their lower leaves at the vegetative stage ($P=0.02$, Figure 3.12.B). The leaf expansion rates of the unsprayed low plants appeared similar, irrespective of leaf position and physiological growth stage (Figure 3.12.B). Similarly, only the lower leaves of the sprayed and unsprayed high plants at their vegetative stage had lower expansion than their middle leaves at the onset of reproduction ($P=0.03$, Figure 3.12.B).

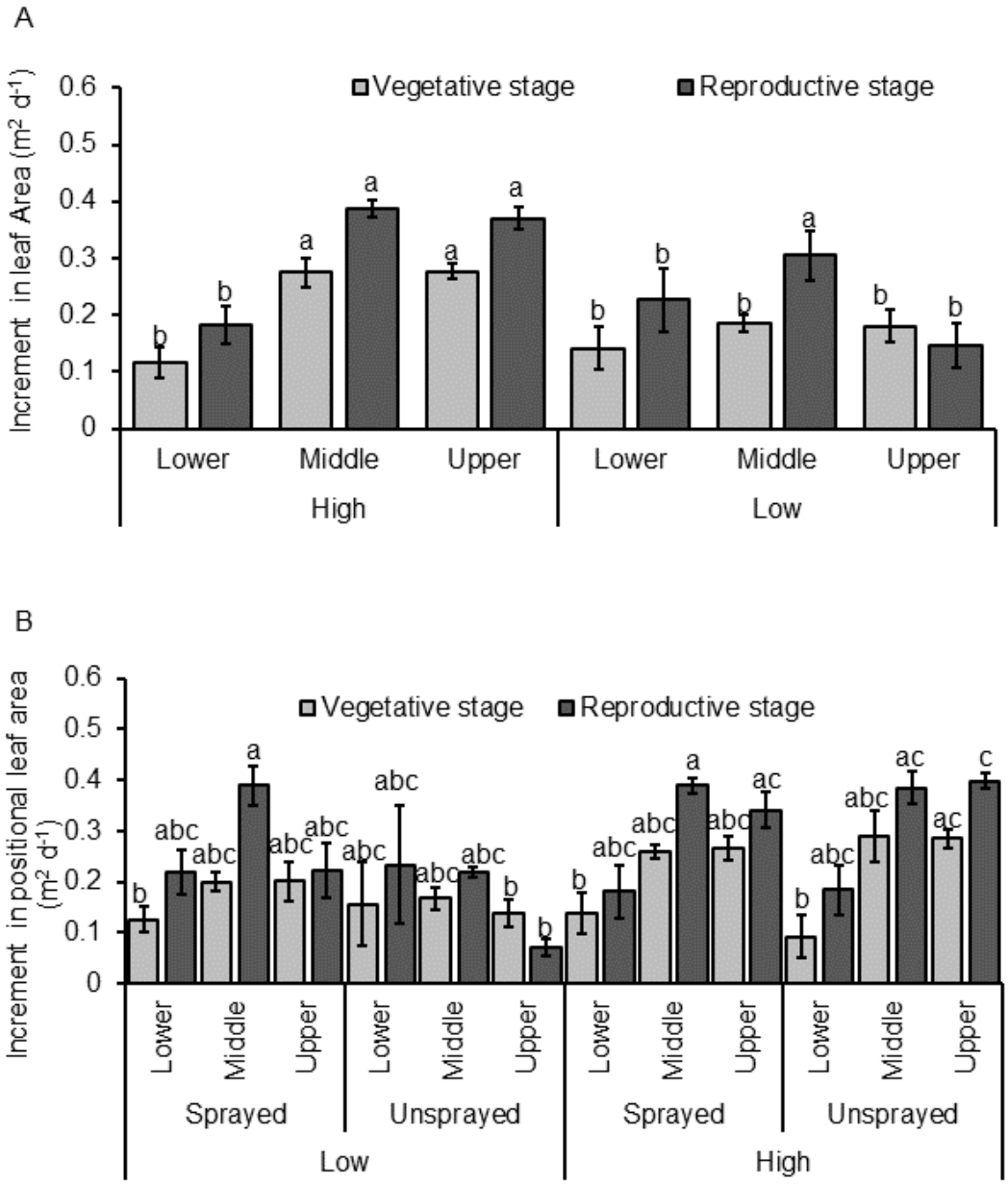


Figure 3.12. Maize plants expand their middle and upper canopy leaves during high nitrogen availability, especially at the onset of reproduction. Plants were either grown on adequate (High) or limited (Low) nitrogen background. They were either treated with high nitrogen(Sprayed) or not treated (Unsprayed). Measurable leaves were grouped into 3 as follows: 4th-6th leaves (Lower), 7th-9th leaves (Middle) and 10th-13th leaves (Upper). Bars are averages(Sprayed:n=3 leaves x4 plants each; Unsprayed:n=3 leaves x1 plant each). Light grey bars represents vegetative stage (21-42) days post germination, While the dark grey bars represents the onset of reproductive stage (49-56) days after germination. Different lowercase letters denote statistically significant difference from 5% downward.

Table 3.2. Statistical differences of 4th – 13th leaves of all plants as compared to the 8th leaves of sprayed low N plants during the high nitrogen resupply experiment, in terms of leaf area duration and chlorophyl levels of leaves at each position on the stem nodes (Leaf area duration: Figure 3.11; Chlorophyll: Figure 3.13).

			Low		*High*	
		Leaf number	Sprayed	Unsprayed	Sprayed	Unsprayed
Leaf Area Duration (m^2 d^{-1})	Vegetative stage	4th	NS	NS	NS	NS
		5th	NS	NS	NS	NS
		6th	NS	NS	NS	NS
		7th	NS	NS	NS	NS
		8th	___	___	___	___
		9th	NS	NS	NS	NS
		10th	NS	NS	NS	NS
		11th	NS	NS	NS	NS
		12th	NS	NA	NS	NS
		13th	NS	NA	NS	NS
	Reproductive stage	4th	***	***	***	***
		5th	***	**	***	*
		6th	NS	NA	**	NS
		7th	NS	NS	NS	NS
		8th	___	___	___	___
		9th	NS	NS	NS	NS
		10th	NS	***	NS	NS
		11th	***	NS	NS	NS
		12th	***	***	NS	NS
		13th	***	***	**	NS
Chlorophyll level	Vegetative stage	4th	NS	NS	***	NS
		5th	NS	NS	***	NS
		6th	NS	NS	***	NS
		7th	NS	NS	NS	NS
		8th	___	___	___	___
		9th	NS	NS	NS	NS
		10th	NS	NS	NS	NS
		11th	NS	NS	NS	NS
		12th	NS	NS	NS	NS
		13th	NA	NA	NS	NS
	Reproductive stage	4th	***	NS	NS	NS
		5th	NS	NS	***	NS
		6th	NS	NS	***	NS
		7th	NS	NS	***	NS
		8th	___	___	___	___
		9th	NS	NS	***	NS
		10th	NS	NS	***	NS
		11th	NS	NS	***	NS
		12th	NS	NS	***	NS
		13th	NS	NS	***	NS

NS = not significantly different at 5% level, * = significant at 5% level, ** = significant at 1% level, *** = highly significant ($P < 0.001$).

3.4.2.6. Relative chlorophyll levels were influenced by growth condition.

High nitrogen background triggered the build-up of more chlorophyll as compared to the chlorophyll contents of low nitrogen plants ($P<0.001$, Figure 3.13. and Figure 3.14). However, additional supply of high N was significantly noticed only in the sprayed low N plants ($P<0.001$) and not the sprayed high N plants ($P=0.2$). The overall chlorophyll contents of individual leaves were higher in the sprayed low plants than the unsprayed ($P<0.001$, Figure 3.13). The chlorophyll contents of both the sprayed and unsprayed high plants increased significantly from the vegetative stage to the onset of reproduction ($P<0.001$, Figure 3.13, Figure 3.14). When these chlorophyll contents were compared among treatments during the two physiological growth stages, it was seen that the chlorophyll contents of the 4^{th}, 5^{th}, 6^{th}, 7^{th}, and 8^{th} leaves of the sprayed low N plants were significantly higher during their vegetative stage which significantly decreased at the onset of reproduction ($P=0.002$, Figure 3.13, Figure 3.14). Only the 4^{th} and 9^{th} leaves of the unsprayed low plants appeared higher during the vegetative stage as compared to their reproductive stage. when the chlorophyll content of all leaves were compared with that of the 8^{th} leaves of sprayed low N plants at the vegetative and reproductive stages,

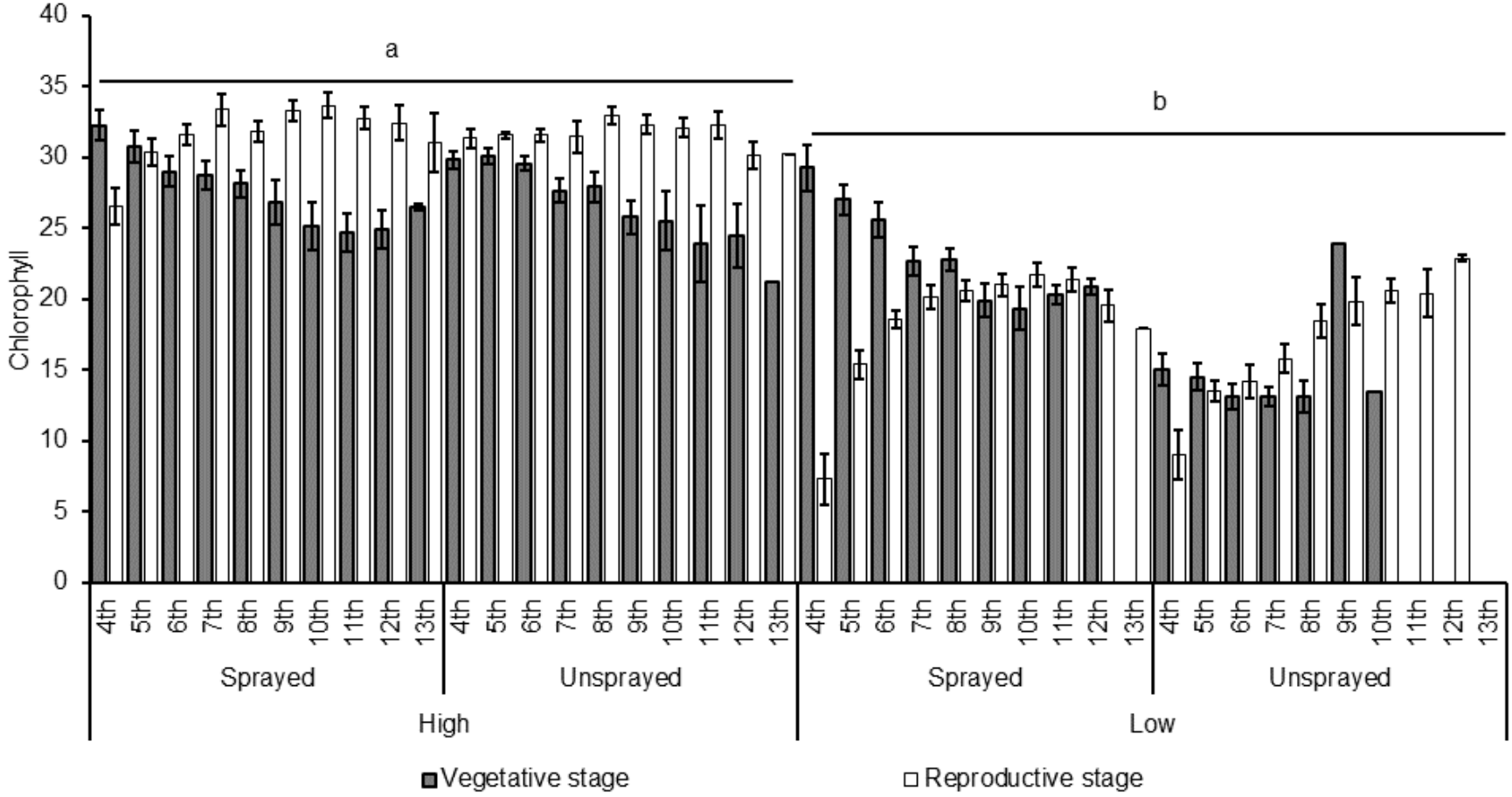


Figure 3.13. Chlorophyll values of all measurable leaves at the vegetative and reproductive stage, following nitrogen pulse application. Plants were either grown on adequate (High) or limited (Low) nitrogen background. They were either given high nitrogen pulse (Sprayed) during high N resupply experiment(first experiment) or not given (Unsprayed). Gray bars represents increase during vegetative stage (21-42) days post germination, while yellow bars represents increase at the onset of reproductive stage (49-56) days post germination. Bars are averages (Sprayed=1x4 plants each; Unsprayed=1x1plant each). Different lowercase letters denote significant main effects,(Table 3.2 for statistical differences). Error bars are the standard error of the means.

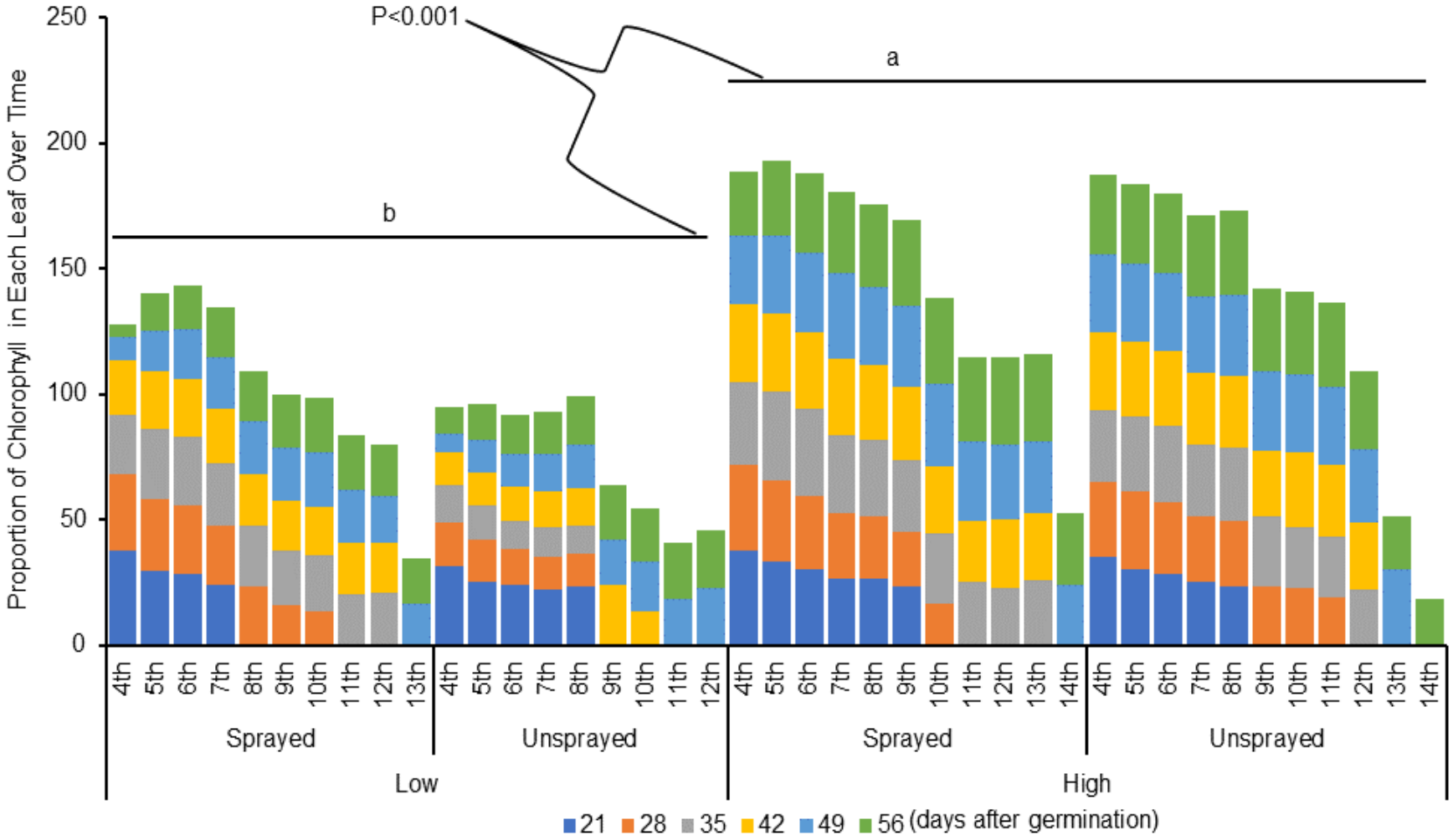


Figure 3.14. Chlorophyll contents of lower leaves in low nitrogen plants decreased over time while adequate nitrogen supply maintained leave chlorophyll content. Plants were either grown on adequate (High) or limited (Low) nitrogen background. They were either given a high nitrogen pulse (Sprayed) or not (Unsprayed) during the high N resupply experiment (first experiment). Bar components are averages (Sprayed: n=1x4 plants each, Unsprayed: n=1x1 plant each. Colours in the bars represents the chlorophyll levels in individual leaves over time in days after germination.

3.5. Discussion

It is unclear the minimum amount of nitrogen fertiliser that is required to increase maize productivity with reduced environmental hazard and economic cost. This creates a necessity to understand the shoot response of maize growing on low and high nitrogen background to additional nitrogen supply. This is important because the short and long-term interaction between nitrogen assimilation and photosynthesis is central for plant growth and development. This study monitored the short- and long-term physiological response of maize plants to nitrogen treatment using microdialysis (Chapter 2). This is to enable understanding of maize nitrogen budget in regulating physiological processes during low nitrogen condition and when additional nitrogen is supplied. Plants from the factorial nitrogen resupply were monitored for short term responses to nitrogen. Plants from the high nitrogen resupply were allowed to reach tasselling (reproductive) stage to understand the effect of one-time high nitrogen supply on growth performance of maize growing on low nitrogen background. This Chapter hypothesized that additional supply of nitrogen to low nitrogen maize plants will trigger dynamic changes in shoot photosynthetic parameters and growth rates, and the changes will be greater when the concentration of the applied nitrogen is increased as a short- term response. It was also hypothesized that the growth rates of shoot functional organs will be greater during the vegetative stage as compared to the reproductive stage in sprayed low N plants (long term response). Thus, the short-term shoot response to high and low nitrogen resupply will be discussed followed by the long-term shoot response to high nitrogen resupply to plants growing on low and high nitrogen background.

3.5.1. Soil nitrogen levels and the chlorophyll content of maize leaves:

Nitrogen availability affects the chlorophyll contents of leaves in maize and measurements of chlorophyll is widely accepted as an estimate for the nitrogen content of leaves (Fageria, 2014; Nadeem et al., 2018; Schlüter et al., 2012). The low N plants whose rhizosphere were sprayed with high N had an increase in chlorophyll content shortly after treatment, as captured by Dualex (Chlorophyll and Nitrogen balance index). This was maintained for the 3 days of the experiment, implying that one pulse of high N was sufficient to cause an upward shift in the concentration of leaf nitrogen. Although the upward shift did not differ statistically with the leaf chlorophyll level of low N plants that were sprayed with low N or the unsprayed, the upward shift may increase photosynthesis. This is because the response of leaf photosynthesis to irradiance depends primarily on the leaf nitrogen concentration (Fageria, 2014; Garnett et al., 2013; Nadeem et al., 2018; Schlüter et al., 2012), and an increase in leaf nitrogen contents can result in a rapid production of photosynthetic enzymes including phosphoenolpyruvate carboxylase (PEPC) and pyruvate orthophosphate dikinase (PPDK)

which eventually leads to increased biomass (Engels. et al., 2012; Gastal. et al., 2015; Urban et al., 2021). Previous studies reported a strong correlation between nitrogen supply and chlorophyll level of maize leaves (Fageria, 2014; Garnett et al., 2013; Nadeem et al., 2018; Schlüter et al., 2012).

3.5.2. Soil nitrogen level and maize leaf temperature differential:

The water status of leaf and transpiration rates affects the leaf temperature, while the leaf temperature also correlates with the air temperature (Zhang et al., 2019). Thus, the leaf temperature differential, which is the difference between the leaf temperature and the ambient temperature, can be used to predict hydraulic conductivity in response to nutrient availability. Transpiration rates have a direct influence on nitrate uptake by exerting effect on radial transport through the apoplast or indirectly by influencing the nutrient supply to the plasma membrane of root cells (Houshmandfar et al., 2018). In this study, the low and high N plants whose rhizosphere were sprayed with high N solution had a lower rise in leaf temperature differential (more negative values) as compared to those sprayed with low N solution after 48 and 72 hours. Could this suggest lesser demand for nitrogen uptake as compared to the plants sprayed with low N? This needs to be evaluated by manipulating the soil moisture content and nitrogen background in a factorial experiment. Possible reason may mean that, in attempt to meet the plants nitrogen demand, the sprayed low N plants captured more N sources in the first day of N pulse since the concentration of applied nitrogen was small. Thus, leading to lower transpiration rates (warmer canopy) on the second and third day, since studies showed that up to 20% reduction in transpiration rates is associated with a major decline in nitrate flux (White, 2012b), and that the nitrate transporter ZmNRT 2.1 which is known for its primary nitrate response role during limited N supply is involved in roots hydraulic conductivity (Hole et al., 1990; White, 2012).

Plants supplied with limited N have been shown to respond to a shortage of available water in substrate (a decrease in the substrate water potential) by a more rapid closure of the stomata than those supplied with adequate N (Engels et al., 2012). This response of stomata closure is mediated by reduced cytokinin level and elevated level of ABA in the xylem sap during N limitation (Engels. et al., 2012). Previous studies with field grown maize found a relationship between stomatal conductance and ABA levels of the xylem sap, but not the immediate leaf water status nor ABA level in the bulk leaf (White, 2021b). The exact interaction between nitrate and ABA in stomatal function is still unclear, but NRT 1.2 was said to be an ABA transporter mediating cellular uptake (Andrews et al., 2013). Accordingly, low N supply can affect the root hydraulic conductance of aquaporins which reduce transpiration (Engels. et al., 2012) and by extension photosynthesis. Aquaporins such as the plasma membrane intrinsic proteins (PIPs) were shown to lead to an increase of root hydraulic conductivity

in response to nitrate resupply (Muratore et al., 2021). The implication of this finding is that intermittent supply of high N to low N plants could enhance nutrient uptake and reduce excessive use of nitrogen fertiliser for improved photosynthesis and plants growth.

3.5.3. Soil nitrogen level and photoprotection (ϕNPQt):

Light energy absorbed by leaves could either be used for photosynthesis or dissipated as heat or can be re-emitted as chlorophyll fluorescence (Mu et al., 2017; Murchie and Lawson, 2013). The non-photochemical quenching emanates from non-radiative dissipative process that regulates the quantum yield of photosystem II and operates to prevent damage to the photosynthetic apparatus by excess light (Guidi et al., 2019; Khamis et al., 1990; Tóth et al., 2002). In this study, the lack of significant difference in ϕNPQt between high and low N plants during the process of microdialysis on the first day, could mean that more time was required for the photosynthetic process of the plants to adjust to changes in the concentration of external nutrient provided even in low N plants. This may be evident as only 24 hours after nitrogen pulse, the low N plants dissipated higher amount of excess light energy, presumably to avoid damage to their photosynthetic apparatus. This finding is line with previous maize studies where higher thermal diffusion and chlorophyll fluorescence were found in plants on low N treatments relative to those on adequate nitrogen treatments (Mu et al., 2017; Tóth et al., 2002).

The ϕNPQt is comprised of energy-dependent, zeaxanthin-dependent, and photoinhibition quenching mechanisms. The energy-dependent and zeaxanthin dependent mechanisms are critical for photoprotection due to their ability to swiftly respond to a change in condition (Guidi et al., 2019). The zeaxanthin-dependent and energy-dependent photoprotection mechanism could be implicated for the elevated ϕNPQt in the low N plants in this study. In this case, acidification of the thylakoid lumen is thought to activate the protonation of the photosystem II subunits S protein, which activate violaxanthin epo-oxidase, which in turn converts violaxanthin into zeaxanthin (Guidi et al., 2019; Kromdijk et al., 2016; Tóth et al., 2002). Both the ϕPSII Subunits S proteins and zeaxanthin acts as triggering regulators that enhance the sensitivity of light-harvesting complex II (LHCII) to the lumen protons and activating the energy dependent ϕNPQ (Guidi et al., 2019; Kromdijk et al., 2016; Tóth et al., 2002). However, the ϕNPQt represent part of the absorbed light energy that was not used for photosynthesis and carbohydrate production. Analysis of the partitioning of the total absorbed light energy by photosystem II will give better understanding on the fate of the absorbed light energy in view of nitrogen utilisation efficiency by maize plants during low nitrogen condition.

3.5.4. Nitrogen availability and the relative allocation of energy:

The elevated ϕPSII (photosynthetic efficiency of photosystem II) from day one to day three in low N plants whose rhizosphere received a high N pulse suggested increased activities of the photosystem II. This is likely due to increase in amino acid contents of the leaves from the synthesis of nitrogen provided on the first day. Additional supply of low nitrogen did not increase the photosynthetic efficiency of the photosystem II of low N plants. It is notable that an additional high N pulse enabled the photosynthetic process and carbohydrate production (ATP and NADPH) of low N plants to be at similar rates as the high N plants. Thus, depicting high nitrogen uptake capacity in low N plants and high photosynthetic nitrogen use efficiency (PNUE) per leaf area for each additional dose of nitrogen. In their review, Urban et al., (2021) reported that low nitrogen conditions in maize lead to increased rubisco content with a simultaneous decrease in the ratio of proteins allocated to phosphoenolpyruvates carboxylase (PEPC) and phosphor pyruvate dikinase (PPDK). This they said designates priority for Rubisco during N limitation by compensating for the lower CO_2 assimilation capacity resulting from shortage of enzymes (Urban et al., 2021). Whether this was the case or not with the low N plants in this study remained to be unravelled through further research.

The ϕNO (proportion of energy dissipated as free energy that can damage the photosystem) in low N plants whose rhizosphere received high N pulse increased on the second day of root- soil nitrogen interaction as compared to day one, while the ϕNPQ (proportion of energy quenched in the leaves for protection in event of excess light) increased in all low N plants in day one as compared to high N plants in day 3, suggesting a link between nitrogen availability and carotenoids of the xanthophyll cycle during the process of photosynthesis of low N plants, as was seen in the non-photochemical quenching section above. There is need for further research on the effect of nitrogen (supply and concentration) on the xanthophyll cycle for an in depth understanding that could be manipulated to improve CO_2 assimilation per unit nitrogen and unit leaf area. Also, since light intensity has profound influence on the nitrogen budget during photosynthesis, more study is required on the interaction between nitrogen and water availability under varying light intensity.

3.5.5. Nitrogen availability and the linear electron flux of maize leaves:

The absorbed light energy is converted to chemical energy by a flow of electrons through the pigment system embedded in the thylakoid membrane of the chloroplast (Engels. et al., 2012; Fageria, 2014). The thylakoid membrane nitrogen is shared between proteins associated with bioenergetics (Cyt b/f and Cf_1/Cf_0 involved in photosynthetic electron transport and photophosphorylation) and light harvesting proteins such as photosystem I, photosystem II, and light harvesting complex II which is associated with photosynthesis (Mu et al., 2016, 2017; Urban et al., 2021). In this study, the similarity

in the average linear electron flux (the number of molecules of electron per seconds moving from the reaction centre chlorophyll (P680) to the primary quinone acceptor of photosystem II (Q_A) for sugar production) across treatments during the process of microdialysis and days after, suggested that the low N plants had an increase in physiological nitrogen utilization efficiency by increase of photosynthetic rates per unit leaf nitrogen. This is because there is no difference in the rate of electron transport in relation to growth condition or the concentration of nitrogen pulse received in the rhizosphere. Because the electron transport capacity of plants is directly related to the expression of cytochrome b/f and ATP synthase network (Evans and Clarke, 2019), the low N plants could have maximised nitrogen use efficiency via allocating more of thylakoid nitrogen to bioenergetics rather than the light harvesting process, to enable the stabilization of electron transfer and phosphorylation for the maximisation of quantum yield, as reported in previous studies (Mu et al., 2016; Urban et al., 2021). This is because C4 plants under adequate nitrogen supply allocate 80% of their thylakoid nitrogen to light harvesting proteins while the remaining percentage goes to bioenergetics, leading to a strong positive relationship between photosynthetic capacity and nitrogen contents per leaf area (Mu et al., 2016).

The downward transport of 3 electrons is involved in the regeneration of NADPH (nicotiamide adenine dinucleotide phosphate) and establishment of an electrochemical gradient across the thylakoid membranes to produce one mole of ATP (adenosine triphosphate), of which the NADPH and ATP are used as energy sources during each step of CO_2 reduction and carbohydrate production (Engels. et al., 2012; Fageria, 2014). The nitrogen depletion studies (Chapter 2) also confirmed faster depletion of N sources in the rhizosphere of low N plants as compared to high N plants. This is in line with previous reports that maize plants increase their nitrogen use efficiency by increasing their N uptake efficiency and/or N utilisation efficiency during condition of nitrogen limitation (Hawkesford et al., 2012; Hirel et al., 2007; Moll et al., 1982; Mu et al., 2016). Time may be required for any adjustment to electron movement in response to external nitrogen depletion during adequate nitrogen supply. This is suggested by the reduction of the linear electron flow three days after the rhizosphere of high N plants were sprayed with high nitrogen. But further research involving nitrogen rates in two or more contrasting genotypes could elaborate this understanding. Specifically, physiological traits related to photosynthesis such as light saturation points, light compensation points, maximum photosynthesis, photoinhibition and how to effectively minimise its effects and occurrence, total protein contents including the ratio of chlorophyll a and b is worth quantifying under limited and adequate nitrogen condition in two or more contrasting genotypes.

3.5.6. Nitrogen availability and the efficiency of photosystem II:

The similarity in the level of Fv'/Fm' (the maximum quantum yield of the photosystem II) between the high and low N plants in this study helps to understand the varied photosynthetic efficiency of the low N plants. The Fv'/Fm' ratio has been widely used as a sensitive indicator of plant photosynthetic performance, where a decrease shows a reduction in photosystem II efficiencies (Guidi et al., 2019). Previous studies found nitrogen-limited plants to have lower CO_2 assimilation power but showed little or no changes in the maximal efficiency of photochemistry, rate of Q_A reduction, and the diversity of photosystem II reaction centre, suggesting that nitrogen limitation has only slight effect on photochemistry and photoinhibition even under high irradiance (Lu and Zhang, 2000). In low nitrogen conditions there modification of photosystem II photochemistry under steady state photosynthesis, possibly by downregulating photosynthetic electron transport to enable the production of ATP and NADPH at equilibrium with reduced demand in the calcium cycle of low N plants (Lu and Zhang, 2000). Low N plants have been shown to be more efficient in CO_2 assimilation per photosynthetic units (Khamis et al., 1990), but will this photosynthetic nitrogen use efficiency and efficacy be reflected in biomass accumulation and subsequent grain yield? Chapter 4 of this thesis will examine biomass accumulation and partitioning among functional organs.

3.5.7. Nitrogen availability and the flavonoid content of maize leaves:

The flavonoid (phenolic compounds and secondary metabolites possessing antioxidant properties) contents of high N plants were lower than that of low N plants in this study. However, the immediate increase in leaf flavonoid of low N plants after receiving high N pulse could suggest an increased production of reactive oxygen species in response to external high N concentration in the plasma membrane of roots and in the soil medium. Reactive oxygen species (ROS) are generated from uncontrollable leakage of electrons unto O_2 from the electron transport activities of the plasma membrane, chloroplast, mitochondria, or as a by-product of various metabolic pathways within cellular sites (Sharma et al., 2012). The disruption of cellular homeostasis enhances the formation of reactive oxygen species due to osmotic stress (Sharma et al., 2012). Thus, the high N pulse given to low N plants seems to immediately alter the concentration of nitrogen related secondary metabolites leading to increase in leaf flavonoid as a defence mechanism to neutralise the adverse effect of reactive oxygen species. The plants given high N pulse had an 82% rise in flavonoid immediately after spraying as compared to prior levels, while the low N plants given a low N pulse maintained the same level of flavonoid contents prior to spraying.

The connection between nitrogen metabolism and the biosynthesis of flavonoids is through the shikimate pathway (Deng et al., 2019), of which nitrogen availability was thought to control the biosynthesis of flavonoid probably by controlling the allocation of carbon between primary and

secondary metabolism (Deng et al., 2019). Nitrate uptake is also influenced by carbon produced during photosynthesis in relation to balancing the levels of root nitrate uptake against the availability of carbon metabolites (Urban et al., 2021; Vidal et al., 2020). The shikimate pathway catalyses the carbohydrates that resulted from glycolysis and the pentose phosphate pathway to synthesize aromatic amino acids such as phenylalanine, tyrosine, and tryptophan (Deng et al., 2019; Fritz et al., 2006), of which the biosynthesis of flavonoids is catalysed by a sequence of enzymes such as the phenylalanine ammonia lyase (PAL), cinnamate 4-hydroxylase, 4-coumaroyl, CoA-ligase, chalcone synthase (Schneider et al.) and flavanone 3-hydroxylase (FHT) (Deng et al., 2019). Flavonoid formation is regulated by N allocation through the photosynthetic allocation among different biochemical pathways (Deng et al., 2019). The strength, lifetime and size of reactive oxygen species signalling pool lies on the equilibrium between the oxidant production and removal by antioxidant such as flavonoids (Sharma et al., 2012). This fit with the increase in flavonoid contents of low N plants whose rhizosphere were sprayed with high N being short lived as the flavonoid levels decreased to similar levels of other sprayed and unsprayed low N plants on the second and third day, suggesting the return of steady state in the photosynthetic process and nitrogen metabolism. This further explains the resilience of the low N plants in adjusting to the changes in their environment.

3.5.8. Nitrogen availability and the relative growth rates of maize plants:

A framework based on both empirical relationships and theory showed that crop growth potential and critical nitrogen concentration controls the demand for nitrogen by plants (Gastal. et al., 2015). The crop growth rate is also influenced by the photosynthetic capacity of the crop at successive growth stages and regulates biological yield (Fageria, 2014). Thus, the relative growth rate provides information on the rate of increase in growth per unit increase in plant size over time. This is important when initial plant size contributes to increases in size and provides a convenient integration of more than one component process that contributes to the performance of the whole plant (Hunt, 2017). Normally plant dry weight is used to obtain the relative growth rates but the equation can be extrapolated to whole plants growth rates as leaves, stems and roots (Hunt, 2017; Price. and Munns., 1999).

In this study, the relative growth rate obtained from plant height was highest and similar in all plants at 21 days after germination. There was a peak in growth rate at 3 weeks after germination which could be an indicator of appropriate timing for fertiliser application, which should be after 2 weeks of germination to coincide with the peak in growth rate. This will ensure improved nitrogen uptake by the plants since the demand for nitrogen by crops is a function of crop growth rate and the critical amount of nitrogen required to accumulate maximum plant mass at each ontogenetic phase (Gastal

et al., 2015). Just after one week of high N pulse, all plants increased in height at similar but reduced rates as compared to the week before, suggesting that nitrogen uptake and utilization efficiency rates are not the same at each growth stage of individual plant and may be decreasing as the plants ages. This is an important consideration when growing maize on the fields, as this experiment is transferable to the field where nutrient availability varies with space and time. Equally important to note, is that both the sprayed and unsprayed high N plants started tasselling (on-set of reproductive phases) without a noticeable decrease in relative growth rate at 35 days post germination. While the sprayed low plants started tasselling at 42 days post germination with decreasing relative growth rate as the unsprayed low N plant began tasselling after 49 days post germination, suggesting that the high N pulse given to low N plants induced the reproductive stage earlier than the unsprayed low N plants.

Accordingly, the relative growth rates across treatments based on their ontogenetic phases shows that the relative growth rate of the low N plants whose rhizosphere were sprayed with high N were significantly greater at the vegetative stage as compared to their reproductive stage, as well as both stages in the unsprayed low N plant. This suggests that the low N plants were able to efficiently capture and use the one-time high N supply to optimise their photosynthetic process for biomass production. The net gain in plant height is a consequence of CO_2 assimilation by leaves minus respiratory loss by the entire plants. Thus, the increase in plant height and increase in leave area over time could give understanding of the net assimilation rates.

3.5.9. Nitrogen availability and the estimated net assimilation rates of maize leaves:

The net assimilation rate represents the net photosynthetic effectiveness in light harvesting, CO_2 assimilation and storage of the photo assimilates of the whole plant. Significant differences in net assimilation rates can emanate from differences in canopy architecture, interception of irradiance, photosynthetic activities of the leaves, respiration, transport of photo assimilates and storage ability of sink as well as the chemical nature of the stored product of photosynthesis (Díaz-López et al., 2020; Price. and Munns., 1999; Sun et al., 2019). In this study, the 19% and 38% increase in net assimilation rates by low and high N plants respectively, after 2 weeks of high N pulse suggests an efficient utilization of captured nitrogen in low N plants as they were able to take in about half of the estimated CO_2 assimilation rates of high N plants, within two weeks of receiving a pulse of high N. Furthermore, the higher net assimilation rates during the vegetative stage of the low N plants whose rhizosphere received high N pulse and the high N plants as compared to their reproductive stage suggests that maize plants require nitrogen supply more during vegetative stage as compared to their reproductive stage. This may be the resultant effect of the crop growth potential and critical nitrogen demand that corresponds with nitrogen uptake rates. The higher crop growth potential of plants during the

vegetative stage has been attributed to higher protein metabolism which includes cell division, whereas the reproductive phase requires relatively higher carbohydrate metabolism (Fageria, 2014). The vegetative and reproductive stages of unsprayed low N plants had similar net assimilation rates, suggesting nitrogen economy during nitrogen limitation. As the plant partition available systemic nitrogen for photosynthetic and other non-photosynthetic activities. A trade-off between leaf nitrogen content and CO_2 assimilation is anticipated in the unsprayed low N plant, as a quadratic relationship between leaf nitrogen content and CO_2 assimilation in maize was reported (Fageria, 2014).

3.5.10. Nitrogen availability, ontogeny, and the leaf area duration:

The leaf area contributes substantially towards whole plant growth. The leaf growth and its integral constituents of the photosynthetic apparatus are directly influenced by nutrients availability. Evaluating the proportion of biomass invested in leaf area over time will give additional understanding of plants performance under different growth conditions. Specifically, the leaf area duration provides an estimate of the duration of time in which the source leaves supply photosynthates to the sink sites (Engels. et al., 2012; Fageria, 2014). In this study, additional supply of high nitrogen to low N plants enabled increase in leaf area duration and chlorophyll level of leaves more than the high N plants and give more than the unsprayed low N plants. This demonstrates that just a one-time supply of high nitrogen delayed senescence in low N plants and enabled gradual leaf expansion and increased nitrogen content for light harvesting and synthesis of carbohydrate for growth and development. Gastal et al., (2015), reported that an increase in leaf area must lead to a decrease in the chlorophyll level of maize leaves. The suggested trade-off between leaf area and chlorophyll levels of leaves can be concluded to occur in all the low N plants.

As with other plant growth processes, the leaf area duration differed with treatment and ontogenetic phase. Additional supply of high N to low plants enabled expansion of middle leaves at remarkably similar rates to both sprayed and unsprayed high plants during the onset of reproduction. However, the leaf expansion rates of the unsprayed low plant were lower as compared to other plants and leaf growth rate was similar across leaf positions and in both vegetative and reproductive stage. The implication of this is that the middle leaves of the maize canopy are well positioned and equipped with more nitrogen contents for the interception of photosynthetically active radiation for increase biomass.

3.6. Conclusion

This study provides evidence that the photosynthetic efficiency of maize plants is not reduced, even during low nitrogen condition. Plants will continue to intercept photosynthetically active radiation for the synthesis of carbohydrate and construction of structural organs that will help to facilitate the process. Plants have evolved mechanisms for dissipating excess intercepted radiation through thermal dissipation or a quenching mechanism to prevent damage to their photosystem (Engels et al., 2012). This Chapter established that, additional supply of high nitrogen to maize plants growing on low nitrogen soils can lead to an immediate increase in chlorophyll level, transpiration rates and the efficiency of the photosystem II. Over a longer period, the relative growth rate of maize is highest at 21 days after germination which can be an ideal time for fertiliser applications (although genotypic differences may exist). The leaf area duration, which is important for the length of the sink filing period (nutrient remobilization from older leaves to younger leaves) and a major determinant of yield in maize can be extended by a one-time application of high dose of nitrogen fertilizer on maize growing on low nitrogen soils.

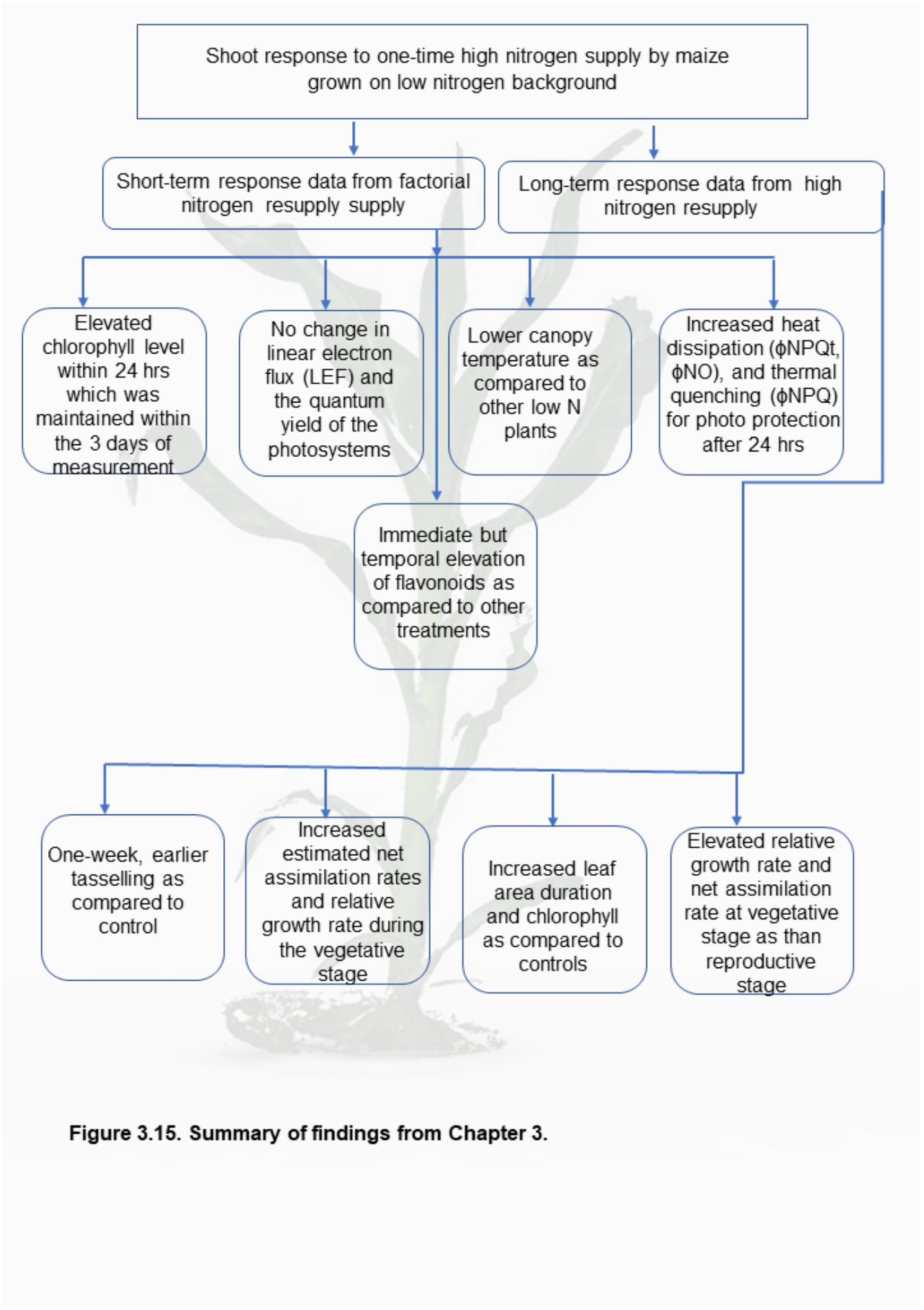


Figure 3.15. Summary of findings from Chapter 3.

3.7. References

Andrews, M., Raven, J. A., and Lea, P. J. (2013). Do plants need nitrate? The mechanisms by which nitrogen form affects plants: Do plants need nitrate? *Annals of applied biology* **163**, 174-199.

Dechorgnat, J., Francis, K., Dhugga, K. S., Rafalski, J. A., Tyerman, S. D., and Kaiser, B. N. (2018). Root Ideotype Influences Nitrogen Transport and Assimilation in Maize. *Frontiers in Plant Science* **9**, undefined-undefined.

Deng, B., Li, Y., Xu, D., Ye, Q., and Liu, G. (2019). Nitrogen availability alters flavonoid accumulation in Cyclocarya paliurus via the effects on the internal carbon/nitrogen balance. *Scientific reports* **9**, 2370-2370.

Díaz-López, E., Aguilar-Luna, J. M. E., and Loeza-Corte, J. M. (2020). Net Assimilation Rate and Agronomic Efficiency of Nitrogen in Tartago (Ricinus communis L.) (Euphorbiaceae) in Dry Climate. *Scientifica (Cairo)* **2020**, 1-7.

Engels., C., Kirkby., E., and White., P. (2012). Mineral nutrition, Yield and Source-sink relationship. *In* "Marschner's Mineral Nutrition of Higher Plants" (P. Marschner, ed.). Academic Press, United States of America.

Evans, J. R., and Clarke, V. C. (2019). The nitrogen cost of photosynthesis. *Journal of experimental botany* **70**, 7-15.

Fageria, N. K. (2014). "Nitrogen Management in Crop Production," First edition/Ed. CRC Press, Boca Raton.

Fritz, C., Palacios-Rojas, N., Feil, R., and Stitt, M. (2006). Regulation of secondary metabolism by the carbon–nitrogen status in tobacco: nitrate inhibits large sectors of phenylpropanoid metabolism. *The Plant journal : for cell and molecular biology* **46**, 533-548.

Garcia-Parra, M., Stechauner-Rohringer, R., Roa-Acosta, D., Ortiz-GonzÁLez, D., Ramirez-Correa, J., Plazas-LeguizamÓN, N., and Colmenares-Cruz, A. (2020). Chlorophyll fluorescence and its relationship with physiological stress in Chenopodium quinoa Willd. *Notulae botanicae Horti agrobotanici Cluj-Napoca* **48**, 1742-1755.

Garnett, T., Conn, V., Plett, D., Conn, S., Zanghellini, J., Mackenzie, N., Enju, A., Francis, K., Holtham, L., Roessner, U., Boughton, B., Bacic, A., Shirley, N., Rafalski, A., Dhugga, K., Tester, M., and Kaiser, B. N. (2013). The response of the maize nitrate transport system to nitrogen demand and supply across the lifecycle. *New Phytologist* **198**, 82-94.

Garnett, T., Plett, D., Conn, V., Conn, S., Rabie, H., Rafalski, J. A., Dhugga, K., Tester, M. A., and Kaiser, B. N. (2015). Variation for N Uptake System in Maize: Genotypic Response to N Supply. *Frontiers in plant science* **6**, 936.

Gastal., F., Lemaire., G., Durand., J.-L., and Louarn., G. (2015). Quantifying crop responses to nitrogen and avenues to improve nitrogen-use efficiency. *In* "Crop Physiology

Applications for Genetic Improvement and Agronomy" (V. O. S. a. D. F. Calderini, ed.), pp. 161-206. Academic Press, United Kingdom.

Gorban, A. N., Pokidysheva, L. I., Smirnova, E. V., and Tyukina, T. A. (2011). Law of the Minimum Paradoxes. *Bulletin of mathematical biology* **73**, 2013-2044.

Guidi, L., Lo Piccolo, E., and Landi, M. (2019). Chlorophyll Fluorescence, Photoinhibition and Abiotic Stress: Does it Make Any Difference the Fact to Be a C3 or C4 Species? *Frontiers in plant science* **10**, 174-174.

Hawkesford, M., Horst, W., Kichey, T., Lambers, H., Schjoerring, J., Moller I.S., and White, P. (2012). Functions of Micronutrients. (P. e. Marschiner, ed.), Vol. 3, pp. 135-189. Academic Press, Mineral Nutrition of Higher Plants.

Hirel, B., Le Gouis, J., Ney, B., and Gallais, A. (2007). challenge of improving nitrogen use efficiency in crop plants: towards a more central role for genetic variability and quantitative genetics within integrated approaches. *Journal of experimental botany* **58**, 2369-2387.

Hole, D. J., Emran, A. M., Fares, Y., and Drew, M. C. (1990). Induction of nitrate transport in maize roots, and kinetics of influx, measured with nitrogen-13. *Plant physiology (Bethesda)* **93**, 642-647.

Houshmandfar, A., Fitzgerald, G. J., O'Leary, G., Tausz-Posch, S., Fletcher, A., and Tausz, M. (2018). The relationship between transpiration and nutrient uptake in wheat changes under elevated atmospheric CO2. *Physiologia plantarum* **163**, 516-529.

Hunt, R. (2017). Growth Analysis, Individual Plants. *In* "Encyclopaedia of applied plant sciences" (B. Thomas, D. J. Murphy and B. G. Murray, eds.), pp. 421-429. Academic Press, London.

Khamis, S., Lamaze, T., Lemoine, Y., and Foyer, C. (1990). Adaptation of the Photosynthetic Apparatus in Maize Leaves as a Result of Nitrogen Limitation: Relationships between Electron Transport and Carbon Assimilation. *Plant physiology (Bethesda)* **94**, 1436-1443.

Kromdijk, J., Głowacka, K., Leonelli, L., Gabilly, S. T., Iwai, M., Niyogi, K. K., and Long, S. P. (2016). Improving photosynthesis and crop productivity by accelerating recovery from photoprotection.

Lu, C., and Zhang, J. (2000). Photosynthetic CO 2 assimilation, chlorophyll fluorescence and photoinhibition as affected by nitrogen deficiency in maize plants. *Plant science (Limerick)* **151**, 135-143.

Maxwell, K., and Johnson, G. N. (2000). Chlorophyll fluorescence—a practical guide. *Journal of Experimental Botany* **51**, 659-668.

McAusland, L., Atkinson, J. A., Lawson, T., and Murchie, E. H. (2019). High throughput procedure utilising chlorophyll fluorescence imaging to phenotype dynamic photosynthesis and photoprotection in leaves under controlled gaseous conditions. *Plant methods* **15**, 109-15.

Moll, R. H., Kamprath, E. J., and Jackson, W. A. (1982). Analysis and interpretation of factors which contribute to efficiency of nitrogen utilization [Zea mays]. *Agronomy journal* **74**, 562.

Mu, X., Chen, Q., Chen, F., Yuan, L., and Mi, G. (2016). Within-Leaf Nitrogen Allocation in Adaptation to Low Nitrogen Supply in Maize during Grain-Filling Stage. *Frontiers in plant science* **7**, 699-699.

Mu, X., Chen, Q., Chen, F., Yuan, L., and Mi, G. (2017). A RNA-Seq Analysis of the Response of Photosynthetic System to Low Nitrogen Supply in Maize Leaf. *International journal of molecular sciences* **18**, 2624.

Muratore, C., Espen, L., and Prinsi, B. (2021). Nitrogen Uptake in Plants: The Plasma Membrane Root Transport Systems from a Physiological and Proteomic Perspective. *Plants (Basel)* **10**, 681.

Murchie, E. H., and Lawson, T. (2013). Chlorophyll fluorescence analysis: a guide to good practice and understanding some new applications. *Journal of experimental botany* **64**, 3983-3998.

Nadeem, F., Ahmad, Z., Wang, R., Han, J., Shen, Q., Chang, F., Diao, X., Zhang, F., and Li, X. (2018). Foxtail millet [setaria italica (L.) beauv.] grown under low nitrogen shows a smaller root system, enhanced biomass accumulation, and nitrate transporter expression. *Frontiers in plant science* **9**, 205-205.

Pandey., R., Das., V. P. M., Meena., M., and Meena., R. C. (2017). Plant Growth Analysis. *In* "Manual of ICAR Sponsored Training Programme on “Physiological Techniques to Analyze the Impact of

Climate Change on Crop Plants” , " Division of Plant Physiology, IARI, New Delh.

Paris, Q. (1992). The return of von Liebig's "law of the minimum". *Agronomy journal* **84**, 1040-1046.

Plett, D. C., Holtham, L. R., Okamoto, M., and Garnett, T. P. (2018). Nitrate uptake and its regulation in relation to improving nitrogen use efficiency in cereals. *Seminars in Cell and Developmental Biology* **74**, 97-104.

Power, J. F., Willis, W. O., Grunes, D. L., and Reichman, G. A. (1967). Effect of Soil Temperature, Phosphorus, and Plant Age on Growth Analysis of Barley1. *Agronomy Journal* **59**, 231-234.

Price., C., and Munns., R. (1999). Growth analysis a quantitative approach. *In* "Plants in Action: Adaptation in Nature, Performance in cultivation." (D. B. J. Atwell., D. P. E. Kriedemann. and D. C. G. N. Turbull., eds.). Macmillan Education, Melbourne, Australia.

Rees, M., Osborne, C. P., Woodward, F. I., Hulme, S. P., Turnbull, L. A., and Taylor, S. H. (2010). Partitioning the Components of Relative Growth Rate: How Important Is Plant Size Variation? *The American naturalist* **176**, E152-E161.

Schlüter, U. U., Mascher, M. M., Colmsee, C. C., Scholz, U. U., Bräutigam, A. A., Fahnenstich, H. H., and Sonnewald, U. U. (2012). Maize Source Leaf Adaptation to Nitrogen Deficiency Affects Not Only Nitrogen and Carbon Metabolism But Also Control of Phosphate Homeostasis1[W][OA]. *Plant physiology (Bethesda)* **160**, 1384-1406.

Schneider, H. M., Postma, J. A., Kochs, J., Pflugfelder, D., Lynch, J. P., and van Dusschoten, D. (2020). Spatio-Temporal Variation in Water Uptake in Seminal and Nodal Root Systems of Barley Plants Grown in Soil. *Frontiers in plant science* **11**, 1247-1247.

Sharma, P., Jha, A. B., Dubey, R. S., and Pessarakli, M. (2012). Reactive Oxygen Species, Oxidative Damage, and Antioxidative Defense Mechanism in Plants under Stressful Conditions. *Journal of botany* **2012**, 1-26.

Sheng, M., Tang, M., Chen, H., Yang, B., Zhang, F., and Huang, Y. (2008). Influence of arbuscular mycorrhizae on photosynthesis and water status of maize plants under salt stress. *Mycorrhiza* **18**, 287-296.

Sun, J., Gao, J., Wang, Z., Hu, S., Zhang, F., Bao, H., and Fan, Y. (2019). Maize canopy photosynthetic efficiency, plant growth, and yield responses to tillage depth. *Agronomy (Basel)* **9**, 3.

Tóth, V. R., Mészáros, I., Veres, S., and Nagy, J. (2002). Effects of the available nitrogen on the photosynthetic activity and xanthophyll cycle pool of maize in field. *Journal of plant physiology* **159**, 627-634.

Urban, A., Rogowski, P., Wasilewska-Dębowska, W., and Romanowska, E. (2021). Understanding Maize Response to Nitrogen Limitation in Different Light Conditions for the Improvement of Photosynthesis. *Plants (Basel)* **10**, 1932.

Vidal, E. A., Alvarez, J. M., Araus, V., Riveras, E., Brooks, M. D., Krouk, G., Ruffel, S., Lejay, L., Crawford, N. M., Coruzzi, G. M., and Gutiérrez, R. A. (2020). Nitrate in 2020: Thirty years from transport to signaling networks. *The Plant cell* **32**, 2094-2119.

White, P. J. (2012). Ion Uptake Mechanisms of Individual Cells and Roots: Short-distance Transport. *In* "Marschner's Mineral Nutrition for Higher Plants" (P. Marschner, ed.). Academic Press, United States of America.

York, L. M., Silberbush, M., and Lynch, J. P. (2016). Spatiotemporal variation of nitrate uptake kinetics within the maize (Zea mays L.) root system is associated with greater nitrate uptake and interactions with architectural phenes. *Journal of experimental botany* **67**, 3763-3775.

Zhang, R., Zhou, Y., Yue, Z., Chen, X., Cao, X., Ai, X., Jiang, B., and Xing, Y. (2019). The leaf-air temperature difference reflects the variation in water status and photosynthesis of sorghum under waterlogged conditions. *PloS one* **14**, e0219209-e0219209.

Chapter 4

Whole plants carbon allocation as influenced by nitrogen availability and ontogenetic phase.

4.1. Abstract

The allometric relationship between plant functional organs enables identification of allocation rules associated with ontogeny and provides a means to estimate traits whose direct measurements would be difficult. This Chapter set out to determine the pattern of carbon allocation to the aerial and hidden half of maize plants in order to understand priorities for carbon partitioning during low nitrogen condition, high nitrogen resupply and during vegetative and reproductive stages.

To understand this whole plant synergetic relationship in carbon partitioning, the fresh and dry weight of biomass data obtained from the high nitrogen resupply and factorial nitrogen resupply experiment were analysed in terms of root-to-shoot ratio, percentage of allocated biomass to the leaves, stem and roots, and moisture contents as influenced by nitrogen availability and ontogenetic phase. The percentage change of root and shoot dry matter between plants grown on low and high nitrogen was also determined, while the specific root length during the vegetative stage was obtained from root image analysis. The leaf anatomical characteristics such as the specific leaf area, specific leaf weight, leaf area ratio and leaf weight ratio, were also determined from leaf dry weights and leaf area data in relation to nitrogen availability.

The root-to-shoot ratio was similar across treatments but higher during the vegetative stage as compared to the reproductive stage. Low N plants allocated more biomass to the roots during the vegetative stage and more to the stem during reproductive stage, while high N plants shared more biomass to the roots during the vegetative stage but distributed a similar quantity of biomass to the roots, leaves and stem during the reproductive stage. Moisture content of the shoot was higher than the roots during the vegetative stage but was similar during the reproductive stage irrespective of growth condition. The specific leaf area increased under low nitrogen condition while the specific leaf weight increased with increased nitrogen availability.

In conclusion, nitrogen rates influenced the pattern of biomass allocated to functional organs during the vegetative stage. This implies that nitrogen resources could be adjusted in terms of rates and timing of supply to maximise uptake and utilization efficiency. It would be important to further test these relationships in a factorial trial using different combinations of nitrogen and water quantity. Further study could also consider nitrogen, phosphorus, potassium, and water rate because root traits required to efficiently capture mineral sources differs and may stimulate a change in biomass allocation patterns. This understanding will enable identification of traits and molecular players which could be manipulated for improved resource capture and utilization efficiency.

4.2. Introduction

The short- and long-term interaction between nitrogen assimilation and photosynthesis during nitrogen nutrition affects biomass production and crop yield. Nitrogen supply upregulates the expression of photosynthetic genes, while soluble sugar facilitates the expression of nitrate assimilation genes. This interaction is important for biomass production and crop yield (Ueda et al., 2017). Biomass production was found to be related to the amount of intercepted radiation and efficient conversion (Massignam et al., 2011) and nitrogen-dependent photosynthetic enzymes (phosphoenolpyruvate carboxylase (PEPC) and pyruvate orthophosphate dikinase (PPDK)) (Ueda et al., 2017), and amount of radiation intercepted by source leaves (Engels. et al., 2012; Fageria, 2014; Gastal. et al., 2015). Nitrogen availability stimulates changes in leaf anatomy (Urban et al., 2021), carbon and nitrogen partitioning between shoot and roots (Gastal and Lemaire, 2002). This partitioning of plant organs controls yield (Engels. et al., 2012).

The allometric relationship (mathematical relationship) between organ parts and body size of organisms enables identification of allocation rules associated with ontogeny and provide proxies to estimate traits whose direct measurements would be difficult (Lehnebach et al., 2018). The allocation of biomass among plant organs enhances the capacity of plants to take up CO_2, water, and nutrients for future use (Poorter et al., 2012). Vascular plants possess leaves for carbon fixation, stem for mechanical support and hydraulic channels and roots that provides anchorage and acquisition of soil resources (Poorter et al., 2012). This functional roles of roots, leaves and stems are regulated to maximise uptake and utilization of limited resources (Kramer-Walter et al., 2016). It was hypothesised that leaves, stems and roots are functionally regulated by heritable traits (factors including genes and other molecular players involved in the transfer of traits characteristics) and environmental cues (nutrient availability, soil type, soil moisture conditions, solar radiation, atmospheric temperature and humidity including the level of CO_2) in which the simultaneous functions of the 3 organs is critical for rapid resource acquisition and metabolism (Kramer-Walter et al., 2016).

Considering the above, it is important to understand how much carbon is allocated to the aerial and subterranean parts of maize plants during low nitrogen nutrition and ontogenetic phases. This will help in understanding the allometric relationships of maize functional organs during low nitrogen availability, with the goal of identifying of key traits and pathways that could be manipulated to enhance nitrogen uptake and utilization for improved nitrogen use efficiency in maize. The fresh and dry weights data from plants used for the microdialysis experiments in Chapter 2 (to determine the spatio-temporal dynamics of applied nitrate and ammonium), were utilised to understand the

allometric relationships between aerial and below ground maize organs during low nitrogen condition including the vegetative and reproductive stage.

Aim and Objectives:

To evaluate carbon allocation to functional pools on account of growth conditions, N-pulses and ontogenetic phase.

Hypothesis:

Supply of high nitrogen to nitrogen limited plants will trigger more allocation of carbon to their shoot as compared to the roots; this is in response to plant increased photosynthesis resulting from a high internal nitrogen concentration to carbon ratio.

More carbon allocation will be channelled to the roots of low nitrogen plants as compared to their shoots during their vegetative stage, as an adaptation for further soil mining of nitrogen sources.

4.3. Methods

To evaluate whole plant carbon allocation from nitrogen treatment and resupply, this chapter combines the root and shoot biomass-related measurements form either fresh or dry weights of plants samples.

Plants growth and treatments (Refer to Chapter 2)

4.3.1. Root and shoot photographs for image analyses:

Immediately after completion of each of the 3 experiments in Chapter 2 of this thesis, the intact plants were carefully removed from the rhizoboxes and their roots carefully shaken by hand for about 5 minutes each, to remove attached soil particles. Although soil removal from the root was less stressful due to the soil texture being sandy, care was given to minimise the loss of fine roots while removing the soil. Each individual whole plant was spread on a pre-laid black or white plastic sheet. A measuring tape was placed beside each plant for scale. Photographs were taken using a digital camera (Exmor R, Sony, Japan). Thereafter, the roots were detached from shoots and photographs taken. The pictures were analysed to measure shoot and root length (cm) on Image J.

4.3.2. Fresh and dry weights (Biomass)

The harvested plants were then weighed for shoot and root fresh weight on a digital scale. The plants were dried to constant weight before weighing.

4.3.3. Root to shoot ratio:

Comparisons of root:shoot ratios were analysed according to developmental stage. In the experiments described in Chapter 2, the first (all sprayed with high N solution) was grown to eight weeks after germination which was reproductive stage. The third experiments (factorial nitrogen resupply) were grown for three weeks after germination which corresponds to vegetative growth stage. From here on the experiments will be discussed with vegetative stage first and reproductive stage experiment second.

To calculate root to shoot ratios: the root weight was divided by the shoot weight. This was conducted on each of fresh and dry weight.

Root to shoot ratio = weight of plants roots (g)/weight of plants shoots(g) Equation 1.

To calculate percentage change in root to shoot biomass as influenced by nitrogen nutrition, the root biomass and/or shoot biomass of low N plants were divided by the root biomass and/or shoot biomass of high N plants as given below:

The percentage change in root biomass = Root biomass of low N plants (g)/Root biomass of high N plants (g) x100 Equation 2a.

The percentage change in shoot biomass = Shoot biomass of low N plants (g)/Shoot biomass of high N plants (g) x100 Equation 2b.

The moisture contents of plants shoots, and roots were obtained as

$MC=(W_w-W_d/W_w)$ x100 Equation 2c.

Where W_w and W_d are fresh weight and dry weights respectively.

Specific Root length:

Root length/Root dry weight ($cm\ g^{-1}$) Equation 3.

4.3.4. Calculation of Percentage biomass allocation to functional organs:

Root = (Root dry weight/Total plant dry weight) x100 Equation 4a.

Stem=(Stem dry weight/Total plant dry weight) x100 Equation 4b.

Leaves= (Leaf dry weight/Total plant dry weight) x100 Equation 4c.

4.3.5. Calculation of leaf anatomical traits:

The leaf anatomic characteristics as influenced by growth conditions was also evaluated:

The specific leaf area as a measure of leaf density was obtained as

SLA =Leaf area/Leaf dry weights ($cm^2\ g^{-1}$) Equation 5a.

The specific leaf weight as a measure of leaf thickness was obtained as

SLW= Leaf dry weights/Leaf area ($g^{-1}\ cm^2$) Equation 5b.

The leaf area ratio gives the size of the assimilatory apparatus and was obtained as

LAR= Leaf area per plant/Plants dry weight ($cm^2\ g^{-1}$) Equation 5c.

The leaf weight ratio gives the index of plants on weight bases and was obtained as

LWR= Leaf weight/Plants weight (dimensionless) Equation 5d.

All equation 3 were obtained from (Sudhakar et al., 2016).

4.3.6. Data Analysis

Statistical differences between nitrogen treatments and between the vegetative and reproductive stage were tested using two-way ANOVA followed by Tuckey's post hoc test.

4.4. Results

To understand the partitioning of biomass at whole plant level after N-pulses were administered, plants from the high nitrogen resupply experiment were harvested at 8 weeks post germination for biomass analysis. These data were also used to determine biomass allocation to shoot, and roots grown to reproductive stage. Meanwhile plants from the factorial nitrogen resupply experiment were harvested at 4 weeks post germination for biomass analysis at vegetative stage.

4.4.1. Biomass analysis of maize plants after 5 weeks of high N-pulse:

4.4.1.1. The spray of high N on the rhizosphere of low nitrogen plants did not reduce their root to shoot ratio.

Addition of high N to low N plants (reciprocal N-pulse) did not lead to a significant change in their root-to-shoot ratio as compared to the unsprayed low N plants (P=0.9, Figure 4.1.A). There was also no statistical difference between the root-to-shoot ratio of the unsprayed plants to any other treatment (either grown on low or high N) sprayed with high plants (P=0.9, Figure 4.1.A), or between the root-to-shoot ratio of unsprayed low N plants to that of unsprayed high N (P=0.3, Figure 4.1.A).

4.4.1.2. The water contents of shoot and roots among treatments was influenced by N resupply.

The tissue water content was measured to determine if low nitrogen cause a reduction in plants moisture contents. Findings showed that the sprayed roots of low N plants had less moisture contents than the shoots of high plants (P=0.008, Figure 4.1.B). However, the interaction between nitrogen background and nitrogen resupply in terms of the water content of roots and shoots were not statistically significant (P=0.6, Figure 4.1.B). Likewise, that of the unsprayed shoots and roots were not significantly different from each other (P=0.4, Figure 4.1.B).

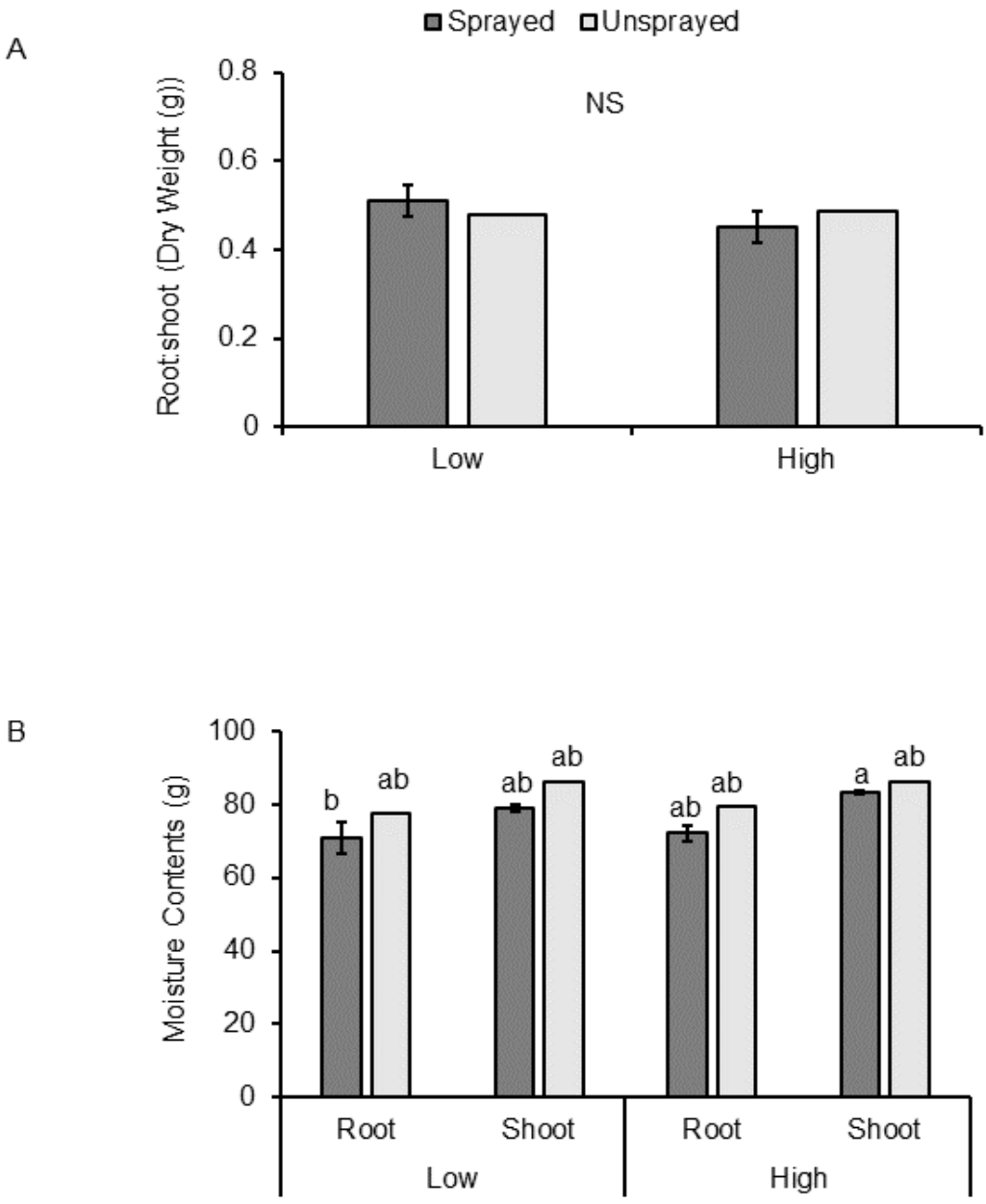


Figure 4.1. Nitrogen resupply did not affect the root : shoot ratio or moisture contents. Plants were grown on either adequate nitrogen supply (High) or nitrogen limited condition (Low). The plants were sprayed with high nitrogen solution at 21 days post germination (Sprayed) while some were left unsprayed (Unsprayed). All bars are averages (Sprayed n=1x4 plants each, Unsprayed n=1x1plant each). Data obtained from the high N resupply experiment where plants were allowed to grow for additional 5 weeks after spraying high N. Different lowercase letters denotes statistical difference from 5% level.

4.4.1.3. The spray of high N on the rhizosphere of low nitrogen plants enabled allocation of more tissue to the stem than the leaves and roots.

The low sprayed plants grew more stem tissues compared to the leaves or roots (Leaves: $P<0.001$, Roots: $P=0.02$, Figure 4.2.), while the unsprayed low plants increased the stem biomass as compared to the leaves but not roots (Leaves: $P=0.05$, Roots: $P=0.5$, Figure 4.2.). Similarly, the sprayed high plants also grew more stem tissues as compared to the leaves but were not significantly different to the root biomass (Leaves: $P<0.001$, Roots: $P=0.9$, Figure 4.2.), while there was no significant difference between any organ (stem, leaves or roots) for unsprayed high N plants (Leaves: $P=0.4$, Roots: $P=0.9$, Figure 4.2.).

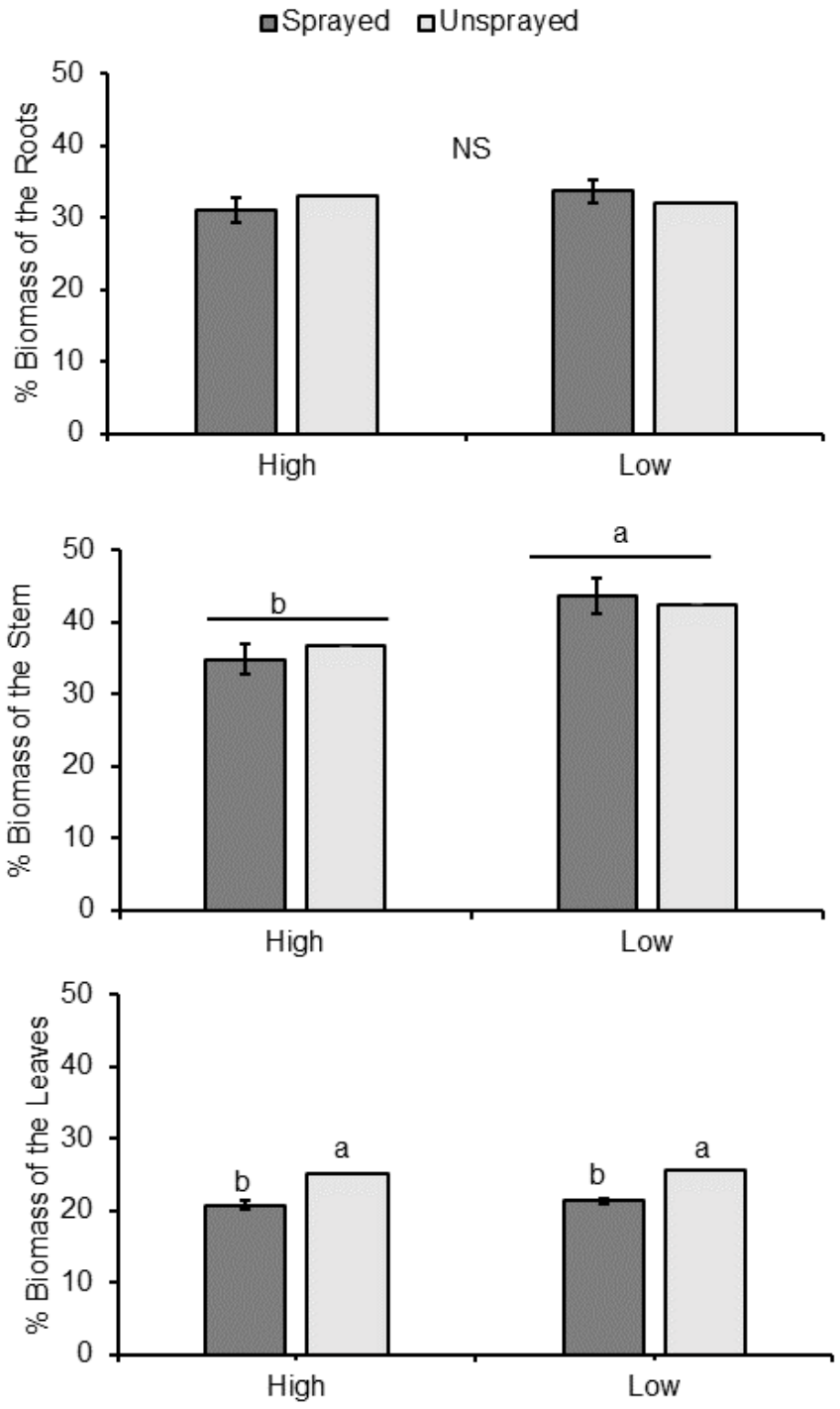


Figure 4.2. Low nitrogen background increased the stem biomass while high N resupply reduced the leaf biomass as compared to the unsprayed plants regardless of nitrogen background. Plants were grown on either adequate nitrogen supply (High) or limited nitrogen condition (Low). The plants were sprayed with high nitrogen solution at 21 days post germination (Sprayed) while some were left unsprayed (Unsprayed). All bars are averages (Sprayed n=1x4plants each, Unsprayed n=1x1plant each). Different lowercase letters denotes statistical difference from 5% level, NS means not statistically significamt. The data consist of one biorep that was obtained from the high N resupply experiment after plants were allowed to grow for additional 5 weeks .

4.4.1.4. The specific leaf area responded negatively with increasing nitrogen background and nitrogen pulse.

The specific leaf area represents the leaf density per plant. Findings showed that low nitrogen strongly triggered increase in specific leaf area (P<0.001, Figure 4.3.A) while nitrogen pulse applications slightly reduced the specific leaf area (P=0.02). In particular, the specific leaf area of the sprayed low plants was 1.5-fold higher than the sprayed high plants (P=0.005).

4.4.1.5. The specific leaf weight was affected by growth conditions.

The specific leaf weight represents the relative thickness of the leaves. The specific leaf weight was lower in low nitrogen plants (P=0.002, Figure 4.3.B) while nutrient spray application gradually increased it (P=0.05). Accordingly, the relative thickness of the leaves of the sprayed high plants was 1.3-fold higher than that of the sprayed low plants (P=0.009), and 1.6-fold higher than the unsprayed low plants (P=0.02).

4.4.1.6. Nitrogen rate and nitrogen pulse influenced the leaf area ratio.

The leaf area ratio represents the proportion of the plants engaged in photosynthetic process, or the size of the assimilatory apparatus. Here we found that the leaf area ratio at 56 days post germination was strongly affected by nitrogen rate and nitrogen pulse (P=0.004, Figure 4.3.C). The leaf area ratio was found to be 1.4-fold higher in the unsprayed low plant as compared to the sprayed low plants (P=0.03, Figure 4.3C), while the unsprayed high plant tended to be higher than the sprayed high plants but was not statistically significant (P=0.1). The leaf area ratio was also found to be 1.3-fold higher in the sprayed low plants as compared to the sprayed high plants (P= 0.04).

4.4.1.7. The leaf weight ratio was influenced by growth conditions.

The leaf weight ratio represents the index of plants on weight bases. The leaf weight ratio at 56 days post germination was affected by growth condition (P=0.006, Figure 4.3.D). Specifically, the leaf weight ratio was 1.2-fold higher in the unsprayed low plant than the sprayed low plants (P=0.01), while the unsprayed and sprayed high plants were similar (P=0.06).

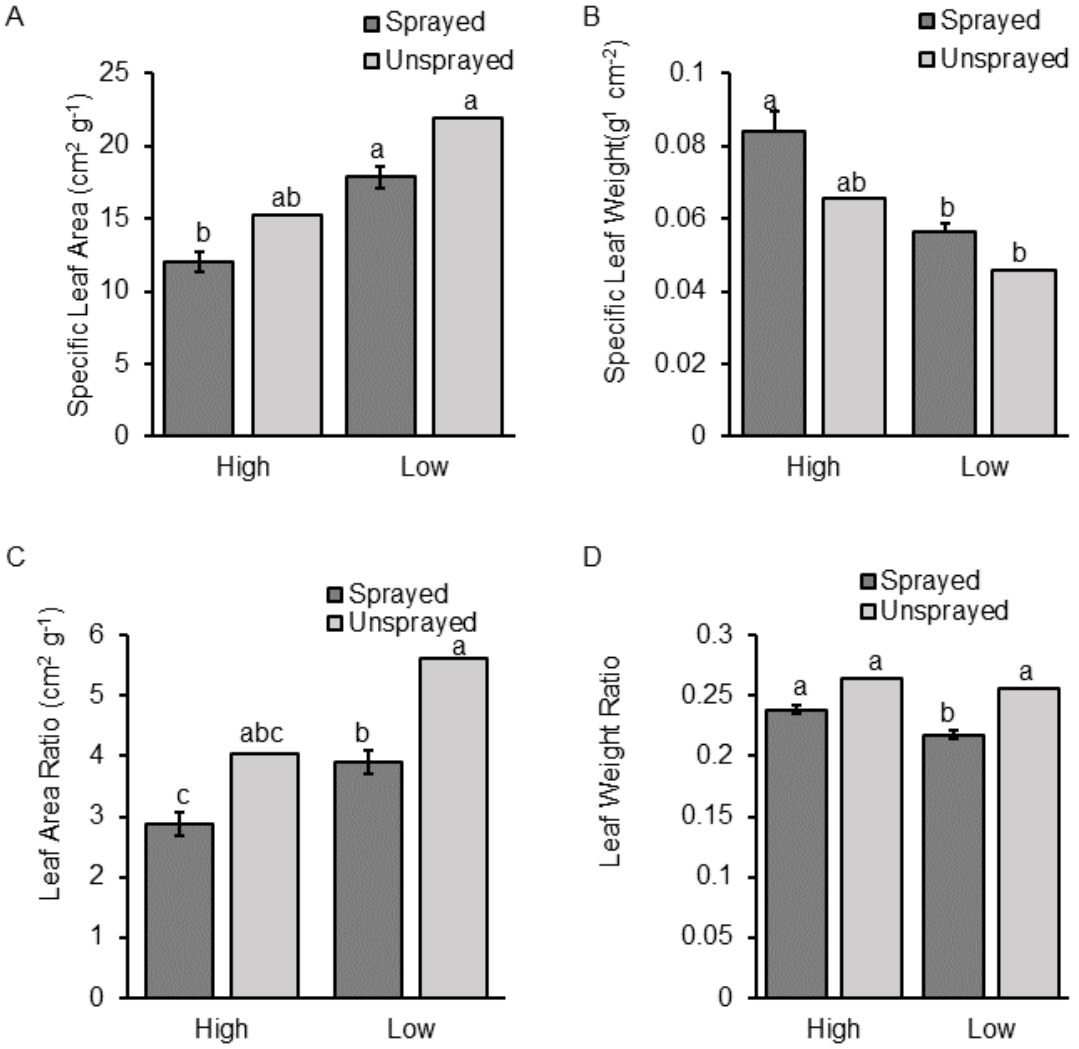


Figure 4.3. Nitrogen background and high nitrogen pulse influenced the anatomical properties of maize assimilatory systems. Plants were either grown on adequate (High) or limited (Low) nitrogen background for up to 56 days post germination. The dark grey bars were treated with high nitrogen (Sprayed) and the light grey bars were not treated (Unsprayed). Bars are averages (Sprayed:n=1x4 plants each; Unsprayed:n=1x1 plant each). The specific leaf area (A) represents the leaf density and was positively related to nitrogen stress, the specific leaf weight (B) represents the leaf thickness and was negatively related to nitrogen stress, the leaf area ratio (C) represents the capacity factor and was positively related to nitrogen stress, the leaf weight ratio (D) represents the leaf dry weight divided by the plant dry weight and was found to be reduced by nitrogen pulse application. Different lowercase letters denote significant difference from 5% downward. Data is from one biorep.

4.4.2. Biomass partitioning to organs during the two ontogenetic phases:

For the vegetative stages the plant biomass from the factorial nitrogen resupply was used while the reproductive stages were biomass data from the high nitrogen resupply experiment. It's important to note as a reminder that the high nitrogen resupply plants were grown on low or high N backgrounds and then all sprayed with high N and left to grow for 5 weeks while for the factorial nitrogen resupply plants were grown on low or high N background and then sprayed with either high or low nitrogen solution and harvested after 3 days of nutrient resupply which was the end of the experiment.

4.4.2.1. The shoot and root fresh weight were significantly affected by nitrogen background during the reproductive stage.

The fresh weight of both the shoot and root of high N plants were greater than that of the low N plants at the reproductive stage ($P<0.001$ each, Figure 4.4.B). But at the vegetative stage, the shoot and root fresh weight of high N plants were like that of low N plants (shoot: $P = 0.07$; root: $P = 0.6$; Figure 4.4.A&B.).

4.4.2.2. The shoot and root dry weight were significantly affected by nitrogen background during the reproductive stage.

The shoot and root dry weight of high N plants at reproductive stage was significantly higher than that of low N plants at the reproductive stage ($P<0.001$ each, Figure 4.4.C&D.). But no statistical difference between the shoot and root dry weight of high N plants as compared to that of low N plants at the vegetative stage respectively.

4.4.2.3. The root-to-shoot ratio of low N plants increased during the vegetative stage.

The root-to-shoot ratio using fresh weight (FW) or dry weight (DW) of low N plants was higher during the vegetative stage compared to their reproductive stage ($P=0.04$ (FW), Figure 4.5.A; $P<0.001$, Figure 4.5.A&B), and compared to both stages in high N plants (FW: Vegetative $P=0.004$, Reproductive $P=0.002$, Figure 4.5.A; DW: Vegetative ($P=0.006$), reproductive ($P<0.001$)). However, the average root-to-shoot ratio of low N plants during their reproductive stage was not different from that of the high N plants at the same stage (FW: $P=0.7$; DW: $P=0.9$, Figure 4.5.B).

4.4.2.4. The water content of roots was influenced by ontogenetic phase rather than growth condition.

The average water content of the roots of low and high N plants at vegetative stage was lower than during the reproductive stage (Low: $P<0.001$, High: $P=0.003$, Figure 4.5.C). The average water contents of the shoot of low and high N plants were not significantly different from each other at the both the vegetative and reproductive stage (Low: $P=0.9$, High: $P=1$, Figure 4.5.C).

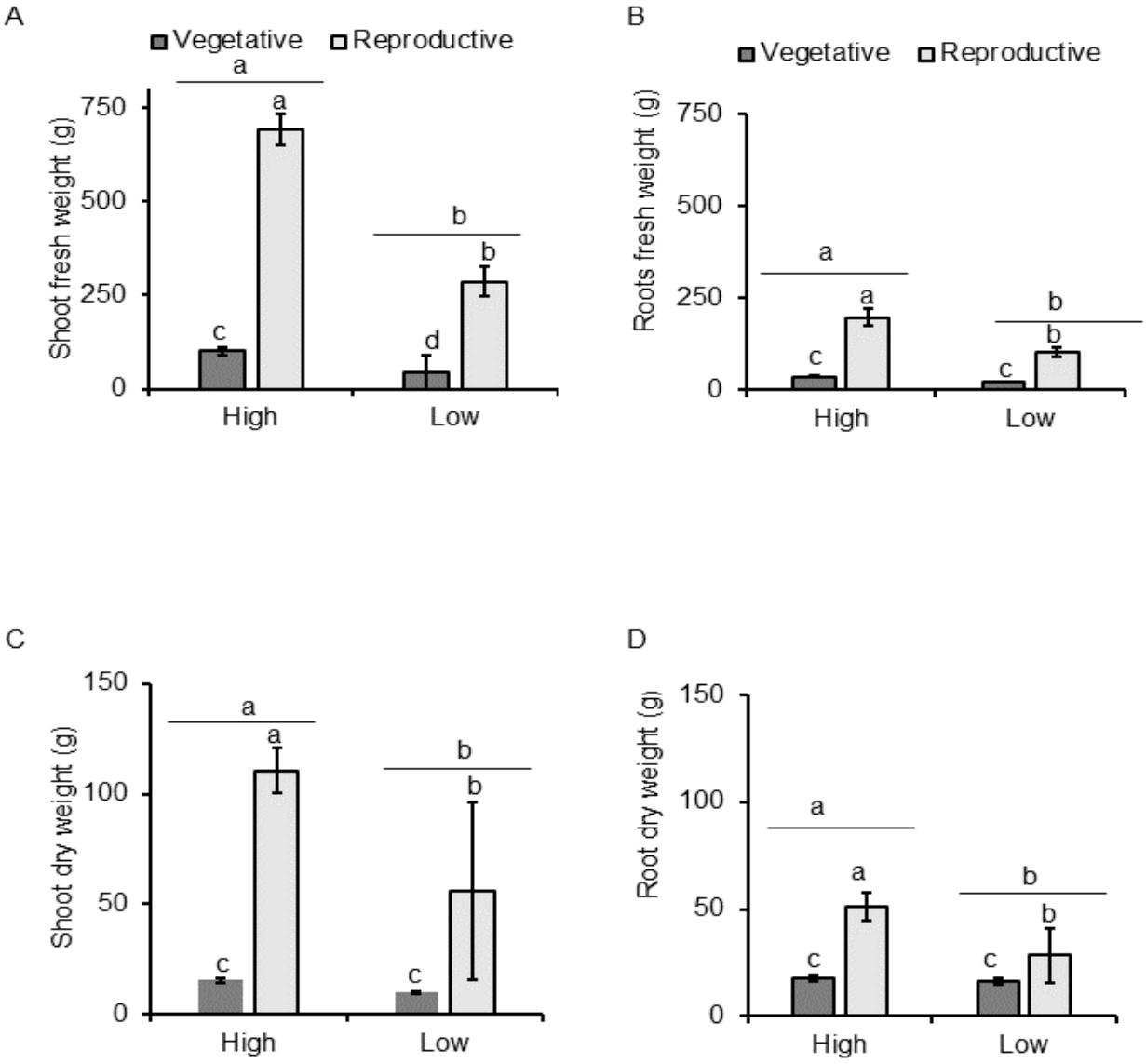


Figure 4.4. Nitrogen background and stage of development affected the shoot and roots respective fresh and dry weights. Plants were grown on either adequate nitrogen supply (High) or limited nitrogen condition (Low). The data consist of two biorep of which the reproductive stage was obtained from the high N resupply experiment, while the vegetative stage was obtained from the factorial N resupply experiment. Plants were harvested at 4 weeks after germination (Vegetative stage) and at 8 weeks after germination (Reproductive stage). All bars are averages (Vegetative: n=1x12 plants each, Reproductive: n=1x5 plants each). Different lowercase letters denotes statistical difference from 1% level.

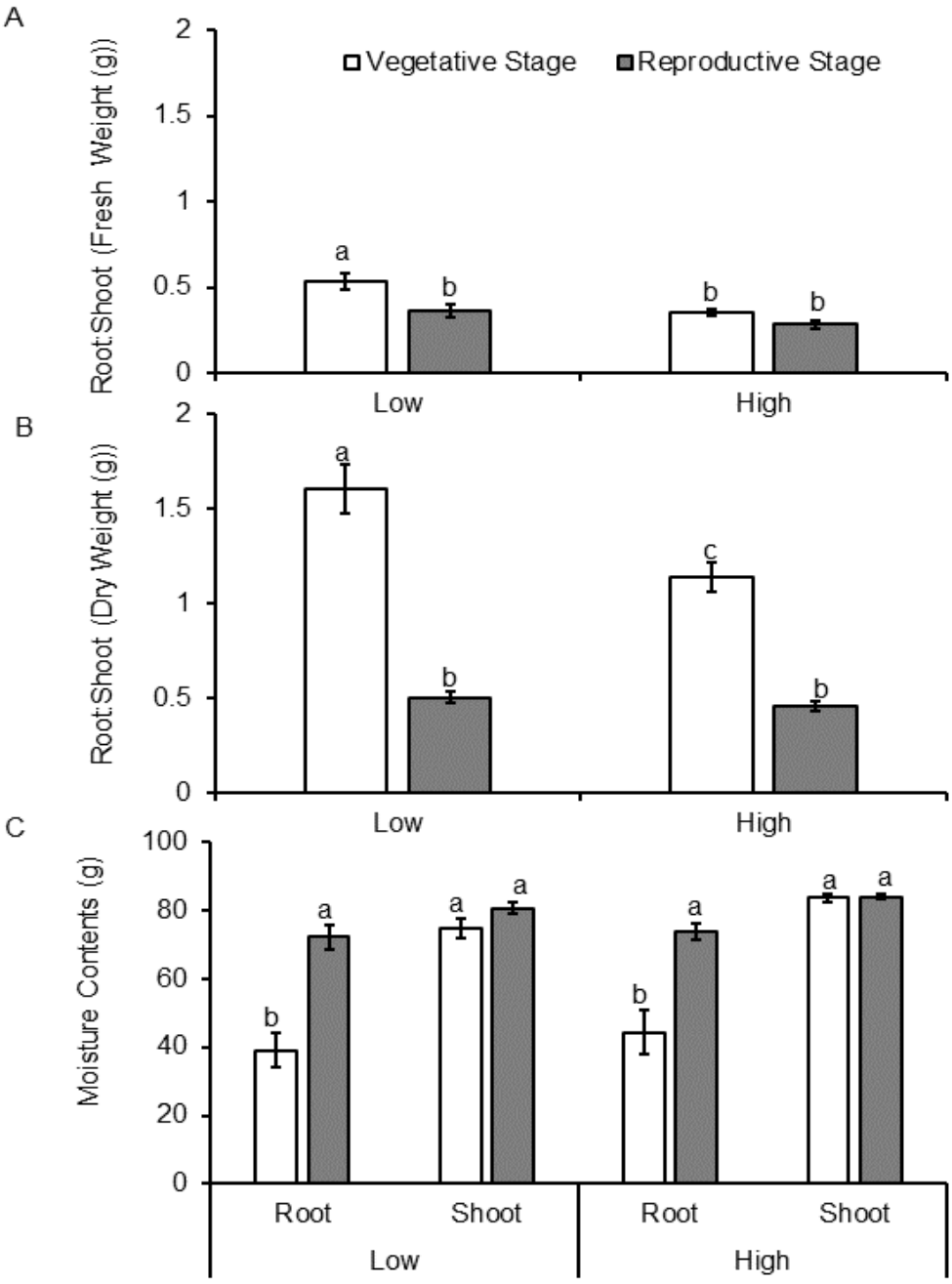


Figure 4.5. Nitrogen stress increased the root:shoot of maize plants during their vegetative stage. Figure (A), represents the root:shoot from fresh weight, Figure (B) represents root:shoot from dry weight, while Figure (C) represents the moisture contents of the biomass. Plants were grown on either adequate nitrogen supply (High) or nitrogen stress environment (Low). Plants were either harvested within 4 weeks after germination (Vegetative stage) or at 8 weeks after germination (Reproductive stage). All bars are averages (Vegetative: n=1x12 plants each , Reproductive: n=1x5 plants each). Different lowercase letters denotes statistical difference from 5% level.

The specific root length during the vegetative stage was not affected by growth condition:

The specific root length (ratio of the root length to their dry mass) of high N plants during the vegetative stage did not differ significantly from that of the low N plants at reproductive stage (P=0.2, Figure 4.6.A).

The percentage reduction of biomass from adequate N supply to limited N condition was influenced by ontogenetic phase:

The average percentage reduction in root biomass from adequate nitrogen supply to low nitrogen condition during the vegetative stage was significantly higher than that obtained at the reproductive stage (P=0.02, Figure 4.6.B). The average percentage reduction of the shoot biomass during the vegetative stage was not significantly different from that at the reproductive stage (P= 0.5, Figure 4.6.B).

Higher percentage of biomass were allocated to the roots during the vegetative stage:

Analysis of biomass allocation shows that both the low and high N plants channelled a greater part of their photosynthate (CH_2O) pool to the roots during the vegetative stage at the expense of the leaves and stems (P<0.001 each, Figure 4.6.C). During the reproductive stage, the stem had more biomass at the expense of the leaves in low N plants (P<0.001, Figure 4.6.C).

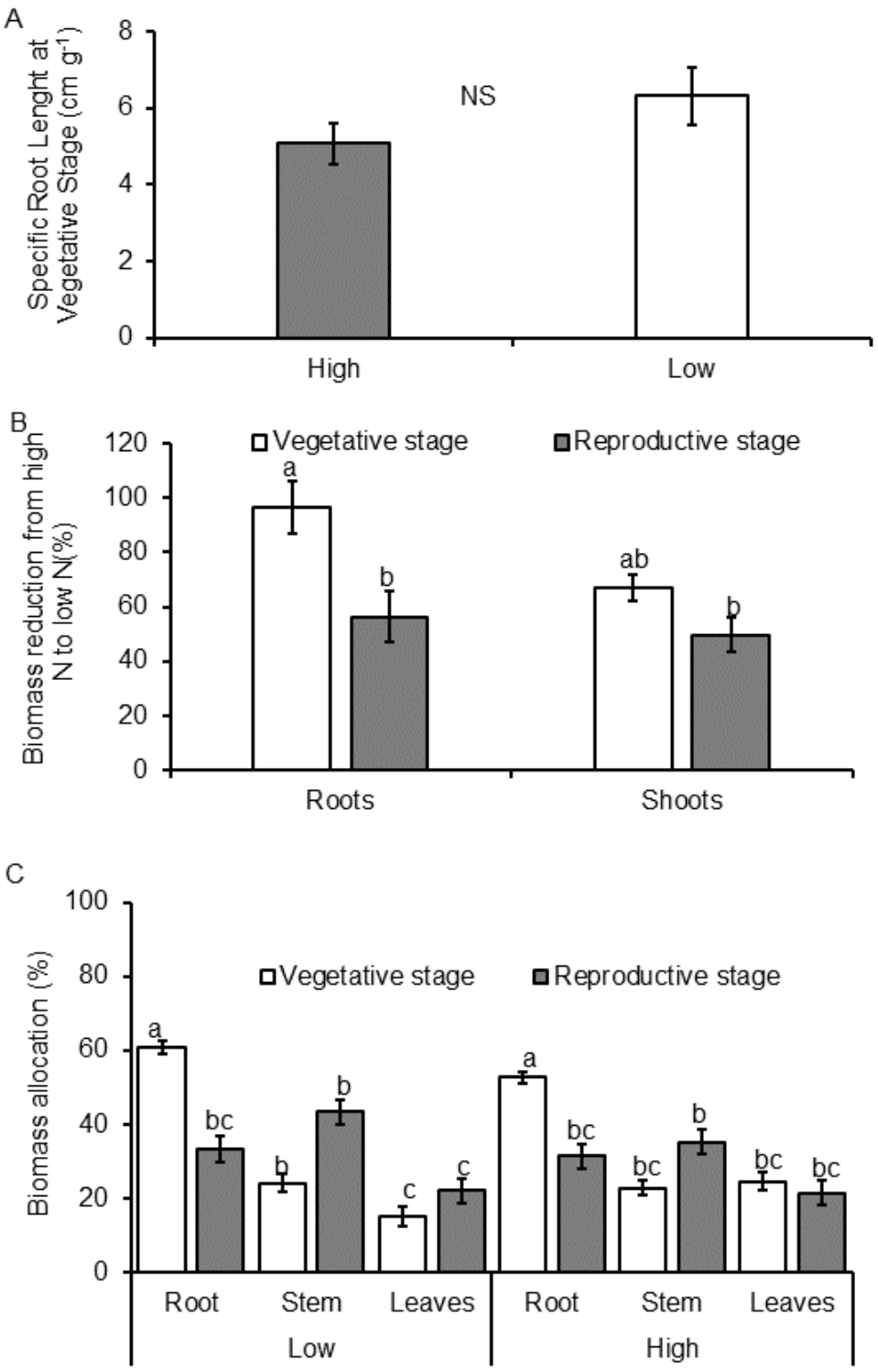


Figure 4.6. Effects of limited nitrogen supply on biomass partitioning Figure (A) represents the root specific root length, Figure (B) represents the percentage reduction of roots and shoot biomass from adequate nitrogen supply to nitrogen stress condition. while Figure (C) represents the fraction of biomass allocated to the 3 principal organs. Plants were grown on either adequate nitrogen supply (High) or nitrogen stress environment (Low). Plants were either harvested within 4 weeks after germination (Vegetative stage) or at 8 weeks after germination (Reproductive stage). All bars are averages (Vegetative: n=1x12plants each , Reproductive: n=1x5plants each). Different lower case letters denotes statistical difference from 5% level, NS means not statistically significant.

4.5. Discussion

Not all mechanism is known about the allometric relationship between the different maize functional organs as influenced by nitrogen rate and resupply, as well as developmental stages. The allometric relationship in the form of biomass partitioning of photosynthates, is the resultant effects of plant responses to changes in internal and external environments. The objective of this chapter was to explore the allometric relationship between the aerial and subterranean parts of maize in response to nitrogen rates and resupply, as well as to quantify this relationship during vegetative and reproductive stages. It was hypothesized that the supply of high nitrogen to nitrogen limited plants will trigger more allocation of carbon to their shoot as compared to their roots, this is in response to plant increased photosynthesis resulting from a high internal nitrogen concentration to carbon ratio. It was also hypothesised that more carbon allocation will be channelled to the roots of low nitrogen plants as compared to their shoots during their vegetative stage, as an adaptation for further soil mining of nitrogen sources. The plants from the first microdialysis experiments (Chapter 2) were used to determine the biomass partitioning after 35 days of applying high N pulse to low N plants. Those plants were used to study biomass partitioning during the onset of reproduction. The plants from the third microdialysis experiment (Factorial nitrogen resupply) (Chapter 2) were used to represent the vegetative stage. The discussion presented the influence of additional supply of high nitrogen followed by comparing the partitioning of biomass at the vegetative and reproductive stage on each of the findings in the results section.

4.5.1. The root-to-shoot ratio:

The concentration of available nitrate affects root development, root architecture and root-to-shoot ratio (Fageria, 2014; Kant et al., 2011; Lynch et al., 2012). The root-to-shoot ratio is a proxy for assessing plant performance based on the pattern of carbon allocation to aerial and below ground organs. Normally a high root-to-shoot ratio depicts a greater need for soil exploration to mine more nitrogen according to the theory of functional equilibrium (Gastal. et al., 2015) however, in this study, there was no significant difference in the root-to-shoot ratio of low and high N plants based on additional supply of a high N pulse. This is not in line with the general understanding of maize physiology (Asibi et al., 2019). It would have been expected that the root-to-shoot ratio of low N plants would be higher than that of the high N plants. The data from this study implies that the low N plants were utilising available nitrogen in similar manner as the high N plants in terms of partitioning biomass to aerial and below the ground parts which can be seen as a form of nitrogen use efficiency. Moll et al., (1982) stated that variation in nitrogen use efficiency of experimental plants during low N condition is associated with nitrogen utilization efficiency. Whereas, during adequate nitrogen

condition, nitrogen uptake efficiency is an ideal trait for nitrogen use efficiency (Reviewed in (Hirel et al., 2007)). The lack of significant difference in the root-to-shoot ratio by growth condition or nitrogen resupply in my experiments could be due to the ontogenetic phase of the plants.

When the root-to-shoot ratio was compared between the ontogenetic phase, the vegetative stage had a higher root-to-shoot ratio compared to the reproductive stage in both low and high N plants. This suggests preference for nitrogen uptake over photosynthesis during the vegetative stage. This is because the roots and shoots serve as sink organs for storage of nitrogen during vegetative stage from which these nutrients are remobilised to the seeds and fruits during the reproductive stage (Gastal. et al., 2015; Hirel et al., 2007; Kant et al., 2011). Thus, the driving force of plant growth rate potential in relation to nitrogen uptake demand could have influenced a proportionately higher biomass to the roots during the vegetative stage as compared to the reproductive stage. This is clearly shown here by the higher root-to-shoot ratio in plants grown on low nitrogen background as compared to high N plants during the vegetative stage. Previous study on maize in underground pot soils in the greenhouse found greater root-to-shoot ratio when nitrogen supply decreased in maize plants and velvetleaf (Bonifas et al., 2005). The high root-to-shoot ratio during vegetative stage can be attributed to the need for initial establishment (Asibi et al., 2019). The competition for photosynthates between the shoot and the roots is the main factor reducing root growth and activities during the reproductive stage (Lynch et al., 2012).

The high root-to-shoot ratio during low nitrogen conditions can be linked to hormonal regulation. The nitrate-dependent regulation of the root system is mediated by auxin and is crucial for several plant growth and developmental processes (Ishaya and Rasmussen, 2021; Vega et al., 2019). Low nitrogen conditions increased shoot-to-root auxin transport, leading to the acidification process of the cell wall that promote cell elongation (Sun et al., 2020). Other variables such as carbohydrate and light also control root nitrate uptake by co-ordinating NRT2.1, NRT2.4 and NRT1 upregulation through the oxidative pentose phosphate pathway-dependent signalling mechanism (Sakr et al., 2018; Vidal et al., 2020). Also, the basic leucine zipper (bzip) transcription factor HY5, which plays vital role in shoot to roots communication upon light illumination, triggers the upregulation of NRT2.1 for nitrate uptake and root growth (Vega et al., 2019; Vidal et al., 2020). HY5 interacts with cytokinin and auxin signalling including light responsive carbon and nitrogen metabolism to regulate shoot and root growth (Vega et al., 2019).

4.5.2. Percentage biomass allocation to functional organs:

The optimal partitioning theory predicted that plants would optimize overall growth rate by making all resources equally limiting and changing their biomass allocation patterns to obtain the most limited

resource in response to resource gradient (Bonifas et al., 2005; Hilbert, 1990). In this study, the low N plants whose rhizosphere was sprayed with high N had more biomass in the stem followed by the roots before the leaves. While the unsprayed low N had nearly similar biomass tissues in the root, stem, and leaves. This suggests that the supply of high N enabled the low N plants to initiate adjustment in allocation pattern that closely relates with the high N plants. This because the high N plants that received additional N pulse had higher stem and root biomass tissues but less leaf tissues. The reason for this pattern of allocation could be due to the need to translocate N sources from older tissues of roots and stem to young developing leaves on the upper canopy. The unsprayed low N plants showed the need to take up more N sources and at the same time needed to remobilise nutrients to the sink while maintaining the ability to intercept photosynthetically active radiation for carbohydrate synthesis.

The differences in biomass allocation pattern between the sprayed and the unsprayed low N plants can be defined as a mechanism to maximise resource efficiency. The sprayed low N plants had more leaves and a greater leaf area and so may have a greater need to translocate more photo assimilates from source (older leaves) to the sink (young developing leaves), than to the leaves rather than the roots. Previous study showed that the leaf-to-stem ratio of wheat and canola greatly reduce in response to N deficiency while maize was not affected (Gastal. et al., 2015). The scholars further stressed that sorghum and maize responded to nitrogen stress by decreasing nitrogen allocation to stems per unit leaf area index, this would allow the plants maintain high leaf nitrogen level and leaf area for radiation use efficiency. However, Gastal et al., (2015) reported a hypothesis that the reduction of N allocation to the stem could negatively affect N remobilization to reproductive meristems. This hypothesis needs to be tested by planning and executing research involving factorial treatments and 2 to 3 bioreps in the glasshouse and the fields. This is to enable a broader understanding of the allometric relationship as influenced by nitrogen availability in maize.

The biomass allocation pattern differed between ontogenetic phases. During the vegetative stage where there is increased cellular activities including cell divisions and nitrogen and carbon metabolism, the roots had more biomass as compared to the shoots in both low and high N plants. This can lead to enhanced nutrient acquisition to meet plant demand and growth rate potential. This situation is further evident in the lack of significant difference between treatments in their specific root lengths at vegetative stage. However, during the reproductive stage, the stem and roots had more biomass as compared to the leaves in low N plants while there was no difference in the proportion of roots, leaves and stem biomass in high N plants. Previous reports suggest that abiotic stress reduces leaf growth and causes a shift of shoot growth to the stem fraction (Ratjen et al., 2018) as stem reserve was said to be critical when sink demand is higher than the photosynthetic source

capacity (Olmedo Pico and Vyn, 2021). However, the leaf:stem ratio was said to decrease as the crop mass increases and more C is allocated to the stem as the plant ages (Gastal. et al., 2015; Lemaire et al., 1992). Whether this was the case or not regarding the findings from this study remained to be researched in more detail.

The percentage change in biomass from high nitrogen treatment to low nitrogen was not significant between the vegetative and reproductive stages. However, it is possible with further replicate over longer times that the root biomass could be higher during the vegetative stage This may mean that investment of carbon towards capture of nutrients was more important than radiation interception when soil nitrogen was scarce. This finding is in line with previous report that mild nitrogen deficiency increased the proportion of root biomass (Schneider et al., 2021; Xiaoli et al., 2020).

4.5.3. Moisture contents of shoot and roots:

The plant moisture contents or water status is coordinated by stomata-mediated photosynthetic gas exchange and expansion growth (Bramley et al., 2013; Brodribb et al., 2015). These activities depend on the established equilibrium between water supply from the roots into the leaves and its loss by transpiration (Brodribb et al., 2015). In this study, a high nitrogen pulse did not influence the moisture contents of roots or shoots in low N plants relative to the unsprayed low N plant. Even though the response of maize to soil water availability is strongly dependent on the nitrogen level of the soil (Bennett et al., 1989), the long-term effect of the applied high N pulse on plants moisture contents can be had to be established. This is because the high nitrogen was applied once during microdialysis (Chapter 2) and subsequent feeding of low N plant was with low N solution, of which the one-time high N pulse cannot instigate a morphological change that will be maintained 35 days after. The long-term response of root hydraulic conductivity to abiotic stress was said to involve changes in anatomy and morphology (Alsina et al., 2011; Brodribb et al., 2015). Also, high root hydraulic conductance was found to relate to new roots rather than changes in individual roots (Alsina et al., 2011; Brodribb et al., 2015). Previous reports and studies suggest that nutrient status of the plants as well as other abiotic stress exert an adjustment in the hydraulic conductivity of roots within minutes or hours (Bramley et al., 2010; Brodribb et al., 2015; Hachez et al., 2006; Lee et al., 2005). Such an immediate response was said to be reversible to match transpiration and relates to activities of aquaporins (water channels involved in membrane transport of water) amongst other mechanisms (Bramley et al., 2013; Brodribb et al., 2015; Hachez et al., 2006; Hachez et al., 2012). Moreso, the plants have crossed from vegetative to reproductive stage at 35 days after the high N supply.

During the vegetative stage in this study, the moisture contents of the shoots was found to be greater than that of roots regardless of growth condition. This is because the vegetative stage involves many

metabolic activities including cell division and cell expansion for multiplication and tissue growth (Fageria, 2014; Wang et al., 2020). Thus, more moisture is conveyed from the roots to the shoots where photosynthesis and gas exchange for carbon gain is highly needed. Although the root growth was highest during the vegetative stage as compared to the reproductive stage, water may only be conveyed first to the tissues with severe need for the internal regulation of whole plant development. This is because the aquaporins abundance are higher in tissues or cells needing greater osmotic control of water flow (Bramley et al., 2013). More than 30 aquaporins have been identified in crop plants including maize, arabidopsis, rice and tomatoes (Bramley et al., 2013; Brodribb et al., 2015; di Pietro et al., 2013; Wang et al., 2020).

In maize, *ZmPIP1;2, ZmPIP2;4,* and *ZmTIP1;1,* were said to be enriched in the root elongation zones (Hukin et al., 2002; Wang et al., 2020) while*, ZmPIP1;1, ZmPIP1;5, ZmPIP2;1, ZmPIP2;5, ZmPIP2;6,* are more at the root tips of which their abundance is developmentally regulated (Hachez et al., 2006; Wang et al., 2020). Furthermore, *ZmPIP1;1, ZmPIP1;2, ZmPIP1;3, ZmPIP2;1,* and *ZmPIP2;2,* are said to be abundant in the leaf elongation zones (Wang et al., 2020). The expression of six *ZmPIP1s* and six *ZmPIP2s* are found to be directly related to cell membrane permeability but coordinated by leaf developmental stage (Wang et al., 2020). Taken together, the localization and roles of this genes are expressed in the region of growth showing that they may be more influential during the vegetative stages where growth rate is faster as compared to the reproductive stage.

During the reproductive stage in this study, the moisture contents of roots and shoots were similar regardless of nitrogen background. This could mean that root uptake of water at this stage is high with a slight reduction in transpiration of the leaves to achieve osmoregulation. Osmoregulation and high-water status are very critical at this stage due to the need for nutrient translocation from the roots and source leaves to the reproductive meristem and young developing leaves (sink). It was proposed that the reproductive stage (post-anthesis) is less sensitive to water deficit due an increasing supply of reserved assimilates later in grain development (Westgate and Grant, 1989). Whether the rhizosphere of mature maize plants gains more water supply through association with microbes to complement water uptake remained to be researched. This because root uptake of soil resources tends to reduce during the reproductive stage.

4.5.4. Nitrogen uptake during maize reproductive phase:

Nitrogen uptake and remobilization are independently inherited traits through desirable alleles and could be considered for breeding to improve nitrogen use efficiency (Asibi e al., 2020). However, environmental factors capable of affecting root growth or root morphology would affects nutrient uptake (Nasielski et al., 2019). When maize N uptake is continues throughout the grain filling period,

less nitrogen is mobilized from the vegetative organs, thus increase the leaf area duration, delaying senescence and enhancing dry matter accumulation ((Asibi et al., 2020). Growth condition influences N uptake in such a way that all conditions favourable for post -anthesis photosynthesis promotes N uptake and reduces N remobilization, while conditions that do not enhances post anthesis photosynthesis reduces the N uptake and increases N remobilization from source leaves to sinks and grains (Asibi et al., 2020). Thus, the corelation between nitrogen uptake and photosynthesis leads to an inverse relationship between N remobilization and N uptake (Asibi et al., 2020). N uptake by maize and N remobilization during the reproductive stage are affected by soil nitrogen supply (Asibi et al., 2020). This implies that maize plants at reproductive stage are also capable of efficient acquisition of soil N during nitrogen supply. But research findings suggest that maize accumulates luxury nitrogen in excess of the requirement for biomass accumulation during the vegetative stage and restricts N uptake during the post- silking stage (Nasielski et al., 2019). This Chapter hypothesize that nitrogen uptake during the reproductive stages of maize is restricted because there is not much N demand orchestrated by shoot growth coupled with the shortage of photo assimilate to the roots and the availability of photosynthates in plant tissues that can be remobilized to support yield.

4.5.5. Nitrogen rates and the structural characteristics of maize leaves.

Leaves are multidimensional and change throughout the leaf ontogeny in response to interaction between genetics and environmental factors (Coneva and Chitwood, 2018). Within maize leaves, the optimization of nitrogen allocation could be an adaptive mechanism to maximize photosynthetic nitrogen for improved grain yield per unit leaf nitrogen (Mu et al., 2016). This can be achieved by increasing the photosynthetic activities of the leaves and/or the photosynthetic area (Engels. et al., 2012; Gastal. et al., 2015).

The specific leaf area, which is a measure of leaf density was found to be higher in low N plants as compared to high N plants in this study. The high and low N plants whose rhizosphere was sprayed with high N solution had lower specific leaf area as compared to the unsprayed. This suggests differences in the rate of sequestration of nitrogenous and carbon compounds as influenced by nitrogen availability in maize leaves. The specific leaf area also varies with non-structural carbohydrates concentration (Gastal. et al., 2015). Mu et al., (2016), found increased cell wall biomass in low N plants presumably due to increase carbohydrate levels of the leaves and allocation to cell wall. The longer chain fatty acid associated with waxes and surface structures were found to increase under prolonged nitrogen limitation (Schlüter et al., 2012). Accordingly, cell wall and lignin biosynthesis were reported to also increase with low nitrogen nutrition in tobacco (Fritz et al., 2006) and maize (Schlüter et al., 2012). The results of the specific leaf area analysis in this study and evidence attributed to the effects of nitrogen limitation from previous studies reported above shows that the

production of carbon rich metabolites which can increase high specific leaf area is preferred to nitrogen rich metabolites during limited nitrogen condition (Schlüter et al., 2012). This could be seen as an adaptation to maximize photosynthesis with reduced impact of high irradiance that could damage the photosynthetic apparatus during low nitrogen nutrition in maize. Previous study found a negative relationship between specific leaf area and leaf thickness (Vile et al., 2005). The specific leaf area is a direct opposite of the specific leaf weight.

The specific leaf weight (leaf thickness) is a strategy for resource capture (water and light) and utilization. This is because the quantity of light absorbed by a leaf and the diffusion channel of CO_2 through the leaf tissue to some extends depends on the leaf thickness (Vile et al., 2005). Also, the leaf thickness is associated with increased cell size which affects water storage (Coneva and Chitwood, 2018). In this study the specific leaf weight increased with increasing rate of nitrogen in all plants, suggesting the role of nitrogen in facilitating photosynthesis. As nitrogen rate increases, the photosynthetic area and photosynthetic capacity increases in leaves of maize plants. This is because allometric studies showed that leaf thickness, and by extension the specific leaf weight, directly relates to the size of the palisade mesophyll cells that increases cell height to enhanced CO_2 assimilation in the mesophyll cells (Coneva and Chitwood, 2018). Accordingly, larger chloroplasts with advance grana, stroma lamellae and starch granule per mesophyll cell were reported in leaves grown on adequate nitrogen (Gao et al., 2020), which increases leaves longevity but with construction cost (Vile et al., 2005) that may not be supported in low N conditions. Previous study also found higher specific leaf weight in plants grown on adequate nitrogen as compared to those grown on limited nitrogen (Vos et al., 2005). Decreasing specific leaf weight (leaf thickness) and smaller chloroplast with no starch granules was linked with leaves of plants limited in nitrogen (Gao et al., 2020).

The leaf area ratio is a measure of the proportion of the plant, which is engaged in photosynthetic process, it gives the relative size of the assimilatory apparatus. In this study, the increase in leaf area ratio in low N plants suggest the intrinsic need to maximise the capture of photosynthetically active radiation during low nitrogen conditions. This is evident as the one-time spray of high N solution on the rhizosphere of low N plants led to decreased leaf area ratio compared to that of the unsprayed high N plant. A study on two maize cultivars showed a uniform increase in individual leaf area from basal positions to leaf 8 and decreased again from 10^{th} leaf towards the apical leaf position, implying that increase in individual leaf area was influenced by fertiliser rates with significant effects on the 10^{th} to 15^{th} leaves (Engels. et al., 2012).

4.6. Conclusion

Biomass partitioning to different plant organs shows the trade-off between resource capture and utilization. From this study, one-time application of high nitrogen pulse to maize plants growing on low N plants enabled an adjustment in biomass partitioning towards more of utilization efficiency as compared to resource capture. This is because the low N plants allocated more biomass to the stem before the roots and the leaves just like the plants growing on high nitrogen. However, the specific leaf weight (leaf thickness) which is important for photosynthesis was reduced under low nitrogen condition. The maize plants allocated more biomass to the roots irrespective of nitrogen background during early vegetative stage. This implies that the vegetative phase could be crucial for early establishment. The implication being that applying nitrogen fertilizer at this stage could facilitate uptake as compared to the reproductive stage. During the reproductive stage, the moisture contents of the plants were higher in the shoot and roots irrespective of nitrogen background. This may be due to nutrient remobilization from the roots and source leaves towards the sink. So, applying foliar nitrogen fertilizer at this time may be helpful since plants roots are unable to efficiently capture soil resource in the reproductive stage.

4.7. Reference

Alsina, M. M., Smart, D. R., Bauerle, T., de Herralde, F., Biel, C., Stockert, C., Negron, C., and Save, R. (2011). Seasonal changes of whole root system conductance by a drought-tolerant grape root system. *Journal of experimental botany* **62**, 99-109.

Asibi, A. E., Chai, Q., and A. Coulter, J. (2019). Mechanisms of Nitrogen Use in Maize. *Agronomy (Basel)* **9**, 775.

Bennett, J. M., Mutti, L. S. M., Rao, P. S. C., and Jones, J. W. (1989). Interactive effects of nitrogen and water stresses on biomass accumulation, nitrogen uptake, and seed yield of maize. *Field crops research* **19**, 297-311.

Bonifas, K. D., Walters, D. T., Cassman, K. G., and Lindquist, J. L. (2005). Nitrogen supply affects root:shoot ratio in corn and velvetleaf (Abutilon theophrasti). *Weed science* **53**, 670-675.

Bramley, H., Turner, N. C., and Siddique, K. H. M. (2013). Water use efficiency: governed by an interactive network of physical. biochemical and hydraulic process. *In* "Genomics and breeding for climate-resilent crops" (C. Kole, ed.), pp. 225-268. Springer-Verlag,, Berlin.

Bramley, H., Turner, N. C., Turner, D. W., and Tyerman, S. D. (2010). contrasting influence of short-term hypoxia on the hydraulic properties of cells and roots of wheat and lupin. *Functional plant biology : FPB* **37**, 183-193.

Brodribb, T. J., Holloway-Philips, M.-M., and Bramley, H. (2015). Improving Water Transport for Carbon Gain in Crops. *In* "Crop Physiology:Applications for Genetic Improvement and Agronomy" (V. O. Sandras and D. F. Calderini, eds.), pp. 251-284. Accademic Press, United Kingdom

Coneva, V., and Chitwood, D. H. (2018). Genetic and Developmental Basis for Increased Leaf Thickness in the Arabidopsis Cvi Ecotype. *Frontiers in plant science* **9**, 322-322.

di Pietro, M., Vialaret, J., Li, G.-W., Hem, S., Prado, K., Rossignol, M., Maurel, C., and Santoni, V. (2013). Coordinated Post-translational Responses of Aquaporins to Abiotic and Nutritional Stimuli in Arabidopsis Roots. *Molecular & cellular proteomics* **12**, 3886-3897.

Engels., C., Kirkby., E., and White., P. (2012). Mineral nutrition, Yield and Source-sink relationship. *In* "Marschner's Mineral Nutrition of Higher Plants" (P. Marschner, ed.). Academic Press, United States of America.

Fageria, N. K. (2014). "Nitrogen Management in Crop Production," First edition/Ed. CRC Press, Boca Raton.

Fritz, C., Palacios-Rojas, N., Feil, R., and Stitt, M. (2006). Regulation of secondary metabolism by the carbon–nitrogen status in tobacco: nitrate inhibits large sectors of phenylpropanoid metabolism. *The Plant journal : for cell and molecular biology* **46**, 533-548.

Gao, L., Lu, Z., Ding, L., Xie, K., Wang, M., Ling, N., and Guo, S. (2020). Anatomically induced changes in rice leaf mesophyll conductance explain the variation in photosynthetic nitrogen use efficiency under contrasting nitrogen supply. *BMC plant biology* **20**, 527-527.

Gastal, F., and Lemaire, G. (2002). N uptake and distribution in crops: an agronomical and ecophysiological perspective. *Journal of experimental botany* **53**, 789-799.

Gastal., F., Lemaire., G., Durand., J.-L., and Louarn., G. (2015). Quantifying crop responses to nitrogen and avenues to improve nitrogen-use efficiency. *In* "Crop Physiology

Applications for Genetic Improvement and Agronomy" (V. O. S. a. D. F. Calderini, ed.), pp. 161-206. Academic Press, United Kingdom.

Hachez, C., Moshelion, M., Zelazny, E., Cavez, D., and Chaumont, F. (2006). Localization and quantification of plasma membrane aquaporin expression in maize primary root: a clue to understanding their role as cellular plumbers. *Plant molecular biology* **62**, 305-323.

Hachez, C., Veselov, D., Ye, Q., Reinhardt, H., Knipfer, T., Fricke, W., and Chaumont, F. (2012). Short-term control of maize cell and root water permeability through plasma membrane aquaporin isoforms. *Plant, cell and environment* **35**, 185-198.

Hilbert, D. W. (1990). Optimization of Plant Root: Shoot Ratios and Internal Nitrogen Concentration. *Annals of botany* **66**, 91-99.

Hirel, B., Le Gouis, J., Ney, B., and Gallais, A. (2007). challenge of improving nitrogen use efficiency in crop plants: towards a more central role for genetic variability and quantitative genetics within integrated approaches. *Journal of experimental botany* **58**, 2369-2387.

Hukin, D., Doering-Saad, C., Thomas, C. R., and Pritchard, J. (2002). Sensitivity of cell hydraulic conductivity to mercury is coincident with symplasmic isolation and expression of plasmalemma aquaporin genes in growing maize roots. *Planta* **215**, 1047-1056.

Ishaya, F., and Rasmussen, A. (2021). Adavances in understanding plant root growth regulators. *In* "Understanding and improving crop root function" (P. Gregory, ed.). Burleigh Dodd Science Publishing Limited, United Kingdom.

Kant, S., Bi, Y.-M., and Rothstein, S. J. (2011). Understanding plant response to nitrogen limitation for the improvement of crop nitrogen use efficiency. *Journal of experimental botany* **62**, 1499-1509.

Kramer-Walter, K. R., Bellingham, P. J., Millar, T. R., Smissen, R. D., Richardson, S. J., Laughlin, D. C., and Mommer, L. (2016). Root traits are multidimensional: specific root length is independent from root tissue density and the plant economic spectrum. *The Journal of ecology* **104**, 1299-1310.

Lee, S. H., Chung, G. C., and Steudle, E. (2005). Gating of aquaporins by low temperature in roots of chilling-sensitive cucumber and chilling-tolerant figleaf gourd. *Journal of experimental botany* **56**, 985-995.

Lehnebach, R., Beyer, R., Letort, V., and Heuret, P. (2018). The pipe model theory half a century on: a review. *Annals of botany* **121**, 1105-1105.

Lemaire, G., Khaity, M., Onillon, B., Allirand, J. M., Chartier, M., and Gosse, G. (1992). Dynamics of Accumulation and Partitioning of N in Leaves, Stems and Roots of Lucerne (Medicago sativa L.) in a Dense Canopy. *Annals of botany* **70**, 429-435.

Lynch, J., Marschner, P., and Rengel, Z. (2012). Effects of Internal and External Factors on Root Growth and Development. *In* "Marschner's Mineral Nutrition for Higher Plants" (P. Marschner, ed.). Accademic Press, United Kingdom.

Massignam, A. M., Chapman, S. C., Hammer, G. L., and Fukai, S. (2011). Effects of nitrogen supply on canopy development of maize and sunflower. *Crop and pasture science* **62**, 1045-1055.

Mu, X., Chen, Q., Chen, F., Yuan, L., and Mi, G. (2016). Within-Leaf Nitrogen Allocation in Adaptation to Low Nitrogen Supply in Maize during Grain-Filling Stage. *Frontiers in plant science* **7**, 699-699.

Nasielski, J., Earl, H., and Deen, B. (2019). Luxury vegetative nitrogen uptake in maize buffers grain yield under post-silking water and nitrogen stress: A mechanistic understanding. *Frontiers in plant science* **10**, 318-318.

Olmedo Pico, L. B., and Vyn, T. J. (2021). Dry Matter Gains in Maize Kernels Are Dependent on Their Nitrogen Accumulation Rates and Duration during Grain Filling. *Plants (Basel)* **10**, 1222.

Poorter, H., Niklas, K. J., Reich, P. B., Oleksyn, J., Poot, P., and Mommer, L. (2012). Biomass allocation to leaves, stems and roots: meta-analyses of interspecific variation and environmental control. *The New phytologist* **193**, 30-50.

Ratjen, A. M., Lemaire, G., Kage, H., Plénet, D., and Justes, E. (2018). Key variables for simulating leaf area and N status: Biomass based relations versus phenology driven approaches. *European journal of agronomy* **100**, 110-117.

Sakr, S., Wang, M., Dédaldéchamp, F., Perez-Garcia, M.-D., Ogé, L., Hamama, L., and Atanassova, R. (2018). The Sugar-Signaling Hub: Overview of Regulators and Interaction with the Hormonal and Metabolic Network. *International journal of molecular sciences* **19**, 2506.

Schlüter, U. U., Mascher, M. M., Colmsee, C. C., Scholz, U. U., Bräutigam, A. A., Fahnenstich, H. H., and Sonnewald, U. U. (2012). Maize Source Leaf Adaptation to Nitrogen Deficiency Affects Not Only Nitrogen and Carbon Metabolism But Also Control of Phosphate Homeostasis1[W][OA]. *Plant physiology (Bethesda)* **160**, 1384-1406.

Schneider, H. M., Yang, J. T., Brown, K. M., and Lynch, J. P. (2021). Nodal root diameter and node number in maize (Zea mays L.) interact to influence plant growth under nitrogen stress. *Plant direct* **5**, e00310-n/a.

Sudhakar, P., Latha, P., and Reddy, P. (2016). "Phenotyping Crop Plants for Physiological and Biochemical Traits."

Sun, X., Chen, H., Wang, P., Chen, F., Yuan, L., and Mi, G. (2020). Low nitrogen induces root elongation via auxin-induced acid growth and auxin-regulated target of rapamycin (TOR) pathway in maize. *Journal of plant physiology* **254**, 153281.

Ueda, Y., Konishi, M., and Yanagisawa, S. (2017). Molecular basis of the nitrogen response in plants. *Soil Science and Plant Nutrition* **63**, 329-341.

Urban, A., Rogowski, P., Wasilewska-Dębowska, W., and Romanowska, E. (2021). Understanding Maize Response to Nitrogen Limitation in Different Light Conditions for the Improvement of Photosynthesis. *Plants (Basel)* **10**, 1932.

Vega, A., O'Brien, J. A., and Gutiérrez, R. A. (2019). Nitrate and hormonal signaling crosstalk for plant growth and development. *Current opinion in plant biology* **52**, 155-163.

Vidal, E. A., Alvarez, J. M., Araus, V., Riveras, E., Brooks, M. D., Krouk, G., Ruffel, S., Lejay, L., Crawford, N. M., Coruzzi, G. M., and Gutiérrez, R. A. (2020). Nitrate in 2020: Thirty years from transport to signaling networks. *The Plant cell* **32**, 2094-2119.

Vile, D., Garnier, E., Shipley, B., Laurent, G., Navas, M., Roumet, C., Lavorel, S., Diaz, S., Hodgson, J. G., Lloret, F., Midgley, G. F., Poorter, H., Rutherford, M. C., Wilson, P. J., and Wright, I. J. (2005). Specific leaf area and dry matter content estimate thickness in laminar leaves. *Annals of botany* **96**, 1129-1136.

Vos, J., Putten, P. E. L. v. d., and Birch, C. J. (2005). Effect of nitrogen supply on leaf appearance, leaf growth, leaf nitrogen economy and photosynthetic capacity in maize (Zea mays L.). *Field crops research* **93**, 64-73.

Wang, Y., Zhao, Z., Liu, F., Sun, L., and Hao, F. (2020). Versatile Roles of Aquaporins in Plant Growth and Development. *International journal of molecular sciences* **21**, 9485.

Westgate, M. E., and Grant, D. L. T. (1989). Water deficits and reproduction in maize. Response of the reproductive tissue to water deficits at anthesis and mid-grain fill. *Plant physiology (Bethesda)* **91**, 862-867.

Xiaoli, N., Hanmi, Z., Xiukang, W., Tiantian, H., Puyu, F., Ting, L., Na, Z., and Dongxue, Y. (2020). Changes in root hydraulic conductance in relation to the overall growth response of maize seedlings to partial root-zone nitrogen application. *Agricultural water management* **229**, 105839.

Chapter 5

The effect of nitrogen and auxin on the emergence and number of maize brace roots and shoot response.

5.1. Abstract

Despite the important of brace roots in providing anchorage and resource capture in maize, not much is known about the effect of nitrogen rates and auxin treatment on the emergence and number of brace roots as well as shoot response to nitrogen treatment. This gap in knowledge may also be associated with the lack of known effective method of administering hormones to intact adult maize plants growing on soil.

To address this gap in knowledge, maize plants were grown on low or high nitrogen in 5 litre pots and rhizoboxes (24 and 18 plants respectively). The containers were filled with sugar beet topsoil in the glasshouse. The potted plants were used to identify for the most effective method of administering hormones using gibberellin stimulation of shoot growth as an indication of successful infiltration. The successful method was then used to apply auxin to rhizobox plants grown to an 8 fully expanded leaf stage. Seven weeks after planting, the test plants were treated with gibberellins, either dissolved in water or mixed with lanoline. Some plants were scratched and applied with water while others were left untouched. The low nitrogen plants treated with 100 µM gibberellin grew taller than other plants after two weeks of treatment. Also, auxin was dissolved in ethanol and diluted with water to 100 µM and applied to the exposed scratched tissues on the stem node. While half of the plants were treated with water only containing 3% ethanol. Weekly measurement taken included plant height, leaf number, leaf length and width as well as chlorophyll levels.

Auxin significantly increased the number of brace root whorls and total brace root number in both low and high N plants as compared to the controls. For the shoot response, the plant heights, leaf area and chlorophyll levels appeared only temporally affected within a week of auxin treatment in both low and high N plants. Only time significant differences were found to have an impact on plant height, leaf area, and leaf area duration while nitrogen balance index (NBI), chlorophyll level significantly increased and decreased variably with time. The percentage dry matter of the shoot was not different across treatments.

In conclusion, exogenous application of auxin (IAA) significantly increased brace roots emergence and number in maize. Further research involving 3 nitrogen rates as no nitrogen, low nitrogen and high nitrogen is required to investigate any interaction between nitrogen and auxin on maize brace root. This could be tested in at least in 2 contrasting maize cultivars and can be extended to involve water as a factor in the factorial design. Understanding this interaction will be important for the manipulation of key genes involved in the nitrogen and auxin pathway for the purpose of optimization of the function of brace root and the root system architecture in maize.

5.2. Introduction:

At the physiological level, phytohormones are the main internal regulatory signals for root system architecture (Vega et al., 2019; Wu et al., 2022). Auxin, cytokinin, gibberellins, ethylene, and abscisic acids are the classical hormones that interact to control the development of root system architecture (Wu et al., 2022; Zhang et al., 2020). Auxin plays a critical role in many developmental activities including cell division, cell extension, cell differentiation, root formation, and tropism (Zhang et al., 2020), and promotes adventitious and lateral root initiation, while decreasing root elongation (Steffens and Rasmussen, 2016). Polar auxin transport via the PIN-FORMED family of auxin efflux carriers is required for the growth of nodal roots in seedlings and adult plants (Steffens and Rasmussen, 2016; Yu et al., 2015), especially in the process of initiation and development of primordia (Liu et al., 2010).

Auxin distribution among plants tissues is controlled by biosynthesis, inactivation, and transport pathways (Ishaya and Rasmussen, 2021). Multiple pathways were proposed to be involved in the biosynthesis of auxin, but the main pathway is the dual step TAA/YUC (Multigene families) route (Blakeslee et al., 2019). Here, tryptophan aminotransferase catalyses YUCCA (YUC), and flavin-dependent monooxygenases to produce auxin in the form of Indole Acetic Acid (IAA) from tryptophan (Blakeslee et al., 2019; Zhang et al., 2022). IAA can also be released from IAA conjugate by hydrolytic division of IAA-amino acids, IAA-sugar and IAA-methyl ester (Zhao, 2010). The location for auxin biosynthesis is still a debate, and different plant species may have unique means of modifying and optimising their auxin production despite shared evolutionary conserved core processes (Zhao, 2010). The polar auxin transport is mediated by three class of proteins: The AUX1/LAX family, the PIN-FORMED (PIN) family, and the P-glycoproteins ABC sub family proteins (Li et al., 2018). The PIN and PGP- mediated auxin transport in maize have been investigated in relation to cellular differentiation during maize embryo genesis, development of endosperm and branching (Li et al., 2018). However, the relationship between polar auxin travel in maize in terms differentially affecting of root type and root system architecture needs more clarity (Li et al., 2018).

Maize root systems include brace roots which are adventitious roots (nodal/shoot-borne roots) that emerge from the stem nodes. Some grow into the soil while others remain aerial all through the life cycle of maize (Sparks, 2023). Brace roots are only present in the Andropogea (maize, sorghum, and sugarcane) and the Paniceae (fox-tail millet, millet, and pearl millet) tribes of the Poaceae family (Hostetler et al., 2021). The brace roots that enter the soil support maize stalks (anchorage) while both underground and aerial brace roots contribute to resource capture (Sparks, 2023). The number of stem nodes in which brace roots emerges relates to the phase of the shoot meristem, implying that

brace roots continue to emerge from successive upper nodes while the lower nodes grow into the soil as the shoot continues to actively grow (Hostetler et al., 2021; Sparks, 2023). The induction and initiation of brace root may be like all shoot-borne roots (Sparks, 2023). It was proposed that brace roots play a distinct role to complement the inabilities of the subterranean roots (Sparks, 2023). The present challenge for researchers is to differentiate between developmental flaws associated with whole plant physiological response and changes in brace roots development due to a direct regulation of root development (Sparks, 2023).

Exogenous application of auxin was reported to lead to the initiation of an adventitious root in Arabidopsis through the coordinated efforts of AUX1 and PIN1, after forming IAA gradient needed for its emergence (Della Rovere et al., 2013; Li et al., 2018). On the same matter, the transient equilibrium of the ARFs was said to regulate adventitious root formation in Arabidopsis (Pacurar et al., 2014). Application of IAA on tomato (*Solanum lycopersicon*) cuttings induced the formation of adventitious roots primordia and promoted elongation (Guan et al., 2019). The IAA treatment was reported to enhance the rate of primordia initiation, showing the involvement of IAA in the inducement of founder cells (Guan et al., 2019). Auxin was also reportedly found to induce adventitious roots in apple (*Malus domestica*) cuttings with success dependence on concentration and type of auxin used (de Klerk et al., 1999). Nitric oxide was reported to be involved in auxin-induced development of adventitious roots in cucumber (*Cucumis Sativus*) (Lanteri et al., 2006). The success of the emergence of adventitious roots from cuttings were said to be affected to some extend by the carbon and nitrogen content of the mother plants (Bellini et al., 2014; da Costa et al., 2013).

Nitrogen availability and form were said to impact on auxin biosynthesis, transport, and transduction (Asim et al., 2020; Yu et al., 2014). As such, the nitrate and ammonium ratio used for growing plants may alter polar auxin transport (Asim et al., 2020). Nitrogen sources from soil are taken up by plant roots through influx systems commonly known as transporters, and these transporters function in a concentration dependent manner (Dechorgnat et al., 2018; Dechorgnat et al., 2019; White, 2012), The nitrate transporter, *NRT 1.1* is a dual affinity transporter involved in nitrate uptake at both low and high concentration and transporting auxin (Ishaya and Rasmussen, 2021; Kant, 2018; O'Brien et al., 2016; Shahzad and Amtmann, 2017). NRT1.1 undergoes phosphorylation to switch from a low affinity (LAT) to a high affinity (HAT) mode during low nitrogen availability (Ishaya and Rasmussen, 2021). It is the phosphorylated NRT1.1 that transports auxin as an efflux carrier to inhibit the formation of lateral roots during low nitrogen conditions (Kiba et al., 2011). While moderate nitrate supply stimulates growth of primary and lateral roots, higher concentration represses root growth (Jia and von Wirén, 2020). For ammonium, the ammonium transporter *AtAMT1.1* found to be involved in lateral roots

during low nitrogen (Asim et al., 2020). The direct impact of nitrogen on the initiation and emergence of adventitious roots requires investigation (Bellini et al., 2014).

Exploring the developmental pattern of roots is important to develop the optimization of the root system for improved nitrogen use efficiency and sustainable of agriculture. No study was reported in the literature regarding the interaction of nitrogen and auxin on the emergence and number of brace roots in maize. Also, it is unclear how to effectively treat auxin to intact adult maize plants growing on soil. This is because most studies applied auxin on seedlings in growth medium (Blizard, 2020; Hetz et al., 1996; Martínez-de la Cruz et al., 2015), nutrient solution (Alarcón et al., 2013; Blizard, 2020; Tian et al., 2008; Yu et al., 2015), lanolin paste (Blizard, 2020; Kaldenhoff and Iino, 1997) and use of needle and thread (Blizard, 2020). Therefore, exploring how hormones can be effectively applied to adult maize plant growing on soil is important for this and future studies.

Aim and Objective:

To explore effective methods of exogenous hormone application in maize, and to understand the influence of auxin (IAA) on maize roots and shoots as it relates to availability of nitrogen.

Hypothesis:

1. Exogenous application of gibberellins using water as a carrier will be more effective because gibberellins are water soluble, and water is transported across cells and tissues of plant. The effect of gibberellins would rapidly elongate the stems of low N plants as compared to and high N plants.

2. Exogenous application of l auxin (IAA) will reduce shoot growth and nitrogen content of low N maize and increase the number of brace roots and brace root whorls in low N plants as compared to high N plants.

5.3. Methods

The experiment was carried out to explore effective methods of exogenous hormone application in maize, and to understand the influence of auxin (IAA) on maize roots and shoots as it relates to nutrient availability.

5.3.1. Gibberellins (GA) trial (Testing for the effective method of hormone application in maize):

5.3.1.1. Experimental design

The GA experiment was a 2 x 12 completely randomised design arranged in 3 columns and 8 rows (Figure 5.1). The main factors were two N levels comprised of adequate N (high N 4 mM) and sub optimum N (low N 0.3 mM). For the hormone treatment, stock solutions were made to 10 mM of GA dissolved in water. The GA solution was diluted to treatment concentration of 50 µM and 100 µM in water or lanoline paste. Two plants were selected at random from each nitrogen level and the stems were gently scratched with sandpaper and treated with the GA of various concentration. A control group were treated with just water and a final set were untouched (not scratching and no treatment) to determine if scratching had an effect.

5.1.1.1. Plant material and growth conditions

A total of twenty-four 5 L plastic pots were filled with sugar beet topsoil (thereafter refers to as 'soil'). The soil pH was 8.2 and contained 5.7% organic matter, 0.29% total nitrogen (Dumas technique) as measured by The British Sugar Company Ltd. The filled containers were watered to field capacity and allowed to drain.

Maize seeds (B73) were sown directly at 1 cm depth. The plants were fed twice a week with the nutrients as described in Chapter 2. Germination was not even so some were resown as a second batch. Three weeks after germination, each plant initial shoot measurements were taken (and taken weekly thereafter) including leaf number, plant height, and Dualex measurements conducted on the 4^{th} and 5^{th} leaves.

Scratched and 50 µm of GA in lanolin was applied	Scratched and 100 µm of GA in water was applied	Unscratched and nothing was applied
Unscratched and nothing was applied	Scratched and only water (0 µm GA) was applied	Scratched and only water (0 µm GA) was applied
Scratched and 100 µm of GA in lanolin was applied	Scratched and 50 µm of GA in water was applied	Scratched and 50 µm in water was applied
Scratched and 100 µm of GA water was applied	Scratched and 50 µm of GA in lanolin was applied	Scratched and 100 µm of GA in water was applied
Scratched and only water (0 µm GA) was applied	Scratched and 50 µm of GA in lanolin was applied	Scratched and only water (0 µm GA) was applied
Scratched and 50 µm of GA in water was applied	Scratched and 100 µm of GA in lanolin was applied	Scratched and 50 µm of GA was applied
Unscratched and nothing was applied	Unscratched and nothing was applied	Scratched and 100 µm of GA in lanolin
Scratched and 50 µm of GA in water was applied	Scratched and 100 µm of GA in lanoline was applied	Scratched and 100 µm of GA in water was applied

Figure 5.1. Experimental design for gibberellin (GA) treatment. The dark green cells ▭ are plants grown on high nitrogen background, while the light green cells▭ are plants grown on low nitrogen background. From each category of nitrogen background, two plants each were treated with 50nm or 100um GA using lanolin as carrier, 50um or 100um GA using water as a carrier. Also, two plants each from each category of nitrogen background were scratched and applied with only water (no GA) while two plants each from low and high N plants were left untouched. Treatment was given at the same day as close as possible.

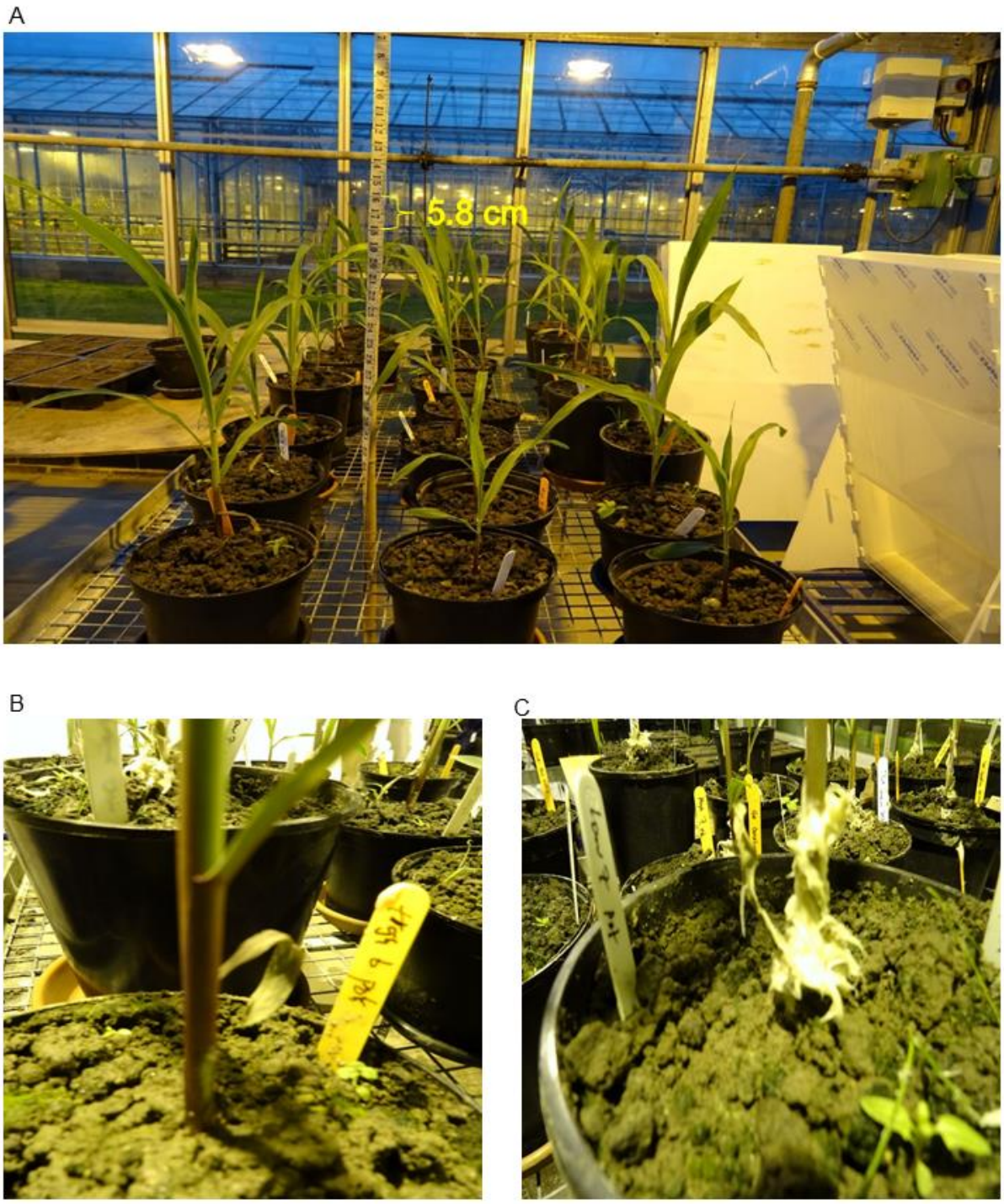


Figure 5.2. Plant height a week after GA treatment (A). The plants with orange label are the high N plants while those with white are the low N plants. The low plants treated with 100 µM GA solution increased in size at the rate that was greater than others and so was adopted as the most effective method of exogenous application on maize for immediate effect. (B) shows the degree of scratching, while (C) shows how the lanolin paste was applied on the scratched stem.

5.3.1.2. Shoot and roots measurement

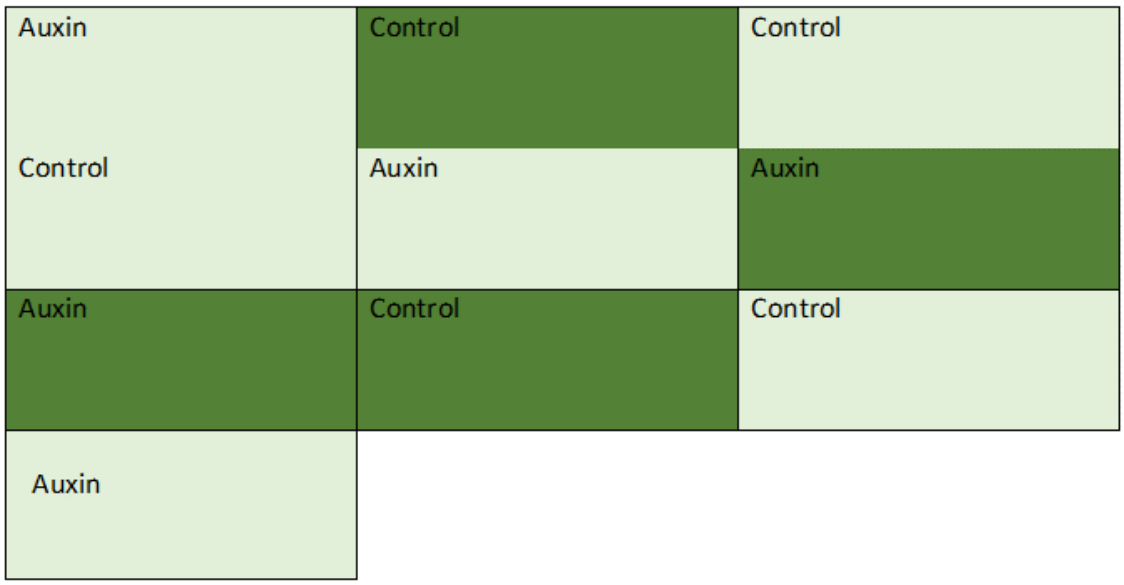
The plant height was used as an indicator of whether GA had entered the plants. This is because GA facilitates stem elongation by degradation of DELLA proteins which are growth inhibitors (Mu et al., 2018; Wang et al., 2020). Because of uneven germination, the percentage change in plant height was determined from the plant height after one week of treatment (Figure 5.2.) and there-after. The leaf number, length and width were also measured weekly. At the end of the experiment number of brace roots and whorls were counted and photos taken.

5.3.2. Auxin trial:

5.3.2.1. Experimental design for auxin trial

The experimental design for the auxin-maize trial was a complete randomised design consisting of two levels of nitrogen (high and low nitrogen) grown in two batches in the interval of one month (Figure 5.3). IAA was dissolved in ethanol (at 3 %) and then distilled water (97 %) was added to make up the stocks at 100 mM final concentration of IAA. This was diluted to 10 mM IAA for treatments. IAA was previously used at 10 µM to induce the emergence of adventitious roots from tomato cuttings (Guan et al., 2019). However, it was also previously reported that auxin may be applied for several days or weeks at lower concentration (micromolar range), or for several seconds or minutes at high concentration (millimolar range) to induce the proliferation of adventitious roots from apple cuttings (de Klerk et al., 1999). For the control, 3 % ethanol was mixed with 97 % water for treatment. The treatments were applied at 56 days after planting (two batches with different germination dates). From 9th Nov – 27th January (batch 1) and a month later for batch 2. The reason for batch 2 is because of poor germination rate experienced with batch 1. To apply treatments, the stems were sandpapered (as Part 1) above node 3 and 1 ml of either 10 mM IAA or control solution (water + 3% ethanol) was slowly applied (approximately 2 minutes) to the scratched area to avoid runoff and maximise uptake. The total number of replicates were 4 and 5 for control or IAA treatments respectively. At the time of application, the plants were at 8-9 fully expanded leaves.

A



B



Figure 5.3. Experimental design and layout of auxin trial. The dark green cells are the plants grown on high nitrogen background, while the light green cells are the plants grown on low nitrogen background in rhizoboxes located in the glasshouse. The first batch are (A) while (B) represents the second batch. Plants were scratched at the second stem node above the ground and were either treated with 1 ml of 10 mM IAA (Auxin) or treated with 1ml of water containing 3% ethanol (Control). All treatment took place the same day when each batch of crops reached 8 fully expanded leaves.

5.3.2.2. Plant material and growth conditions

The rhizoboxes were filled with 85-90 kg of sugar beet topsoil (thereafter refers to as 'soil') and watered to field capacity. B73 seeds were sown as described in Chapter 2. The seeds were allowed to germinate, and fertiliser was provided twice a week (as in part 1 and a detailed description of nutrient solutions in Chapter 2). There were high N and low N grown plants as described in Chapter 2. Glasshouse conditions day/night temperatures 20/15 degrees. Daylength was 14 hours.



Figure 5.4. Growth condition for the hormone trial. The full view of the experimental site showing both the GA (pot plants) and the first batch of plants for IAA application (rhizobox plants) growing simultaneously. All plants were either grown on high or low nitrogen background prior to GA or IAA treatment. The high N plants has the orange colour label while the low N plants had the white label. The nutrient treatment in the form of watering continued twice a week on till harvest. The glasshouse had 14 hrs/10 hrs day/night cycle with average temperatures of 20°C /15°C. Shoot measurement including photosynthetic parameters, chlorophyll levels were measured using Dualex, leaf length and width for leaf area and plant heights were determined using a measuring tape. The weekly shoot measurement started when the plants were at 4-5 leaves stage and lasted till harvest.

A



B



C



Figure 5.5. Degree of scratching the lower stem node for IAA application. The root before scratching (A), The degree of scratching (B), and what the stem nodes looked like after 2 weeks of IAA applications (C).

5.3.2.3. Shoot Measurements

Weekly shoot measurement taken included: The use of Dualex to determine the nitrogen balance index, chlorophyll, and flavonoids from 4 weeks after planting. Plant heights were measured with a tape from the stem base above the soil to the tip of the plants. The leaf length and leaf width were determined, and Leaves were also counted.

5.3.2.4. Final harvest

The shoots were cut above the brace roots and laid on black plastic on the floor with measuring tape and photographed using camera mounted on a tripod stand. Thereafter, each shoot was cut into two and placed in a measuring container mounted on a scale for fresh weight. The cut plant parts were string tied around the stems and were hung from the rafter in the glasshouse to dry (for dry weight). The top panel window of the rhizoboxes were removed and photographed with a measuring tape beside them. Then the roots were cut below soil level and washed before photographing the brace roots section with a ruler beside them. The number of brace roots were counted in each whorl starting at first whorl above soil (whorl one), then next whorl (whorl 2) then next whorl (whorl 3) and so on. The roots were then grouped by treatment in plastic bags for Laser Ablation Tomography.

5.3.3. Data Analysis

Data were imported into R statistical software (R version 4.2.0 (2022-04-22 ucrt)) and were analysed using two-way analysis of variance (2-way ANOVA) and Tukeys post hoc test.

5.4. Results

To determine which GA treatment method would work, gibberellin (GA) was applied either mixed in lanolin or in water at 50 µM or 100 µM concentration to low and high N maize plants grown in pots in the glasshouse. The plant height and leaf area were obtained weekly as an indicator of treatment effect. The percentage increase in plant height after one week of treatment was highest in the low N plants treated with 100 µM GA in water. Therefore, water was employed as a carrier to convey 100 µM of IAA into low and high N maize plants grown in rhizoboxes after they developed 7-8 fully expanded leaves in the glasshouse. Plant height and leaf length and width for leaf area were recorded weekly until the plants began tasselling, making the end of the vegetative stage and onset of reproductive stage.

5.4.1. Effects of gibberellins on maize heights and brace root number:

5.4.1.1. The percentage increase in plant height was influenced by treatment in low N plants:

The low N plants treated with 100 µM GA solution significantly increased in height as compared to all low N plants that received different treatments and the control plants ($P<0.01$, Figure 5.6.A). While there were no significant differences in the GA treatment method in high N plants (Figure 5.6. B).

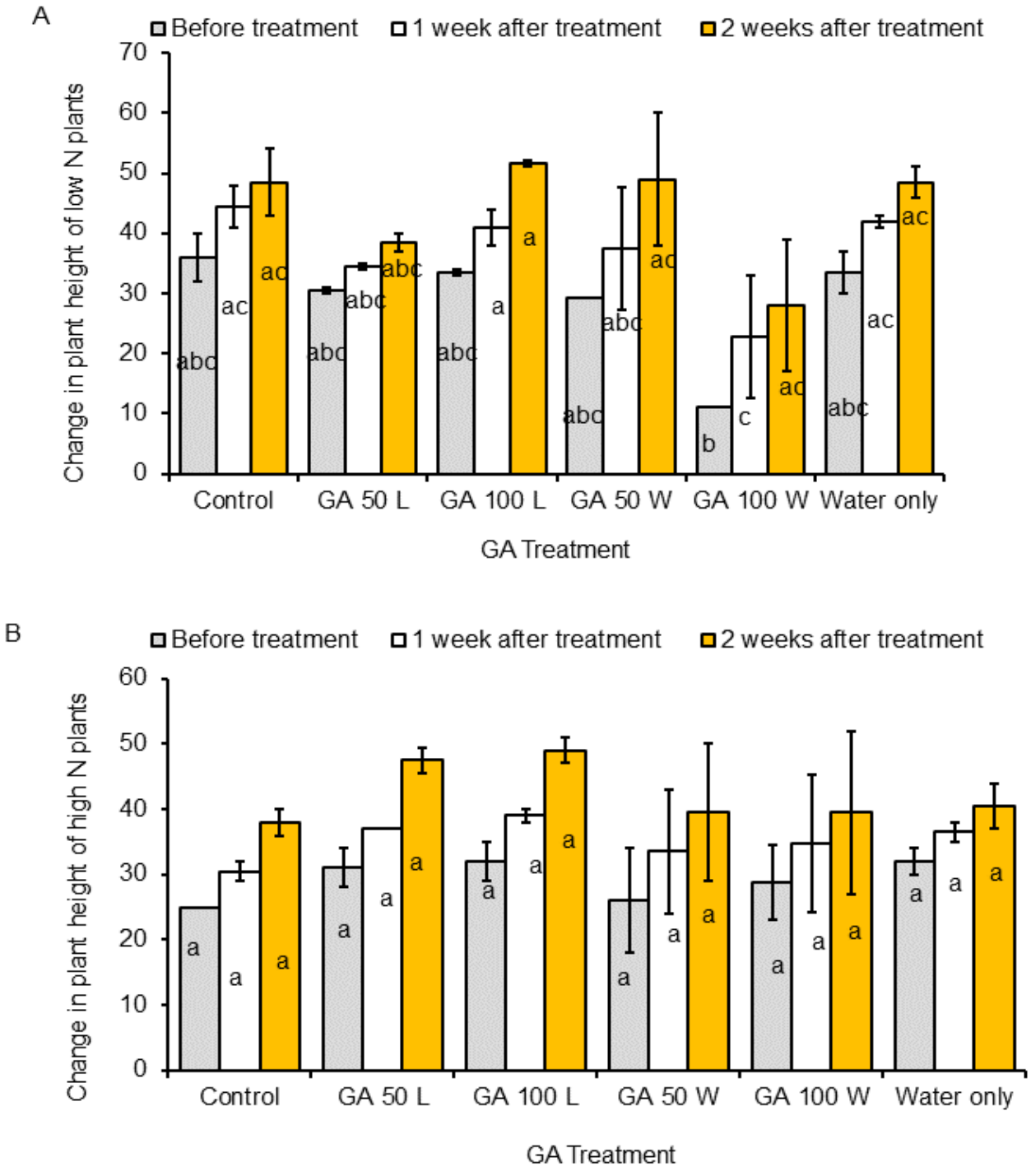


Figure 5.6. The percentage increase in plant height was influence by treatment method in low N plants. Plants were either grown on low nitrogen background (A) or high nitrogen background (B) up to 49 days post germination. GA 50 L, GA100 L, GA 50 W, GA 100 W, represents GA 50 µM or 100 µM dissolved in lanolin (L) or water(W) respectively. Water only are plants that were scratched and treated with water while the controls were the untouched plants. All bars are averages (n=2 plants each) while error bars are standard error of the mean. Different lowercase letters denotes significant differences at 1% level.

5.4.1.2. The number of brace roots per whorls were not influenced by nitrogen background or GA treatment:

There was no significant difference between the number of brace roots of high and low nitrogen GA treated plants (P=0.18, Figure 5.7) or based on the treatment method employed (P= 0.7). However, the number of brace roots in the first whorl was significantly more than the third whorl in Low nitrogen GA treated plants (P=0.05). While the high N plant tended to have more brace root number in the first whorl as compared to third whorl (P=0.07).

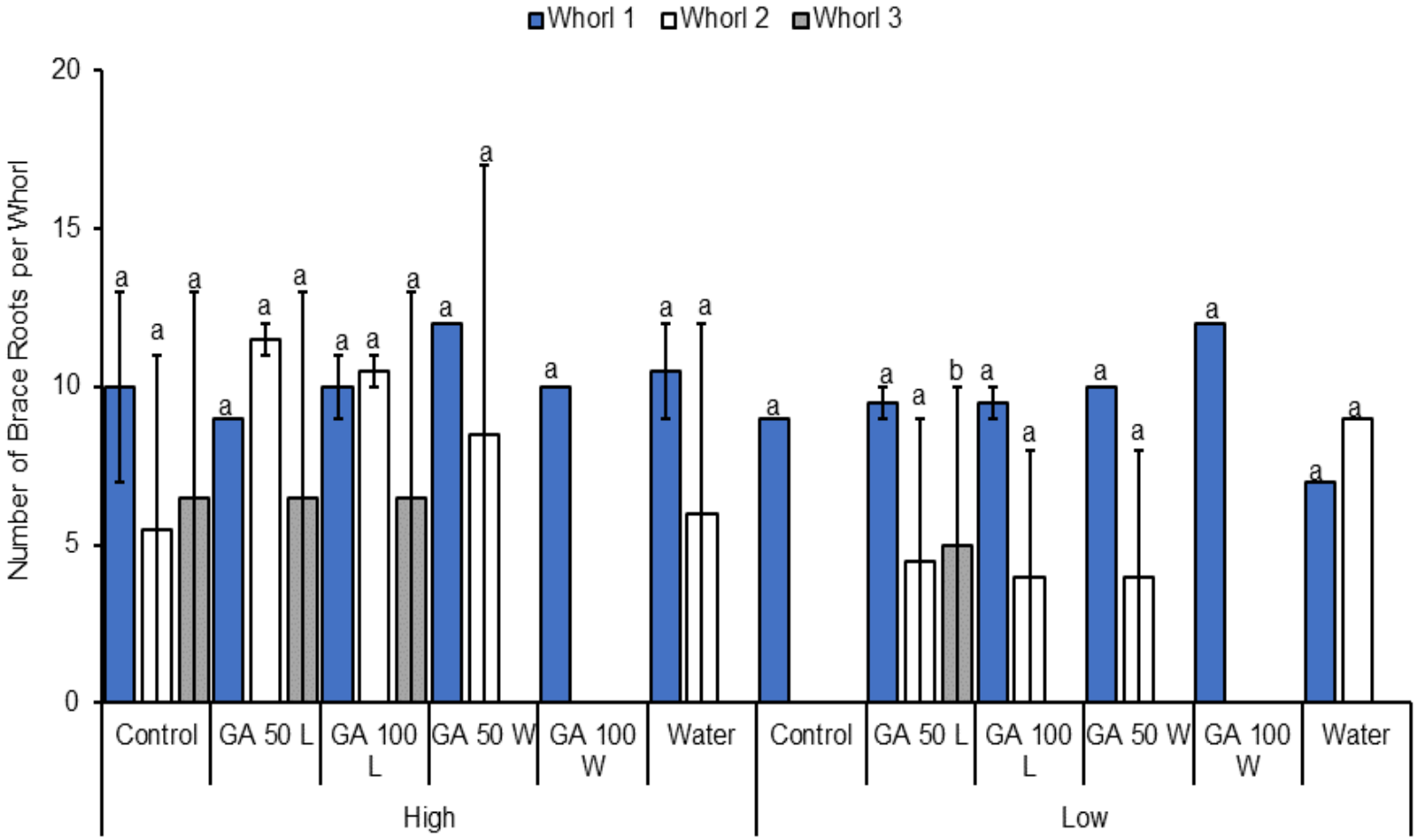


Figure 5.7. The number of brace roots per whorls was not influenced by nitrogen background or treatment application method. Plants were either grown on adequate (High) or limited (Low) nitrogen background for up to 49 days post germination. GA 50 L ,GA 100 L, GA 50 W, GA 100 W denotes GA 50 or 100 µM concentration dissolved in lanolin (L) or (W) water, respectively. Water are plant that were scratch and treated with only water while the controls are the untouched plants. Bars are averages (n=2 plant each), while error bars are standard error of the mean. Different lowercase denotes statistical difference at 5%. There was no significant interaction between nitrogen background and treatment method.

5.4.2. Effects of auxin on maize shoot and brace root number:

5.4.2.1. Plant height was not influenced by nitrogen background or auxin treatment:

There was no significant difference between the plant height of low and high N plants (P=0.3, Figure 5.8.A.), and no significant difference between auxin treated plants and controls (P=0.8) irrespective of nitrogen background. No interaction was significant but as the plants were growing over time, heights were significantly different at each successive time point in all plants (P=0.001).

5.4.2.2. Leaf area was influenced by nitrogen background and slightly by auxin treatment:

The control plants in low nitrogen background had higher leaf area as compared to those on high nitrogen background (P=0.02, Figure 5.8.B). And unsurprisingly, the plants grew more leaves over time irrespective of treatments and nitrogen background. So, leaf area increase was significantly different at each sampling time (P=0.001).

5.4.2.3. Chlorophyll related parameters were not influenced by nitrogen background and auxin treatment:

There was no significant difference between the nitrogen balance index (NBI), chlorophyll level and flavonoid levels of high and low N plants (P=0.4, P=0.5, and P=0.5, respectively; Figure 5.9 A, B&C). Auxin treatment did not significantly change the nitrogen balance index and flavonoid levels in all plants irrespective of nitrogen background (P=0.2, P=0.6, respectively), but auxin treatment led to a reduction in the chlorophyll level of high and low N plants as compared to the control plants (P=0.05, Figure 5.9.B). Both nitrogen balance index and chlorophyll levels of all plants increased with time (P=0.01), however, time was not influential on flavonoid levels of all plants (P=0.06).

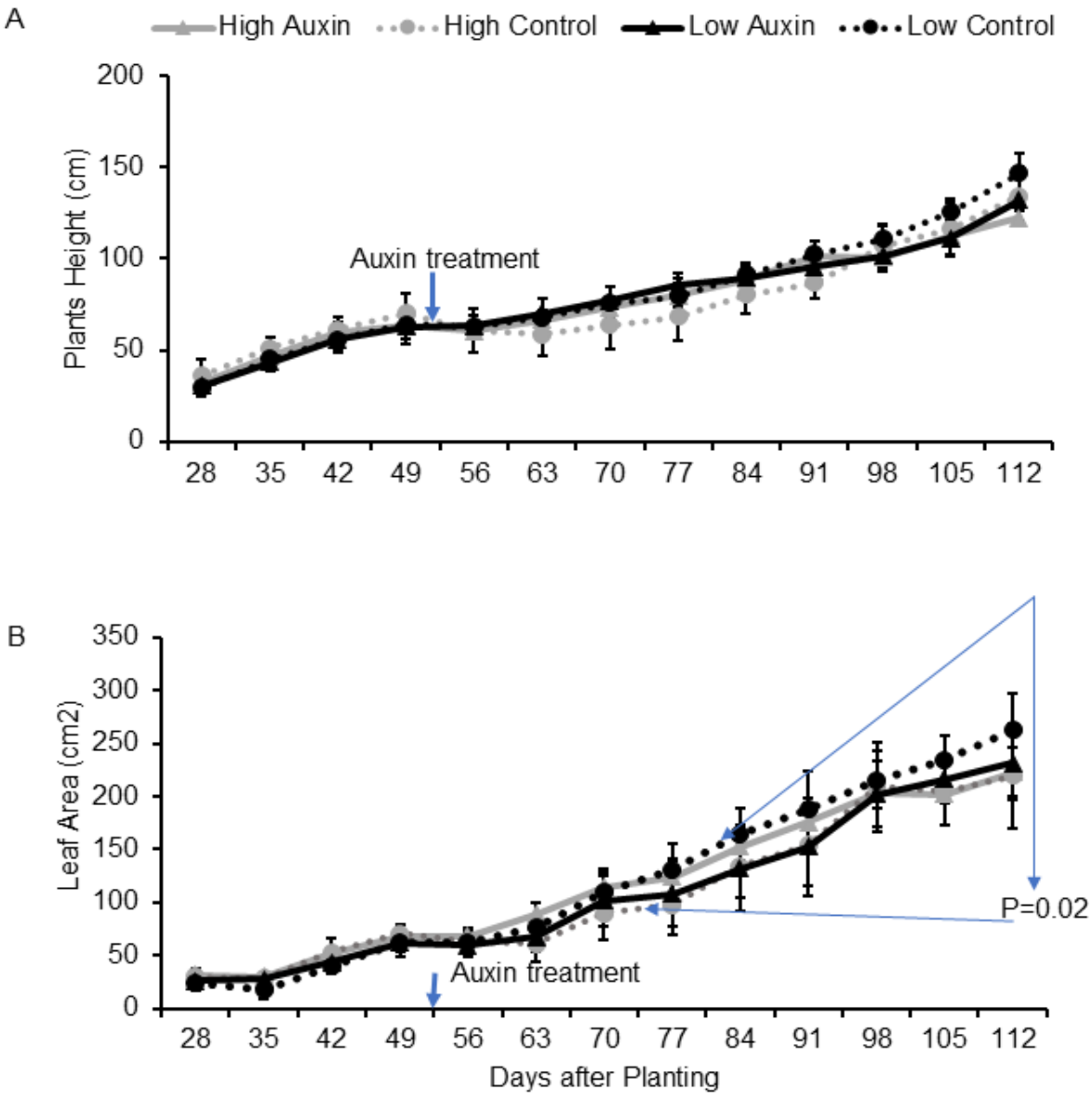


Figure 5.8.Auxin did not influence maize shoot growth irrespective of nitrogen background. High auxin and Low auxin are plants treated with 100ul of IAA solution while High controls and Low controls are the plants treated with water only as control from high and low nitrogen background respectively. Data points are averages (n=5 for plants treated with auxin from each nitrogen background and n=4 for the plants treated with water) only the time point was statistically different from another (P=0.001) for (A) Plant Height, (B) Leaf Area. Error bars are the standard error of the mean.

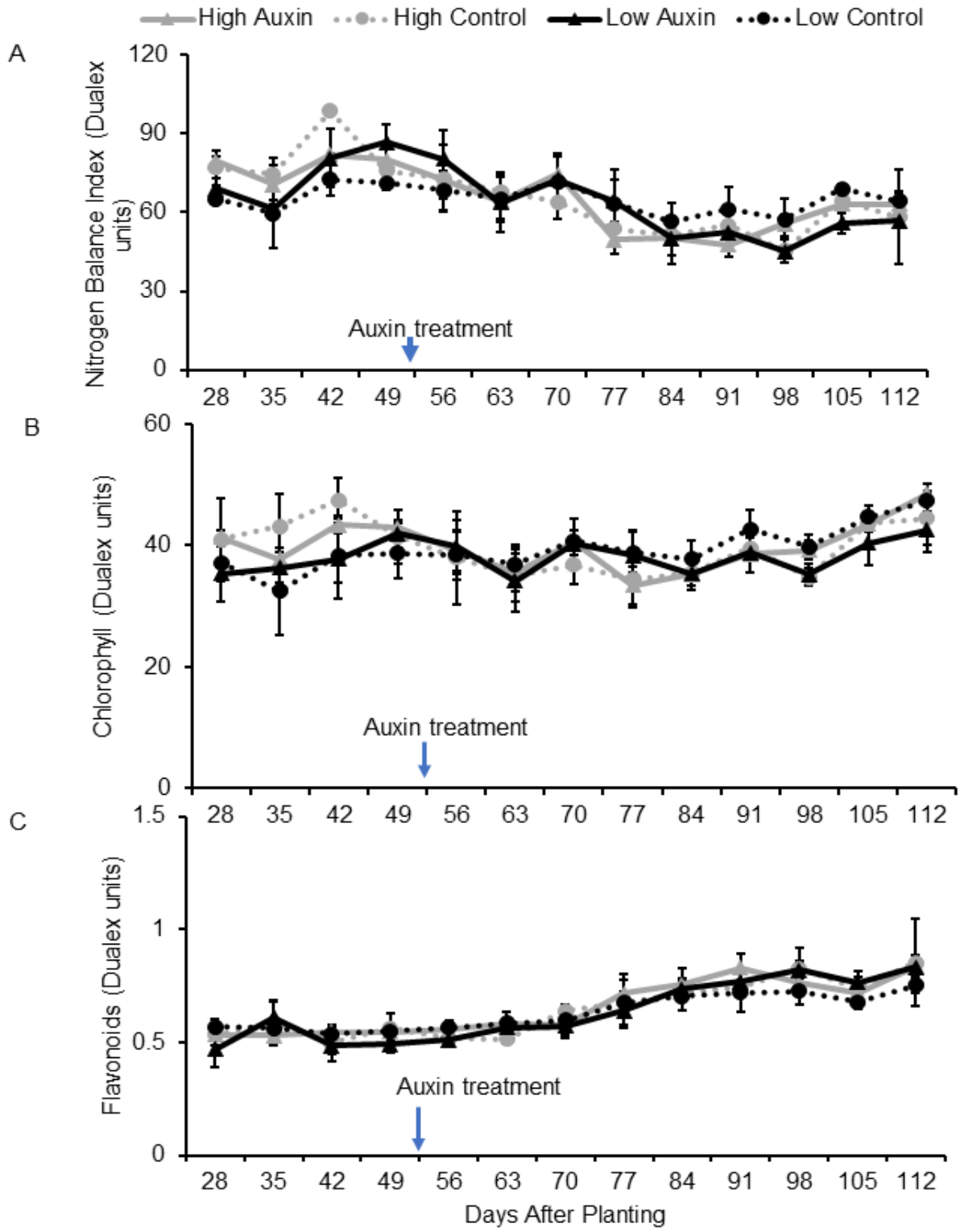


Figure 5.9. Nitrogen background and auxin did not significantly influence nitrogen balance index and flavonoid levels, but auxin changed chlorophyll level over time. High auxin and Low auxin are plants treated with 100ul of IAA solution while High controls and Low controls are the plants treated with water only as control from high and low nitrogen background respectively. Data points are averages (n=5 for plants treated with auxin from each nitrogen background and n=4 for the plants treated with water) only. Each time point (main effect) statistically varied (P=0.001) for nitrogen balance index and chlorophyll level but not flavonoid. Error bars are standard error of the mean.

5.4.2.4. Leaf area duration was not influenced by nitrogen background or auxin treatment.

There was no significant difference between the leaf area duration between high N and low N plants (P=0.2, Figure 5.9.A). Auxin treatment did not increase or reduce the leaf area duration of low or high nitrogen plants (P=0.9). However, the leaf area duration of all plants significantly increased over time (P=0.001).

5.4.2.5. Growth condition and auxin treatment did not change the percentage dry matter content of maize shoots.

The percentage dry matter contents of the shoots were not statistically different from each other irrespective of nitrogen background or treatment received (P=0.8, and P=0.6, for nitrogen background and treatment respectively; Figure 5.9.B).

5.4.2.6. Auxin treatment increased the number and whorls of maize brace roots irrespective of nitrogen background.

The exogenous application of auxin led to both the high and low N plants to increase brace root whorls (P<0.001 each, Figure 5.10), and brace root number (P=0.02, Figure 5.10.). The increase in brace root number was up to 3.4-fold and 1.4-fold in auxin treated high and low nitrogen plants respectively, as compared to the control plants in the same nitrogen background.

5.4.2.7. The 3 dimensional quantitative and qualitative analysis of brace root anatomy

The purpose of the 3-Dimensional view of brace roots is to understand how the internal tissues are organized within the cortex. It is unclear if differences exist between the maize crown and brace roots in terms of the arrangement of internal tissues, since both the crown and brace roots are adventitious roots. The cross-sectional image showed that the arrangement of the internal tissues in the cortex of maize brace roots is like that of maize crown roots. The image showed clearly defined features to include the endodermis, cortex, endodermis, xylem, phloem, metaxylem. However, an unnamed metaxylem-like feature randomly distributed in the centre of the endodermis is only unique to brace root (Figure 5.11.).

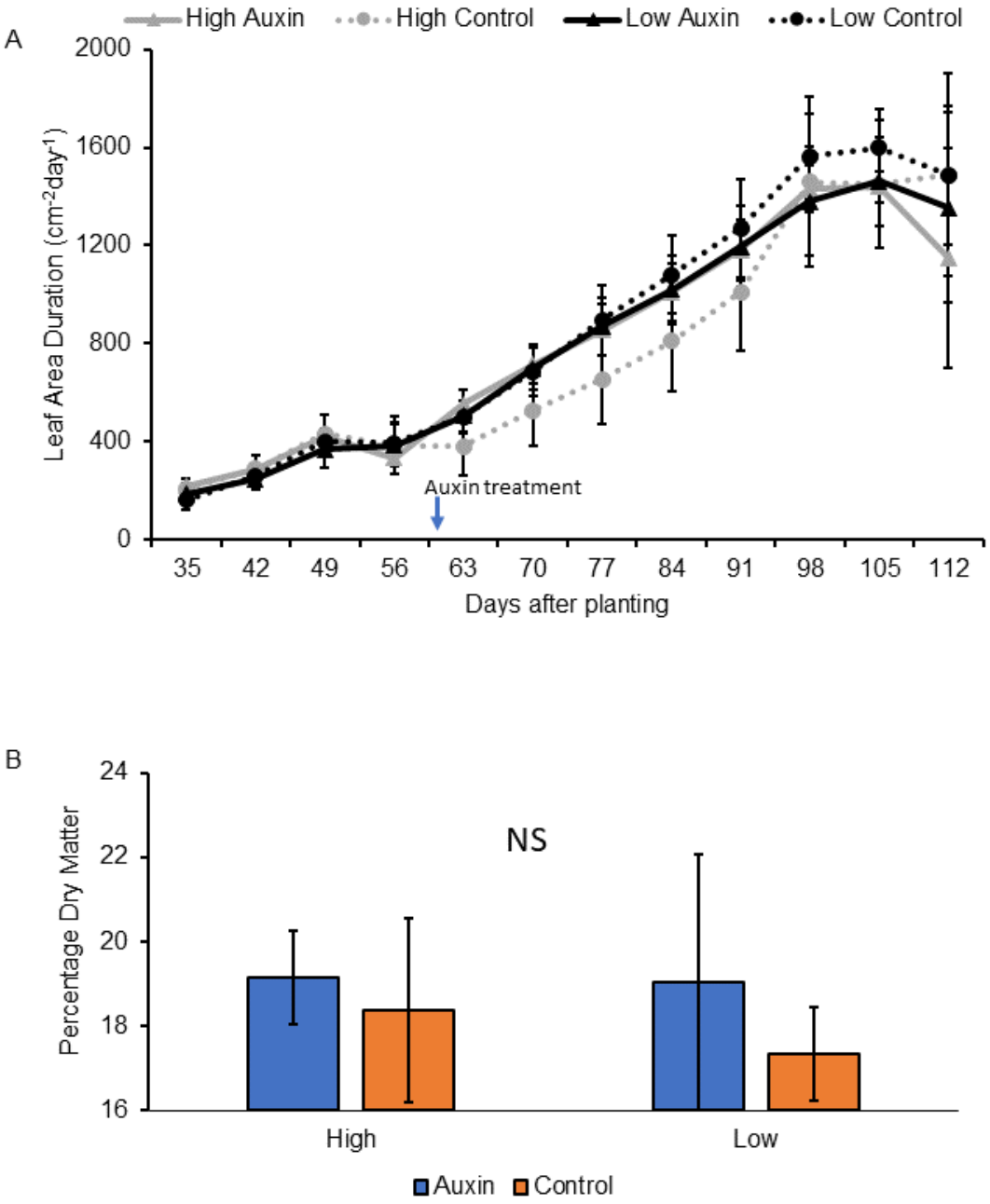


Figure 5.10. There is no effect of auxin on leaf area duration and the plants percentage shoot dry matter. Plants were grown on either adequate (High) or limited (Low) nitrogen background. The plants were treated with 100 uM of IAA solution (Auxin) while (Controls) are plants treated with water only. Data points and bars are averages (n=5 for plants treated with auxin and n=4 for the controls). NS denotes not significantly different from each other at 5 percent level. Error bars are the standard error of the mean.

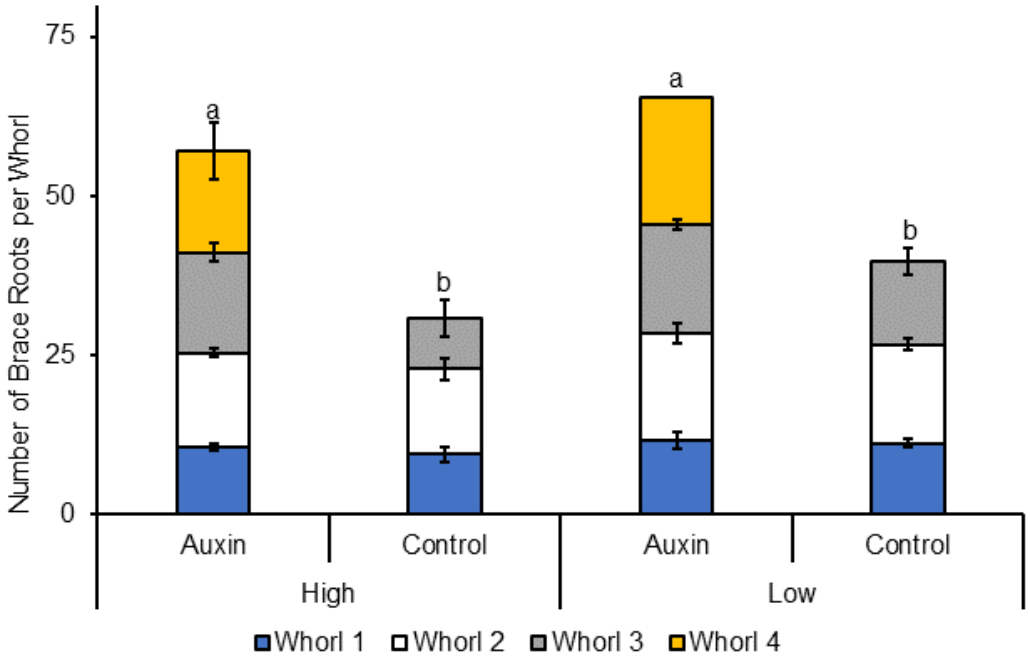


Figure 5.11. Auxin increased the total number and whorls of brace roots in both high and low nitrogen plants. Plants were grown on either adequate (High) or limited (Low) nitrogen background. The plants were treated with 100 um IAA solution (Auxin) while (Control) are plants treated with water only. Bars are averages (n=5 for plants treated with auxin and n= 4 for the controls). Lower case letters indicate significant difference at 0.01. Error bars are the standard error of the mean.

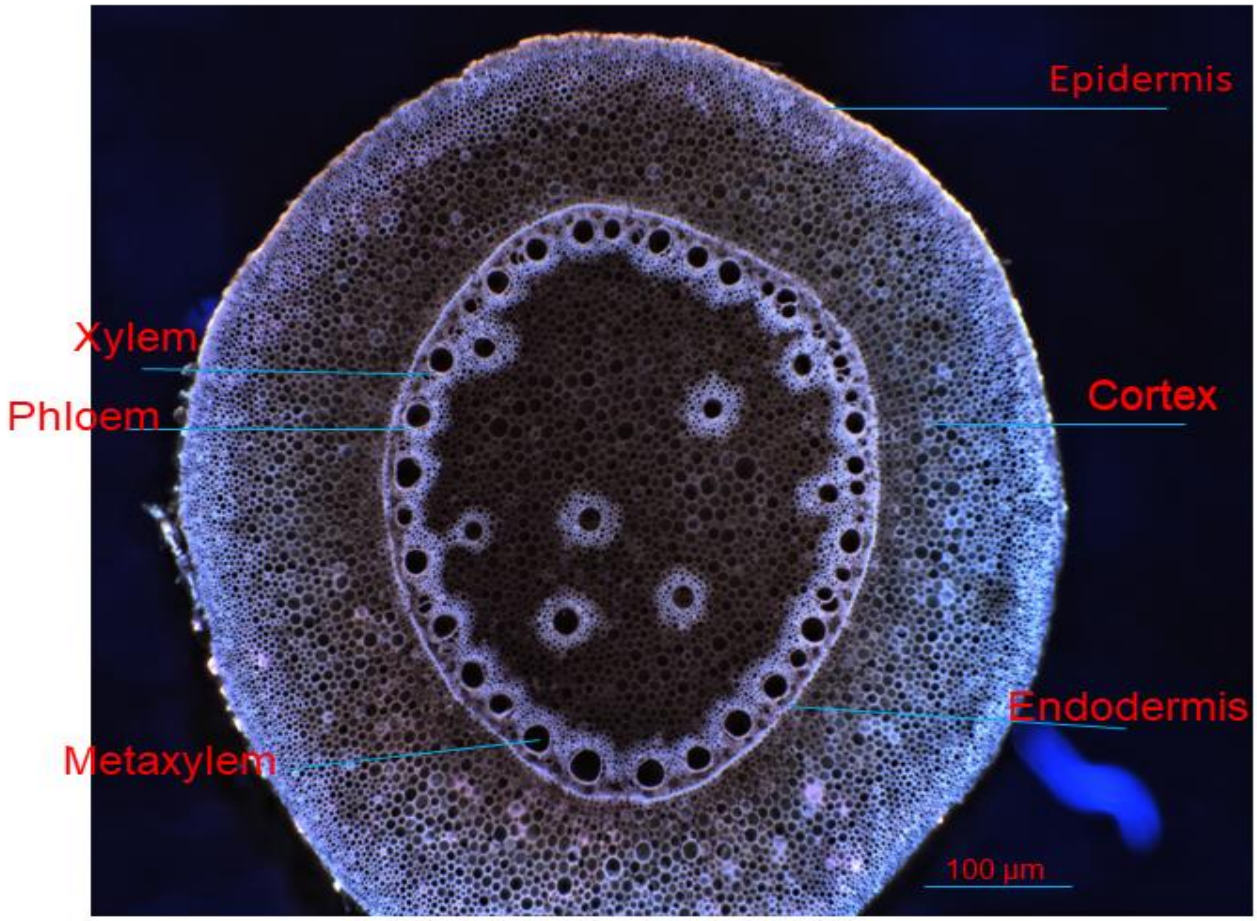


Figure 5.12. The 3-Dimensional qualitative and quantitative view of the internal structure of maize brace roots after emergence.

5.5. Discussion

The difficulty in applying hormones to mature maize plants grown in soil arises from the thick waxy cuticle of maize. This necessitated the development of new technique of applying hormones to mature tissues as a contribution to the understanding of nodal root development in maize. The aim of this study is to explore the effective means of treating plants with hormones for adult maize plant grown in soil. This is for the purpose of inducing development of brace roots which normally emerge later in maize. It was hypothesized that exogenous application of gibberellins using water as a carrier will be more effective, and the effect would be evident in the elongation of the stems of low N plants as compared to other application methods within one week. Secondly, it was hypothesized that the exogenous application of natural auxin (IAA) will reduce shoot growth and nitrogen content of low N maize and increase the number of brace roots and brace root whorls in low N plants, as compared to high N plants. Since gibberellins function to promote stem elongation, it was used to test treatment methods because of the likely earlier evidence of successful application. Then the method of using water as a carrier and application of the hormone solution to a scarified stem base of the plants (the adjudged effective method) was used to apply auxin (IAA). This Chapter thus, explored an effective means of applying hormone in adult maize grown on soil and investigated the effect of nitrogen and auxin on the number of brace root emergence and shoot response. The importance of the new method of phytohormone treatment will be highlighted first, followed by the effect of gibberellins and then the effect of auxin.

5.5.1. The importance of the new phytohormone treatment method uncovered in this study:

The importance of the new hormone application method outlined in this Chapter cannot be over emphasized. Based on literature, there is hardly any report regarding the molecular and physiological regulation of brace root development in relation to nitrogen and phytohormones in maize. This gap in knowledge formed the crux of this Chapter. The maize brace root is potentially critical for resource acquisition in nutrient limited soils, in addition to its known function of preventing lodging by offering firm anchorage to the plants. Recently, the aerial brace roots of a Mexican land race lines were reported to have the ability to synthesize atmospheric gas and convert it to usable form such as ammonium, through the relationship of its mucilage exudate and diazotrophic bacterial (G. Viana et al., 2022; Sparks, 2023; Van Deynze et al., 2018). Field experimentation on low nitrogen soil showed that aerial brace roots of the Mexican landrace contributed 89% of the plant's nitrogen nutrition from atmospheric fixation (G. Viana et al., 2022; Van Deynze et al., 2018). This suggests availability of gene pools in brace roots that needed to be identified and characterized for the optimization of root architecture. More so, the internal structure of the brace roots shows higher probability for nutrient

uptake because of the larger metaxylem, but further studies involving nitrogen rates and transporter activities may help to broaden our understanding.

The hormone application method in this Chapter allows the direct involvement of the molecular players of the shoot meristem, other than the roots to trigger the required changes in the transcriptional regulation of shoot-borne roots especially the brace roots in maize. The sole-involvement of the shoot meristematic activities in leu of the root would enable the manipulation of the shoot derived signalling and gene expression involved in the development of shoot-borne roots for the advancement of maize research. This is important because the maize shoot borne roots (crown and brace roots) are the main roots involved in resource capture and anchorage as compared to the embryonic roots (primary and seminal roots) during the advanced stage of growth and development. This is because the brace roots normally emerge at the juvenile-to-adult transitional phase, during which the leaf cuticles and waxes would have advanced maturity. The situation would create barrier that will prevent the immediate absorption of exogenous hormones. Therefore, the hormone application method will pave the way for advance approaches in studying transcriptional, posttranscriptional and posttranslational activities of molecular players in relation to root development and kinetics of nutrients uptake, through the manipulation of events using exogenously applied phytohormones.

5.5.1.1. Gibberellins concentration of 100 µM in water earlier increased the height of low N plants the most

With the aim of finding the most effective method of applying hormones into the maize plants to lead to a visible immediate effect, gibberellins were used to induce increase in plant height, especially in low nitrogen plants. This is because gibberellins act to bring about stem elongation by degrading the DELLA proteins which are growth inhibitors (Mu et al., 2018; Wang et al., 2020). The percentage increase in plant height per nitrogen and gibberellin treatment was greatest in those plants treated with 100 µM of dissolved gibberellins in water compared to that in lanolin. Even in terms of concentration, plants treated with 50 µM gibberellin solution did not increase in height as much as those injected with 100 µM gibberellin solution, implying gibberellin acts in a concentration dependent manner.

The number of replicates were just two plants per treatment and methods due to poor germination rates. A higher replicate may lead to more significant differences between treatment and methods. Out of 40 pot plants planted, only 24 pot plants germinated and unevenly. This necessitated the use of percentage change in plant height to detect changes in plant growth.

Gibberellins have been shown to interact synergistically with auxin in the control of tissue differentiation and cell expansion (Zhang et al., 2020). Although only gibberellins were applied on this set of plants, it is expected that the natural biosynthesis of auxin will occur especially in low N plants, to facilitate root development. This trial found no significant difference in the number of brace roots in low and high N plants, regardless of gibberellin concentration or the medium used as carrier. It is unclear the reason for the lack of significant difference, but recent review said that gibberellins promote ethylene-triggered adventitious root growth (Steffens and Rasmussen, 2016). This showed that more detailed study is required to unravel the interactive effects of gibberellins, nitrogen and other phytohormones in the regulation of maize shoot borne roots. But the aim of using gibberellins was to find the most effective method of getting hormones into adult maize which was carried out.

5.5.2. Auxin and shoot growth parameters of maize plants

The leaf area, which shows the assimilation capacity (available surface area for capturing the photosynthetically active radiation (PAR)) of maize plants was affected by nitrogen level. This is expected because increased availability of nitrogen triggers N uptake by plants, since both nitrate and ammonium act as signalling molecule to facilitate influx. Higher nitrogen uptake means higher cell divisions, leading to more growth of the shoot meristem to increase the surface area for carbon assimilation and radiation use efficiency. The auxin treatment tended to reduce the rate of increase in leaf area of both low and high N plants within the first seven days after treatment, although this was not statistically significant. This suggest that the imported exogenous auxin (IAA) led to transcriptional changes in gene expression in a manner that suppressed the IPT genes of the cytokinin which normally act to facilitate growth of the arial parts of the plants. It was reported that auxin was shown to prevent the production of cytokinin in the nodes by controlling the expression of *OsIPT* genes in rice (Xu et al., 2015). This temporal suppression within one week after auxin treatment was also observed in plant heights. Previous studies on exogenous application of auxin (IAA) in maize showing overexpression of ZmPIN1a negatively affected plant height, internode length and ear height (Li et al., 2018).

The nitrogen balance index (NBI), which represents the ratio between the chlorophyll and the epidermal flavonoids levels of leaves is an important indicator of crop health. In this study, the lack of significant difference in NBI, chlorophyll, or flavonoids between plants of nitrogen background shows the resilience and nitrogen economy of the low N plant to maintain chlorophyll level for improved radiation capture at an almost similar rate as the high N plants. This could lead to improved nitrogen use efficiency. However, auxin treatment seemed to have decreased the NBI and chlorophyll contents of the leaves within seven days of application. This further indicates the down regulation of the genes

associated with nitrogen metabolism. The regulation of auxin and cytokinin in the shoot also depends on the transcription factors *LONG HYPOCOTYLS 5* (HY5) and *GOLDEN2-LIKE2* (GLK2) which acts antagonistically to control chlorophyll biosynthesis (Kobayashi et al., 2012). The co-ordinated role of HY5 and GLK2 have been shown to act downstream of the light and auxin and cytokinin signalling pathways in the expression of genes involved in chlorophyll biosynthesis in *A. thaliana* (Kobayashi et al., 2012). The polar auxin transport from shoot to roots regulate chlorophyll biosynthesis in roots, via IAA14 AND ARFs7/19 (Kobayashi et al., 2012) and SLR/IAA14 and AXR2/IAA7 (Gangappa and Botto, 2016) and auxin signalling is said to be responsible for the inhibition of chlorophyll biosynthesis in roots of *A. thaliana* (Kobayashi et al., 2012; Wei-Gui et al., 2023). But how HY5 and GLK2 regulate auxin and cytokinin in maize is yet to be elucidated. Therefore, this Chapter hypothesised that the temporal effect of suppressing chlorophyll production immediately after auxin treatment may be attributed to the activities of HY5 suppressing the GLK2 genes as can be seen in the transient increase of flavonoids especially in the low N plants treated with auxin. However further studies involving a follow up application of cytokinin after auxin treatment in a similar factorial design may help elucidate this view.

The leaf area duration is a proxy way to determine changes in dry mater content of plants non-destructively. This is important especially in glasshouse experiment where replication might be limited as compared to field plants (Power et al., 1967), as there exists a strong linear relationship between the leaf area duration and plant dry weight, even with varying treatments (Power et al., 1967). Here auxin treatment did not lead to a significant change in plant shoot biomass irrespective of nitrogen background. This was also seen in the lack of percentage dry matter difference in shoot biomass across treatments or controls at the end of the experiment. It is assumed that because the plants were not nitrogen-stressed (as plants were fed twice a week), the plants nitrogen status reduced the auxin biochemical activities of suppressing shoot growth and enhanced cytokinin signalling. This is due to the fact that auxin is a negative regulator of cytokinin and recent studies and review showed that the antagonistic relationship between auxin and cytokinin is unidirectional in a manner that only auxin regulates the biosynthesis and signalling of cytokinin, and not the reversed case (Kolachevskaya et al., 2021; Kolachevskaya et al., 2017; Kurepa et al., 2019; Kurepa and Smalle, 2022). Furthermore, the nitrate transporter ZmNRT1.1(LAT) may not be phosphorylated at such times and may not be involved in polar auxin transport regulation of ZmNRT2.1 (HAT), which relates to cytokinin signalling for uptake, also contributes to nitrogen uptake even during high nitrogen availability, might be responsible for the lack of difference in biomass accumulation. However, it was previously reported that the mRNA level of NRT2.1 was strongly reduced within hours of auxin and cytokinin supply (Krouk et al., 2011). The allocation of leaf nitrogen to Rubisco (Ribulose 1, 5-biphosphate carboxylase/oxygenase) is

constant in maize irrespective of the leaf nitrogen level but increase in nitrogen leads to a rapid production of phosphoenolpyruvate carboxylase (PEPC) and pyruvate phosphate dikinase (PPDK) (Urban et al., 2021). Biomass production rate was shown to depend on the concentration of PEPC rather than Rubisco (Urban et al., 2021), indicating that even the nitrogen content of low N plants was not significantly lower than that of high N plants. Thus, no significant difference was found in the shoot dry matter. More molecular analysis is required regarding the nitrogen content of maize leaves as influenced by nitrogen rates and frequency of supply, and the interaction of exogenous IAA and cytokinin pathways.

5.5.3. *The influence of auxin (IAA) on brace roots whorls and number*

Polar auxin transport is critical for the formation of local auxin maxima and is important for root morphogenesis (Guan et al., 2019; Li et al., 2018; Liu et al., 2010; Pacurar et al., 2014). The process of brace root formation involves four unique phases, including the induction stage where one or two cells are required to be the founder cells possessing ability to divide, the initiation stage depicting the presence of primordia as an observable morphological change, the emergence stage which is the elongation of the primordia, and root growth which is the last stage that determines the extent of the root system and the number of the nodes containing brace roots that can protrude into the soil (Hostetler et al., 2021). The induction and initiation stages may be similar between crown and seminal roots because they emanate from the stem node, which are comparable anatomically. But the modulation of the emergence and growth is likely influenced by unique environmental circumstances based on the subsoil or aerial environment (Hostetler et al., 2021). This study found that the number of brace roots whorls and brace roots was significantly increased with auxin (IAA) treatment in both low and high N plants. This suggests that IAA accumulated in the stem after application and mediated changes in gene expression involving hormone metabolism and signal transduction that led to the increase in brace roots number.

A closer view of the process of applying the auxin can give a clue about the likely processes that could have occurred to enable the emergence of brace roots in both high and low nitrogen plants. First, the lower nodes on the maize stems already have undergone the induction and initiation stage like it was reported in mung bean (de Klerk et al., 1999), *Salix*, *Populus* and *Jasminum* (Pacurar et al., 2014). These already pre-formed adventitious root initials are likely derived from cells located in the vascular region, of which the specificity of the cell type is unknown (Guan et al., 2019; Hostetler et al., 2021; Pacurar et al., 2014). The next process in the developmental phase is emergence which is regulated by auxin and is the centre of this Chapter. The exogenously applied auxin (IAA) can enter the scratched surface and is taken up by a pH trapping process and influx carriers (da Costa et al., 2013; de Klerk et

al., 1999). This entry is expected to lead to a local auxin gradient that will trigger the activities of the *ZmAUX/LAX* and ZmPIN families which are influx and efflux auxin carriers respectively (Liu et al., 2010). The activities of these importers and exporters is expected to enable the establishment of auxin maxima at the quiescent centre. This is crucial for indeterminate growth of adventitious roots (Pacurar et al., 2014). The determination of the auxin maximum at primordium tip of *A. thaliana* is orchestrated by PIN1 and LAX3 relative to cytokinin, where *WOX5* is restricted (Pacurar et al., 2014). The endogenous auxin synthesis by *YUCCA6* is expected to be maintained and regulate apical auxin accumulation (Guan et al., 2019; Pacurar et al., 2014).

Since IAA can be relatively unstable, excessive IAA around the internal tissues may be degraded by oxidation or by conjugation(reversible) to render it inactive (de Klerk et al., 1999). The wounding effect from scratching the internal tissues during the auxin application is expected to trigger the expression of stress response players including jasmonate (da Costa et al., 2013; Rasmussen et al., 2015), phenolic compounds and endogenous auxin at the scratched base with a transient lower peroxidase activities and formation of sink for carbohydrate in the same zone (da Costa et al., 2013; de Klerk et al., 1999). The increased expression of phenolic compounds will prevent the oxidation of auxin (IAA) (da Costa et al., 2013; de Klerk et al., 1999), which would allow accumulation of a high auxin concentration that is required for the emergence of adventitious roots (Guan et al., 2019; Pacurar et al., 2014; Rasmussen et al., 2015; Steffens and Rasmussen, 2016). The control plants in this experiment were also scratched and 3% ethanol in water was applied, meaning a stress response would also be induced, but the number of brace roots in auxin treated plants was significantly higher regardless of nitrogen background, suggesting that auxin and not just stress response was influential in the number of brace roots that emerged.

The high concentration of auxin is expected to trigger auto modulation mechanism of canalization and maximisation to receptive marked cells (Druege et al., 2016; G. Viana et al., 2022; Zhang et al., 2022). This mechanism involves the modulation of gene expression through $SCF^{TIR1/AFB}$ – *Aux/IAA-ARF* nuclear signalling cascades (Zhang et al., 2022). The signalling cascades requires the F-box proteins *AFB1-AFB5/TIR1* auxin receptors subunits of SCF-Family E3 ubiquitin, which synthetase the activator class *of ARF (AUXIN RESPONSE FACTOR*) transcription factor and the transcriptional repressor Aux/IAA (Ludwig et al., 2013; Luo et al., 2018b; Zhang et al., 2022). Auxin initially binds to *TIR1/AFB* receptors to degrade Aux/IAA proteins that are associated with ARF transcription factor through the 26S proteasome and prevent auxin signalling (Ludwig et al., 2013; Luo et al., 2018a; Luo et al., 2018b; Zhang et al., 2022). It was shown that different *TIR1/AFB-Aux/IAA* protein combinations usually has different auxin binding level, and the concentration of auxin may differ within different tissue and ontogenetic phase (Luo et al., 2018a) This leads to differences in the auxin sensing effect (Luo et al.,

2018a). Also, the kinetics of *Aux/IAA* transcription is unique and depend on the variability of the control of free auxin, the tissue specific auxin receptors and different modulation of transcriptional and post transcriptional event (Ludwig et al., 2013). The tissue and the developmental phase specificity may be one of the reasons for the differences in the developmental programmes between brace root and lateral roots in relation to auxin. This is because the brace roots emerge at the juvenile-adult vegetative phase while lateral roots can develop any time in the vegetative stage of maize.

After the degradation of Aux/IAA, the released ARF protein may employ *SWITCH / SUCROSE NON FERMENTING (SWI / SNF*) to remodel the chromatin into active state, and bind the auxin response DNA cis-element (Aux/REs) to induce alteration in target auxin response gene at the transcriptional level. The rhizogenic actions (processes involved in producing roots) of auxin at the primordium, which is short lived (between 72-96 hrs) (de Klerk et al., 1999), allows the cells within the primordium to differentiate into vasculature tissues that in due course links to the stem vasculature to enable adventitious roots (hopefully brace roots too) to become functional roots (Guan et al., 2019). Subsequently, the primordium emerges from the stem epidermis giving rise to mature adventitious roots (Guan et al., 2019). The final step is the programmed cell death of the epidermal cells for the emergence of new roots (Guan et al., 2019). This process contrasts with the enzymatic separation of cortical and epidermal cells in development of lateral roots ((Guan et al., 2019). This is an important difference between adventitious roots and lateral roots development process and may mean different transcriptional, translational, and post-translational regulation. However, auxin gradients in the formation of adventitious roots were found to be like those observed in the formation of primary and lateral roots (Guan et al., 2019).

Previous studies reported the influence of IAA in the formation of adventitious roots in *A. thaliana* through the stimulation of coordinated influx and efflux activities of *AUX1* and *PIN1* genes (Della Rovere et al., 2013; Li et al., 2018). In maize, there are 34 identified and characterised Aux/IAA genes in line B73 (Ludwig et al., 2013; Luo et al., 2018a). In terms of expression levels (Figure 5.13), a total of 19 Aux/IAA were said to be expressed in maize crown roots (early adventitious roots in maize) as compared to primary roots (Ludwig et al., 2013). The proteins include *ZmIAA1, ZmIAA2, ZmIAA4, ZmIAA5*, *ZmIAA8, ZmIAA13, ZmIAA14, ZmIAA15, ZmIAA16, ZmIAA18, ZmIAA20, ZmIAA21, ZmIAA23, ZmIAA25, ZmIAA27, ZmIAA28, ZmIAA30, ZmIAA33, ZmIAA34.* Among them, *ZmIAA16, ZmIAA27, ZmIAA28*, *ZmIAA30* were said to be highly expressed between crown and seminal roots, *ZmIAA2, ZmIAA13*, *ZmIAA28, ZmIAA30*, were said to be highly expressed between crown and lateral roots (Ludwig et al., 2013). While only *ZmIAA6, ZmIAA9*, and *ZmIAA20,* were found to be more highly expressed in the shoot tissues than

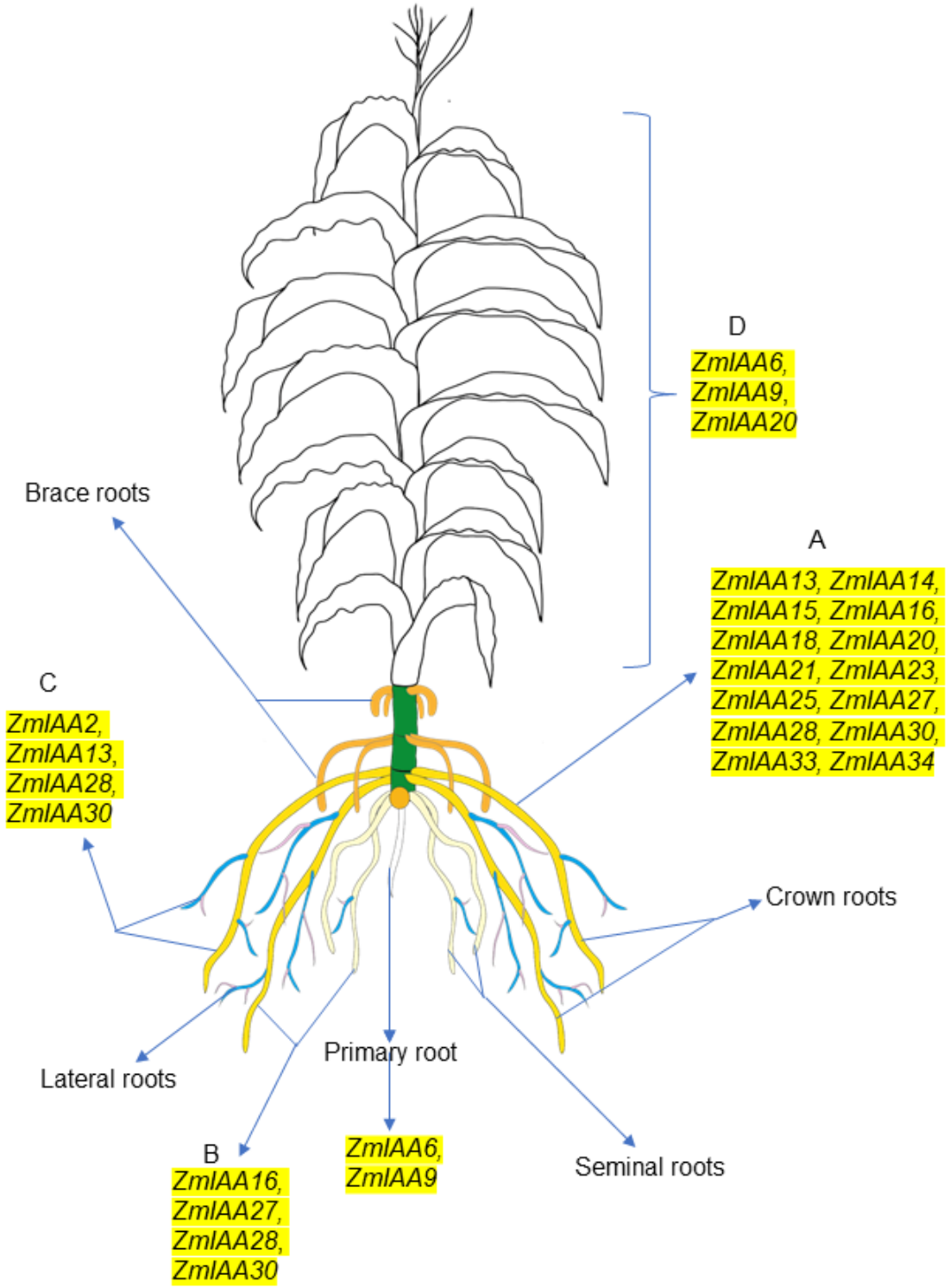


Figure 5.13. Differentially expressed IAA/Aux genes in root types of B73 inbred lines. (A) represents genes with high transcript abundance in crown roots at more than one stage of development, (B) represents genes that are highly expressed between crown and seminal roots, (c) represents genes that were highly expressed between crown and lateral roots , (D) genes with higher transcript abundance in the shoot but not roots. (Based on Ludwig, Y., Zhang, Y., & Hochholdinger, F. (2013).
The maize (zea mays L.) AUXIN/INDOLE-3-ACETIC ACID gene family: Phylogeny, synteny, and unique root-type and tissue-specific expression patterns during development. *PLoS One, 8*(11) doi:https://doi.org/10.1371/journal.pone.0078859.

root tissues (Ludwig et al., 2013). The scholars demonstrated that the expression levels of *ZmIAAs* were greatest in crown roots, followed by seminal roots and then primary root, with lateral roots having the least expression. This suggests that the adventitious roots which includes crown and brace roots may have greater capabilities to carry out root function as compared to embryonic roots due to the myriad of genes that were found highly expressed in crown roots. Since the brace roots are shoot-borne roots, *ZmIAA6, ZmIAA9*, and *ZmIAA20,* may have special roles in the development of brace roots because they were the only set of Aux/IAA that were found to be over express in shoot tissues of B73 maize lines expressed in the shoot tissues may have roles in brace root development.

There are about 14-15 PIN coded genes which were identified in B73 lines and were grouped into separate categories. There are 4 long-loop PINS and 7 and 8 short-loop PINS which were Identified and categorised with the ortholog in Arabidopsis and rice (Forestan et al., 2012; Li et al., 2019). Among those PIN genes, *ZmPIN1a* and *ZmPIN1b* were found to be more responsive to abiotic stress as compared to *ZmPIN1c* and *ZmPIN1d* and their (*ZmPIN1a-d*) transcript abundance are more than the others (Li et al., 2019). According to previous work, the expression levels of *ZmPIN1b* was highest at the shoot meristem while *ZmPIN1a* expression was enriched in the root meristem of transgenic maize over expressing the ZmPIN1a (Li et al., 2019b). Therefore, because the brace roots are short-borne roots, the activities of *ZmPIN1b* may greatly facilitate the basipetal auxin travel to enhance brace root formation. The study (Li et al., 2019b) was on transgenic maize lines that greatly express the PIN1 genes during low phosphate supply, findings may be different when other cultivars are used, and nitrogen was involved in the experimentation.

Previous study found no effect of exogenous NAA and IAA on the elongation of crown, primary and seminal roots of maize seedlings during low nitrate supply. However, high nitrate supply restored elongation of primary and seminal roots (embryonic roots) but not crown roots (adventitious/shoot-borne root) after applying NAA and/or IAA. (Tian et al., 2008). The scholars concluded that low level of auxin as influenced by high nitrogen supply inhibited root growth. Although my research is on brace roots, the crown and brace roots are both adventitious roots. This Chapter have demonstrated that high nitrogen supply did not inhibit the stimulatory effects of auxin resulting to root outgrowth of brace roots, but the rates and type of gene expression my differ between the two maize adventitious roots. This is because the maize crown normally emerges from 10 to 14 days after germination (Hochholdinger et al., 2004), while the brace roots emerge close to the time of transition from vegetative stage to reproductive stage (Hostetler et al., 2021). So, the transcription relating to phase change (juvenile-adult transition phase) might predominate over usual uptake activities in brace roots than in crown roots. The inability of auxin to stimulate the growth of the crown roots during high nitrogen availability in their study (Hostetler et al., 2021) may have to do with tissue specificity. This

is because, in addition to auxin concentration in the plant's tissues, a concentration gradient and the differential sensitivity of various cell types are facilitators for auxin modulated growth (Kiba et al., 2011).

Recent analysis of root-type specific reprograming of maize pericycle transcriptomes revealed a significantly distinct increase in density of emerged lateral roots of brace roots as compared to other maize root types, 20 days after local high nitrate supply (Yu et al., 2016). The scholars showed that the brace roots have larger transversal area, stele, metaxylem area, higher number of cortical cell layers and metaxylem elements than crown, seminal and primary roots. This finding suggests the importance of brace roots in relation to nutrient and water acquisition capability. One can deduct from their findings that brace roots have a greater capability to take up soil resources as compared to other maize root types. One major cause of limited research on brace roots may be because most of the brace roots on an average maize plant are aerial with few subterranean ones, and so from its name, brace roots are mostly seen as a mere structural support system of the maize plants rather than having a resource capture role. Yu and colleagues further investigated the transcriptomic landscape of the pericycle cells between root types, findings that higher number of genes were specifically activated in brace roots by local high nitrate supply as compared to other root types. Their findings were consistent with adult brace roots contributing more to branching response nitrate uptake, more than seedling primary and seminal roots during the whole life cycle. Their findings showed that during uniform low nitrate nutrition, the lateral root densities of each root types appeared similar, showing that the concentration (low and high) and the pattern of nutrients supply (homogenous or heterogeneous) affects transcriptional responses which may be distinct to root types. If their study (Yu et al., 2016) had involved a mix nitrogen nutrition, it would perhaps help understand the resource capture by brace roots when nitrogen nutrition is mixed. This is because nitrogen availability was shown to affect auxin biosynthesis, but there are no studies on the concentration of nitrogen supply and the pattern of supply on the activities of the biosynthesis, transport and signal induction of auxin during initiation of brace in maize. The study of the interaction of nitrogen and auxin on maize root development is mostly on lateral roots from shoot born roots, not the main shoot-borne roots.

Local high nitrogen was shown to promote auxin mediated cell cycle activation (Yu et al., 2015). This finding highlights the importance of nitrogen source in the regulation of gene expression that leads to varying processes for root formation and development. This Chapter experiment employed uniform application of ammonium nitrate as nitrogen source meanwhile Yu and colleagues used only nitrate as nitrogen form. This could bring about difference in the *IAA* transcriptional regulation in low N plants, and is remained to be explored as recent study showed that co-application of nitrate and ammonium could enhance auxin biosynthesis in maize by increasing the levels of phosphoenolpyruvate and

tryptophan, compared to sole nitrate or ammonium in maize (Ishaya and Rasmussen, 2021; Wang et al., 2019). Furthermore, studies showed that nitrate responsive genes are more highly differentially expressed in brace roots than crown, seminal and primary roots in maize (Yu et al., 2016). Moreso, auxin accumulation was shown to be stimulated in rice roots that were grown on mixed nitrogen sources (75/25 ammonium/nitrate) than those grown on sole ammonium (Song et al., 2013; Vega et al., 2019), implying auxin sensitivity to nitrogen forms and rates.

Expert review suggest that the *TRYPTOPHAN AMINOTRANSFERASE RELATED2 (TAR2*) is an important auxin biosynthesis gene involved in the adaptation of root system to low nitrogen conditions (Vega et al., 2019). Under low nitrogen supply, the transcript abundance of *TAR2* was found to be elevated to increase IAA concentration for the development of lateral roots (Sun et al., 2017), showing the modulation of auxin concentration in response to nitrogen availability. Evidence from cell sorting and study of mutant *arf8* in *A, thaliana* suggest that nitrogen signals regulate auxin signalling through the action of miR167a in controlling the initiation of lateral roots (Gifford et al., 2008). This showed the involvement of miRNAs in the post transcriptional regulation of gene expression. Although no miRNA is shown to regulate a specific maize nitrate transporter (Plett et al., 2018), this study hypothesized that miR156 and miR172 are involved in nitrogen signals that control brace root emergence. This is because the miR156-miR172-AP2 pathway were earlier proposed to orchestrate the timing of juvenile-to-adult phase transition and subsequent flower development in maize (LI et al.,2019). And nitrogen availability enables the maize plant to flower earlier than nitrogen deficient plants (Asibi et al., 2019). In the light of the above, more study is required at the physiological and molecular level to show t5he similarities and differences between of brace root and lateral root initiation and development in maize. This is because the process of lateral and adventitious root development shares some common aspect to include the requirement for initial high concentration of auxin followed by subsequent reduction, the participation of auxin influx and efflux carriers, cell wall dynamics and the activities of specific transcription factors in relation to nitrogen availability for these processes.

5.5.4. Optimising maize root system architecture for low nitrogen soil using the findings from this study:

The maize root systems require optimisation based on environmental specificity for improved nutrients uptake and utilization efficiency. The different root types of maize may be improved to adapt to low nitrogen soils for the collective efficiency of the root system (Figure 5.14). For example, the maize crown root being the most efficient root for nitrogen acquisition on soils limited in nitrogen (Dechorgnat et al., 2018; Hochholdinger et al., 2004) could also be modified using a dose of high

nitrogen pulse and exogenous IAA treatment to achieve reduced crown root whorls and number in maize growing on low nitrogen soils. Although the hormone treatment method in this Chapter focused on the emergence of maize brace root rather than crown roots, the idea could be applied to manipulate crown root number and diameter which can invariably affects the crown root angle. The rationale for integrating exogenous IAA with a pulse of high nitrogen lies in the fact that the entrance of IAA into the shoot meristem would cause the initial accumulation of auxin to achieve auxin maxima needed for the differentiation of founder cells required for the initiation of crown root. Whilst the single dose of high nitrogen on low N plants will cause the synthesis of cytokinin. Cytokinin is believed to modulate cell cycle and control nitrogen status of the plant between the root and the shoot via the phosphorelay pathway and stimulate lateral root elongation after initiation have occurred (Nadeem et al., 2023). The action of cytokinin in promoting lateral root elongation may be the same for all root types. These overlapping activities and responses of auxin and cytokinin in relation to other phytohormones would enable reconfiguration of the maize root architecture. This is because the low nitrogen background is expected to stimulate root (crown, seminal, primary and brace) elongation in leu of increased root number. Previous study reported that maize grown in aeroponics showed reduced crown root number which were longer during low nitrogen availability (Dechorgnat et al., 2018; Gaudin et al., 2011). The low soil nitrogen condition could also encourage increased root cortical aerenchyma by replacing living cells with lacunae in other to minimise the metabolic energy cost of soil exploration (Bouranis and Chorianopoulou, 2021). Furthermore, the low nitrogen status of the soil could encourage the penetration of emerged brace roots into the soil for scavenging of topmost nutrient, while maintaining the thinner nature of the seminal roots. However, the dose of high nitrogen is expected to trigger more laterals on the seminal roots.

The dual capacity nitrate transporter NRT 1.1 was reported to trigger the gene expression of the MADS box transcription factor ANRI to enhance lateral root elongation in response to NO_3^- supply in *A. thaliana* (Nadeem et al., 2023). While local nutrient supply was shown to instigate changes in the root development by increasing root branching in areas of rich nutrient patches (Dechorgnat et al., 2018; López-Bucio et al., 2003). The situation may be very similar in the case of maize. The nutrient application method in this study was homogenous but a nutrient gradient would later be created due to the temporal and spatial disposition of soil nitrogen. This may exert similar influence on root branching just like local nutrient. It is important to use mix nitrogen sources such as the ammonium nitrate solution used in this study. This is important because ammonium was believed to promote the proliferation of lateral roots while nitrate facilitate elongation of lateral roots in *A. thaliana* (Liu and von Wirén, 2017; Ravazzolo et al., 2020).

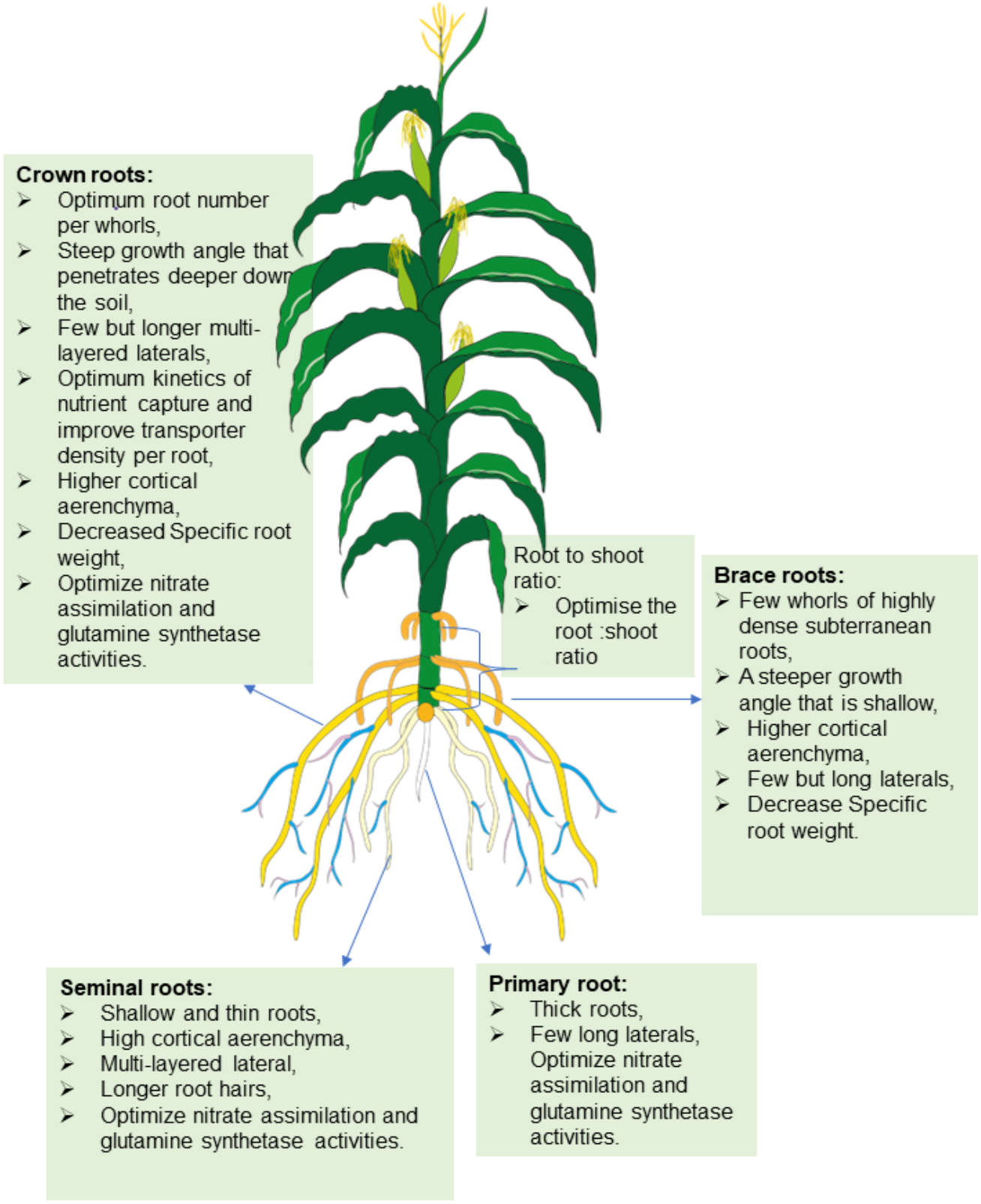


Figure 5.14. Optimized root architecture for improved nutrient uptake. The desirable root traits required for improved nutrients uptake by different root types will help to achieve nutrient uptake efficiency for improved nitrogen use efficiency. Adapted and modified from Jonathan P. Lynch, Steep, cheap and deep: an ideotype to optimize water and N acquisition by maize root systems, *Annals of Botany*, Volume 112, Issue 2, July 2013, Pages 347–357, https://doi.org/10.1093/aob/mcs293

5.6. Conclusion

Dissolving hormones in water and injecting into the stem lower node is an effective means of getting water or ethanol soluble hormones into the maize plant in a concentration dependent manner. Moreso, exogenous application of IAA enabled the maize plants to increase brace root whorls and number as compared to the controls. This was accompanied by a temporal relaxation of shoot growth related parameters that later recovered. This suggests greater possibility of optimizing root architecture through nitrogen and hormone pulses, for the improvement of nitrogen use efficiency in maize.

5.7. References

Alarcón, M. V., Lloret, P. G., and Salguero, J. (2013). Auxin–ethylene interaction in transversal and longitudinal growth in maize primary root. *Botany* **91**, 680-685.

Asim, M., Ullah, Z., Oluwaseun, A., Wang, Q., and Liu, H. (2020). Signalling overlaps between nitrate and auxin in regulation of the root system architecture: Insights from the Arabidopsis thaliana. *International journal of molecular sciences* **21**, 2880.

Bellini, C., Pacurar, D. I., and Perrone, I. (2014). Adventitious roots and lateral roots: similarities and differences. *Annual review of plant biology* **65**, 639-666.

Blakeslee, J. J., Spatola Rossi, T., and Kriechbaumer, V. (2019). Auxin biosynthesis: spatial regulation and adaptation to stress. *Journal of experimental botany* **70**, 5041.

Blizard, S. (2020). Uncovering the Role of Hormones in Maize Brace Roots. Electronic, University of Deleware, Delaware.

Bouranis, D. L., and Chorianopoulou, S. N. (2021). Adavances in understandimg plants roots anatomy and nutrients acquisition. *In* "Understanding and improving crop roots function" (P. Gregory, ed.). Burleigh Dodd Science Publishing, United Kingdom.

da Costa, C. T., de Almeida, M. R., Ruedell, C. M., Schwambach, J., Maraschin, F. S., and Fett-Neto, A. G. (2013). When stress and development go hand in hand: main hormonal controls of adventitious rooting in cuttings. *Frontiers in plant science* **4**, 133-133.

de Klerk, G. J., van der Krieken, W. M., and de Jong, J. (1999). Review: The Formation of Adventitious Roots: New Concepts, New Possibilities. *In vitro cellular & developmental biology. Plant* **35**, 189-199.

Dechorgnat, J., Francis, K., Dhugga, K. S., Rafalski, J. A., Tyerman, S. D., and Kaiser, B. N. (2018). Root Ideotype Influences Nitrogen Transport and Assimilation in Maize. *Frontiers in Plant Science* **9**, undefined-undefined.

Dechorgnat, J., Francis, K. L., Dhugga, K. S., Rafalski, J. A., Tyerman, S. D., and Kaiser, B. N. (2019). Tissue and nitrogen-linked expression profiles of ammonium and nitrate transporters in maize. *BMC plant biology* **19**, 206-206.

Della Rovere, F., Fattorini, L., D'Angeli, S., Veloccia, A., Falasca, G., and Altamura, M. M. (2013). Auxin and cytokinin control formation of the quiescent centre in the adventitious root apex of arabidopsis. *Annals of botany* **112**, 1395-1407.

Druege, U., Franken, P., and Hajirezaei, M. R. (2016). Plant Hormone Homeostasis, Signaling, and Function during Adventitious Root Formation in Cuttings. *Frontiers in plant science* **7**, 381-381.

Forestan, C., Farinati, S., and Varotto, S. (2012). The Maize PIN Gene Family of Auxin Transporters. *Frontiers in plant science* **3**, 16-16.

G. Viana, W., Scharwies, J. D., and Dinneny, J. R. (2022). Deconstructing the root system of grasses through an exploration of development, anatomy and function. *Plant, cell and environment* **45**, 602-619.

Gangappa, Sreeramaiah N., and Botto, Javier F. (2016). The Multifaceted Roles of HY5 in Plant Growth and Development. *Molecular plant* **9**, 1353-1365.

Gaudin, A. C. M., McClymont, S. A., Holmes, B. M., Lyons, E., and Raizada, M. N. (2011). Novel temporal, fine-scale and growth variation phenotypes in roots of adult-stage maize (Zea mays L.) in response to low nitrogen stress. *Plant, cell and environment* **34**, 2122-2137.

Gifford, M. L., Dean, A., Gutierrez, R. A., Coruzzi, G. M., and Birnbaum, K. D. (2008). Cell-specific nitrogen responses mediate developmental plasticity. *Proceedings of the National Academy of Sciences - PNAS* **105**, 803-808.

Guan, L., Tayengwa, R., Cheng, Z. M., Peer, W. A., Murphy, A. S., and Zhao, M. (2019). Auxin regulates adventitious root formation in tomato cuttings. *BMC plant biology* **19**, 435-435.

Hetz, W., Hochholdinger, F., Schwall, M., and Feix, G. (1996). Isolation and characterization of rtcs, a maize mutant deficient in the formation of nodal roots. *The Plant journal : for cell and molecular biology* **10**, 845-857.

Hochholdinger, F., Park, W. J., Sauer, M., and Woll, K. (2004). From weeds to crops: genetic analysis of root development in cereals. *Trends in plant science* **9**, 42-48.

Hostetler, A. N., Khangura, R. S., Dilkes, B. P., and Sparks, E. E. (2021). Bracing for sustainable agriculture: the development and function of brace roots in members of Poaceae. *Current opinion in plant biology* **59**, 101985-101985.

Ishaya, F., and Rasmussen, A. (2021). Adavances in understanding plant root growth regulators. *In* "Understanding and improving crop root function" (P. Gregory, ed.). Burleigh Dodd Science Publishing Limited, United Kingdom.

Jia, Z., and von Wirén, N. (2020). Signaling pathways underlying nitrogen-dependent changes in root system architecture: from model to crop species. *Journal of experimental botany* **71**, 4393-4404.

Kaldenhoff, R., and Iino, M. (1997). Restoration of phototropic responsiveness in decapitated maize coleoptiles. *Plant physiology (Bethesda)* **114**, 1267-1272.

Kant, S. (2018). Understanding nitrate uptake, signaling and remobilisation for improving plant nitrogen use efficiency. *Seminars in Cell and Developmental Biology* **74**, 89-96.

Kiba, T., Kudo, T., Kojima, M., and Sakakibara, H. (2011). Hormonal control of nitrogen acquisition: roles of auxin, abscisic acid, and cytokinin. *Journal of Experimental Botany* **62**, 1399-1409.

Kobayashi, K., Baba, S., Obayashi, T., Sato, M., Toyooka, K., Keränen, M., Aro, E.-M., Fukaki, H., Ohta, H., Sugimoto, K., and Masuda, T. (2012). Regulation of Root Greening by Light and Auxin/Cytokinin Signaling in Arabidopsis. *The Plant cell* **24**, 1081-1095.

Kolachevskaya, O. O., Myakushina, Y. A., Getman, I. A., Lomin, S. N., Deyneko, I. V., Deigraf, S. V., and Romanov, G. A. (2021). Hormonal Regulation and Crosstalk of Auxin/Cytokinin Signaling Pathways in Potatoes In Vitro and in Relation to Vegetation or Tuberization Stages. *International journal of molecular sciences* **22**, 8207.

Kolachevskaya, O. O., Sergeeva, L., Floková, K., Getman, I. A., Lomin, S. N., Alekseeva, V. V., Rukavtsova, E. B., Buryanov, Y. I., and Romanov, G. A. (2017). Auxin synthesis gene tms1 driven by tuber-specific promoter alters hormonal status of transgenic potato plants and their responses to exogenous phytohormones. *Plant cell reports* **36**, 419-435.

Krouk, G., Ruffel, S., Gutiérrez, R. A., Gojon, A., Crawford, N. M., Coruzzi, G. M., and Lacombe, B. (2011). A framework integrating plant growth with hormones and nutrients. *Trends in plant science* **16**, 178-182.

Kurepa, J., Shull, T. E., and Smalle, J. A. (2019). Antagonistic activity of auxin and cytokinin in shoot and root organs. *Plant direct* **3**, e00121-n/a.

Kurepa, J., and Smalle, J. A. (2022). Auxin/Cytokinin Antagonistic Control of the Shoot/Root Growth Ratio and Its Relevance for Adaptation to Drought and Nutrient Deficiency Stresses. *International journal of molecular sciences* **23**, 1933.

Lanteri, M. a. L., Pagnussat, G. C., and Lamattina, L. (2006). Calcium and calcium-dependent protein kinases are involved in nitric oxide- and auxin-induced adventitious root formation in cucumber. *Journal of experimental botany* **57**, 1341-1351.

Li, Z., Li, P., and Zhang, J. (2019). Expression analysis of PIN-formed auxin efflux transporter genes in maize. Plant signaling & behavior. *Plant Signalling and Bhaviour* **14**.

Li, Z., Zhang, X., Zhao, Y., Li, Y., Zhang, G., Peng, Z., and Zhang, J. (2018). Enhancing auxin accumulation in maize root tips improves root growth and dwarfs plant height. *Plant biotechnology journal* **16**, 86-99.

Liu, J., An, X., Cheng, L., Chen, F., Bao, J., Yuan, L., Zhang, F., and Mi, G. (2010). Auxin transport in maize roots in response to localized nitrate supply. *Annals of botany* **106**, 1019-1026.

Liu, Y., and von Wirén, N. (2017). Ammonium as a signal for physiological and morphological responses in plants. *Journal of experimental botany* **68**, 2581-2592.

López-Bucio, J., Cruz-Ramírez, A., and Herrera-Estrella, L. (2003). The role of nutrient availability in regulating root architecture. *Current opinion in plant biology* **6**, 280-287.

Ludwig, Y., Zhang, Y., and Hochholdinger, F. (2013). The maize (Zea mays L.) AUXIN/INDOLE-3-ACETIC ACID gene family: phylogeny, synteny, and unique root-type and tissue-specific expression patterns during development. *PloS one* **8**, e78859.

Luo, J., Zhou, J.-J., and Zhang, J.-Z. (2018a). Aux/IAA Gene Family in Plants: Molecular Structure, Regulation, and Function. *International journal of molecular sciences* **19**, 259.

Luo, Y., Tang, Y., Zhang, X., Li, W., Chang, Y., Pang, D., Xu, X., Li, Y., and Wang, Z. (2018b). Interactions between cytokinin and nitrogen contribute to grain mass in wheat cultivars by regulating the flag leaf senescence process. *The Crop Journal* **6**, 538-551.

Martínez-de la Cruz, E., García-Ramírez, E., Vázquez-Ramos, J. M., Reyes de la Cruz, H., and López-Bucio, J. (2015). Auxins differentially regulate root system architecture and cell cycle protein levels in maize seedlings. *Journal of plant physiology* **176**, 147-156.

Mu, X., Chen, Q., Wu, X., Chen, F., Yuan, L., and Mi, G. (2018). Gibberellins synthesis is involved in the reduction of cell flux and elemental growth rate in maize leaf under low nitrogen supply. *Environmental and experimental botany* **150**, 198-208.

Nadeem, M., Yahya, M., Tong, J., Shah, L., Khan, S. U., Ali, A., Sher, A., Ullah, N., and Waheed, A. (2023). Improving Nitrogen Acquisition and Utilization Through Root Architecture Remodelling: Insight from Legumes. *Journal of plant growth regulation*.

O'Brien, José a., Vega, A., Bouguyon, E., Krouk, G., Gojon, A., Coruzzi, G., and Gutiérrez, Rodrigo a. (2016). Nitrate Transport, Sensing, and Responses in Plants. *Molecular Plant* **9**, 837-856.

Pacurar, D. I., Perrone, I., and Bellini, C. (2014). Auxin is a central player in the hormone cross-talks that control adventitious rooting. *Physiologia plantarum* **151**, 83-96.

Power, J. F., Willis, W. O., Grunes, D. L., and Reichman, G. A. (1967). Effect of Soil Temperature, Phosphorus, and Plant Age on Growth Analysis of Barley1. *Agronomy Journal* **59**, 231-234.

Rasmussen, A., Hosseini, S. A., Hajirezaei, M.-R., Druege, U., and Geelen, D. (2015). Adventitious rooting declines with the vegetative to reproductive switch and involves a changed auxin homeostasis. *Journal of Experimental Botany* **66**, 1437-1452.

Ravazzolo, L., Trevisan, S., Forestan, C., Varotto, S., Sut, S., Dall'acqua, S., Malagoli, M., and Quaggiotti, S. (2020). Nitrate and ammonium affect the overall maize response to nitrogen availability by triggering specific and common transcriptional signatures in roots. *International journal of molecular sciences* **21**, 686.

Shahzad, Z., and Amtmann, A. (2017). Food for thought: how nutrients regulate root system architecture. *Current Opinion in Plant Biology* **39**, 80-87.

Song, W., Sun, H., Li, J., Gong, X., Huang, S., Zhu, X., Zhang, Y., and Xu, G. (2013). Auxin distribution is differentially affected by nitrate in roots of two rice cultivars differing in responsiveness to nitrogen. *Annals of botany* **112**, 1383-1393.

Sparks, E. E. (2023). Maize plants and the brace roots that support them. *The New phytologist* **237**, 48-52.

Steffens, B., and Rasmussen, A. (2016). The physiology of adventitious roots. *Plant Physiology* **170**, 603-617.

Sun, C.-H., Yu, J.-Q., and Hu, D.-G. (2017). Nitrate: A Crucial Signal during Lateral Roots Development. *Frontiers in plant science* **8**, 485-485.

Tian, Q., Chen, F., Liu, J., Zhang, F., and Mi, G. (2008). Inhibition of maize root growth by high nitrate supply is correlated with reduced IAA levels in roots. *Journal of plant physiology* **165**, 942-951.

Urban, A., Rogowski, P., Wasilewska-Dębowska, W., and Romanowska, E. (2021). Understanding Maize Response to Nitrogen Limitation in Different Light Conditions for the Improvement of Photosynthesis. *Plants (Basel)* **10**, 1932.

Van Deynze, A., Zamora, P., Delaux, P.-M., Heitmann, C., Jayaraman, D., Rajasekar, S., Graham, D., Maeda, J., Gibson, D., Schwartz, K. D., Berry, A. M., Bhatnagar, S., Jospin, G., Darling, A., Jeannotte, R., Lopez, J., Weimer, B. C., Eisen, J. A., Shapiro, H.-Y., Ané, J.-M., and Bennett, A. B. (2018). Nitrogen fixation in a landrace of maize is supported by a mucilage-associated diazotrophic microbiota. *PLoS biology* **16**, e2006352-e2006352.

Vega, A., O'Brien, J. A., and Gutiérrez, R. A. (2019). Nitrate and hormonal signaling crosstalk for plant growth and development. *Current opinion in plant biology* **52**, 155-163.

Wang, P., Wang, Z., Pan, Q., Sun, X., Chen, H., Chen, F., Yuan, L., and Mi, G. (2019). Increased biomass accumulation in maize grown in mixed nitrogen supply is mediated by auxin synthesis. *Journal of Experimental Botany* **70**, 1859-1873.

Wang, Y., Yao, Q., Zhang, Y., Zhang, Y., Xing, J., Yang, B., Mi, G., Li, Z., and Zhang, M. (2020). The Role of Gibberellins in Regulation of Nitrogen Uptake and Physiological Traits in Maize Responding to Nitrogen Availability. *International journal of molecular sciences* **21**, 1824.

Wei-Gui, L., Qi-Wen, L., Yi, S., Chao, H., Bei-Xin, M., Yu, Y., and Lang-Tao, X. (2023). Auxin inhibits chlorophyll accumulation through ARF7-IAA14-mediated repression of chlorophyll biosynthesis genes in Arabidopsis. *Frontiers in plant science* **14**.

White, P. J. (2012). Ion Uptake Mechanisms of Individual Cells and Roots: Short-distance Transport. *In* "Marschner's Mineral Nutrition for Higher Plants" (P. Marschner, ed.). Academic Press, United States of America.

Wu, B., Ren, W., Zhao, L., Li, Q., Sun, J., Chen, F., and Pan, Q. (2022). Genome-Wide Association Study of Root System Architecture in Maize. *Genes* **13**, 181.

Xu, J., Zha, M., Li, Y., Ding, Y., Chen, L., Ding, C., and Wang, S. (2015). The interaction between nitrogen availability and auxin, cytokinin, and strigolactone in the control of shoot branching in rice (Oryza sativa L.). *Plant cell reports* **34**, 1647-1662.

Yu, P., Baldauf, J. A., Lithio, A., Marcon, C., Nettleton, D., Li, C., and Hochholdinger, F. (2016). Root Type-Specific Reprogramming of Maize Pericycle Transcriptomes by Local High Nitrate Results in Disparate Lateral Root Branching Patterns. *Plant physiology (Bethesda)* **170**, 1783-1798.

Yu, P., Eggert, K., von Wirén, N., Li, C., and Hochholdinger, F. (2015). Cell Type-Specific Gene Expression Analyses by RNA Sequencing Reveal Local High Nitrate-Triggered Lateral Root Initiation in Shoot-Borne Roots of Maize by Modulating Auxin-Related Cell Cycle Regulation. *Plant physiology (Bethesda)* **169**, 690-704.

Yu, P., White, P., Hochholdinger, F., and Li, C. (2014). Phenotypic plasticity of the maize root system in response to heterogeneous nitrogen availability. *An International Journal of Plant Biology* **240**, 667-678.

Zhang, C., Li, X., Wang, Z., Zhang, Z., and Wu, Z. (2020). Identifying key regulatory genes of maize root growth and development by RNA sequencing. *Genomics (San Diego, Calif.)* **112**, 5157-5169.

Zhang, Q., Gong, M., Xu, X., Li, H., and Deng, W. (2022). Roles of Auxin in the Growth, Development, and Stress Tolerance of Horticultural Plants. *Cells (Basel, Switzerland)* **11**, 2761.

Zhao, Y. (2010). Auxin Biosynthesis and Its Role in Plant Development. *Annual review of plant biology* **61**, 49-64.

Chapter 6

General Conclusion

6.1. Abstract

Maize is widely grown for food, fodder, and bioenergy. Its production requires enormous use of nitrogen fertiliser to boost yield. But it is not clear how long it takes for the applied nitrogen fertiliser to deplete at the background (bulk) soil as compared to the rhizosphere adjacent to maize root types. Moreso, how the whole plant (both shoot and roots) would respond to nitrogen regimes needed to be made clearer in other to influence fertiliser management decision. Knowledge about the actual N uptake or net effect of nitrate and ammonium by intact maize root remained to be elucidated. By and large, the most effective means of successfully applying hormones to adult maize plants growing on soil needed to be explored in other to facilitate more research for advancement of knowledge.

To proffer solution to the problems mentioned above, this research planned to determine the amount of applied nitrogen fertiliser available for uptake by maize roots. This was to be achieved through determining the rate at which nitrate and ammonium from applied nitrogen deplete adjacent to maize crown root, seminal root and the background soil of plants growing on low and high nitrogen background. The research also planned to determine the net uptake by maize crown and seminal roots during low and high nitrogen supply. Furthermore, the maize shoot response to root nitrogen dynamics during microdialysis were determined under short-term and long-term effect. The study also explored methods of applying phytohormones to adult maize plants growing on soil and employed the best method to apply auxin to facilitate brace root emergence.

Maize plants were grown in soil inside rhizoboxes as low and high nitrogen plants in the glasshouse. At 21 days post germination, each rhizobox was opened and sprayed with one litre of either high or low nitrogen solution. Microdialysis technique was used to sample for available nitrate and ammonium ions at the background soil, adjacent to crown root and seminal root at time sequence for up to 44 hours. The samples obtained were later analysed using the calorimetric techniques. The stable isotopes of nitrogen ^{15}N were used to determine net influx/net effects of nitrate and ammonium uptake by intact maize crown and seminal roots. Meanwhile, the photosynQ (Multispec), Dualex and SPAD were used to monitor shoot response to root nitrogen dynamics. Other shoot variables such as plant heights, leaf length and width for leaf area were measured weekly using tape and photographs for image analysis at the end of the experiment. Leaf numbers were counted weekly, shoots and roots fresh and dry weights were obtained at the end of each experiment. Furthermore, techniques associated with allometric relationships were used to determine the biomass partitioning patterns as influenced by nitrogen regimes and the two ontogenetic phases. In order to explore the most effective method of applying hormones to adult maize plant growing on soil, gibberellins were either dissolved in water or lanoline at 50µM and 100 µM and applied to scratched stem of maize plants. The plants

treated with 100 µM GA in water showed the earliest effect and was then used as the concentration to apply auxin to maize stem at 8 fully expanded leaf stage.

The findings showed that ammonium rapidly depleted from the soil surface as compared to nitrate, and occurring earlier plants grown on limited nitrogen. The crown root was found to capture more ammonium during perceived low external nitrogen while the seminal roots capture more nitrate during adequate nitrogen supply. In terms of shoot response, low nitrogen availability caused warmer canopy and enabled dissipation of excess captured solar radiation, but the quantum yield of the photosystem was not affected. The leaf area duration was higher during the onset of reproductive stage as compared to the vegetative stage while high nitrogen enabled allocation of more biomass to the stem as compared to leaves and roots in low N plants. The applied auxin enabled increased number of brace root whorls and number of brace roots that emerged in both low and high nitrogen plants.

In summary, the spatio-temporal dynamics of nitrogen forms influences its availability for plant uptake. This depends on the concentration and frequency of nitrogen application as well as the different maize root types. Also observed was that the maize plants growing on low nitrogen nutrition can photosynthesise at similar rates to plants growing on adequate nitrogen supply. Thus, implying that maize plants can be grown on low nitrogen and supplied with a dose of high N intermittently to boast uptake. The maize crown roots can be optimised for low nitrogen nutrition, while the emergence of brace roots can be enhanced by auxin.

6.2. Introduction

Brief summary of the findings from thesis Chapters.

This thesis provides new insights into nitrogen uptake by maize root types and utilization efficiency (whole plant response) during low nitrogen conditions for improved nitrogen use efficiency. Nitrogen availability affects the spatiotemporal nitrogen dynamics of the soil, the activities of nitrogen influx system, and hormone homeostasis. These chemical and physiological changes can impact on root plasticity, root preferential uptake of nitrogen forms, photosynthetic efficiency and biomass allocation in a manner that shows the plants nitrogen economy. The two forms of inorganic nitrogen common in agricultural soils are nitrate and ammonium which is central to this study. Here, the spatiotemporal dynamics of nitrogen was linked to the nitrogen form and the nitrogen background of the soil containing the growing plants. The frequency and pattern of the depletion of nitrogen forms in the background soil and adjacent to root types varies with the nitrogen background of the soil containing the growing plants and the concentration of nitrogen applied (Chapter 2). These uptake pattern also influenced the nitrogen budget of the plants as regarding photosynthetic efficiency (Chapter 3),

including the size of assimilatory organs and partitioning of internal nitrogen between the photosynthetic (light harvesting, electron transport, carbon reduction) and non-photosynthetic component. Biomass allocation to functional organs (Chapter 4), including roots, stems and leaves during limited nitrogen supply. The role of nitrogen and auxin in shaping root system architecture of plants cannot be over emphasized. This root plasticity role was seen in the increased number of brace roots in plants treated with auxin (IAA) as compared the controls (Chapter 5). By and large, all these varying physiological responses to nitrogen rates could provide insight into the activities of nitrogen responsive genes and other transcriptional factors that interact in the hormonal pathway. Those molecular players could be unravelled through mutant studies and cell sorting approaches. This will enable the optimization of root system architecture for improved uptake of N sources, manipulation of photosynthetic efficiency by nitrogen rates, and manipulation of carbon allocation to varying organs to improve the overall nitrogen use efficiency of the plant and maximise yield at a minimal cost.

The specific objectives and main findings were:

Chapter 2 (Nitrogen uptake); To determine the spatio-temporal dynamics of nitrogen forms in soil during maize growth and to measure nitrogen uptake efficiency of maize root types during suboptimal and adequate nitrogen availability. Findings suggest that ammonium rapidly disappeared from the soil surface as compared to nitrate. The depletion rate was earlier in soils of low nitrogen background and when nitrogen resupply is of lower concentration. Root type comparisons shows that the maize crown root captured more ammonium during perceived low external nitrogen by plants growing on both low and high nitrogen background soils. While the maize seminal roots tended to capture more nitrate during adequate nitrogen supply. The differences in the uptake rate of nitrogen forms by crown and seminal roots did not correlate with the root diameter. Surprisingly the nitrogen uptake ability of maize plants growing on low nitrogen background was lower than plants grown on adequate nitrogen irrespective of the concentration of the nitrogen resupplied. By and large, the use of rhizoboxes for nutrient depletion study may require some modification for effectiveness.

Chapter 3 (Nitrogen Utilization (Photosynthetic nitrogen efficiency)); To determine short-and long-term shoot response to soil nitrogen fluxes after nutrient resupply. Findings suggest that just one-time spray of high N solution slightly elevated the chlorophyll levels of low N plants within the three days of root nitrogen interaction. While low nitrogen availability caused warmer canopy (increased leaf temperature differential) in both low and high N plants 48 hours after spraying low nitrogen. The influence of nitrogen level on the partitioning of captured photosynthetically active radiation (PAR) showed that ϕNPQt (heat dissipation of chlorophyll excitation energy) increased in low N plants after 24 hours of nutrients resupply, ϕNO(Potentially harmful energy) were released by low N plants on the

second day after treatment and more ϕNPQ were quenched in all low N plants irrespective of treatment received for safety reasons. The exchange of electron between photosystem I and photosystem II as well as the quantum yield of the photosystem was not affected by growth condition or nitrogen resupply. However, flavonoid levels (stress response) were temporarily elevated immediately after spraying the rhizosphere of low N plants with high nitrogen. Findings from the long-term effect of a pulse of high nitrogen on low N plants showed that high nitrogen enabled the low N plants to tassel a week earlier, whilst the estimated net assimilation rate increased in the vegetative stage as compared to reproductive stage in low N plants. Collectively, the relative growth rates of all plants peaked at 21 days after germination which was the time that nutrient pulses were applied. The leaf area duration was higher during the onset of reproductive stage as compared to the vegetative stage.

Chapter 4 (Nitrogen utilization (Biomass allocation)); To determine the effect of nitrogen resupply to adequately fed and sub-optimally fed maize over time. Findings suggest that the root to shoot ratio was not affected by growth condition. But a single dose of high nitrogen enabled allocation of more biomass to the stem as compared to leaves and roots in low N plants. Ontogenetic phase analysis showed that the vegetative stage of low N plants had higher root to shoot ratio than the reproductive stage which was also higher than the vegetative stage of high N plants, while the moisture contents of roots were higher during the reproductive stage than the vegetative stage in both low and high N plants. Leaf physical properties such as the leaf thickness and weight were influenced by growth condition.

Chapter 5 (Nitrogen and auxin interaction); To determine the most effective method of hormone application in adult maize, and to measure the influence of auxins on emergence of brace roots as well as whole plants response. Findings suggest that auxin enable increased emergence of brace root whorls and brace roots number in both low and high nitrogen plants, while the chlorophyll related parameters as well as plant height and leaf area were not significantly influenced by auxin.

All in all, this research brought new insight into the variability of soil nitrogen forms in both space and time. Bringing to light an important parameter for decision making regarding the form of nitrogen to be used. This could be in terms of the ratio of nitrate to ammonium that commensurate with the micro-environment where maize will be cultivated. The findings could also be used to modify the existing fertilizer application methods and timing of application, for the purpose of maximising nitrogen uptake and minimising nitrogen losses. The study showed that the uptake abilities of maize root types based on nitrogen forms were influenced by growth conditions. The important findings could assist in the identification of nutrient transporters that are expressing at varying levels in

different root types during homogenous low nitrogen condition. Knowing that will help in modifying the root system architecture towards optimizing transporter activities for improved nitrogen uptake. This will mean either breeding for more or less of specific roots considering the nitrogen cost and benefit of constructing new roots that will enhance nitrogen uptake. The study also looked at photosynthetic efficiency of maize under low nitrogen nutrition with a goal of identifying nitrate responsive genes that interacts with hormones to improve the photosynthetic efficiency of maize plants under low nitrogen conditions. As findings showed a significant increase in chlorophyll levels of plants grown on low nitrogen background after a 24 hours of high nitrogen treatment. This important finding points to the resilience and nutrient efficiency of low N plants that could be adopted to increase the farmers profit margin while minimising environmental pollution. Also, biomass allocation patterns give insight into the structural priority of maize plants during nitrogen budget. The influence of auxin on the emergence of maize brace roots showed that exogenous hormones could be used to influence the root system architecture of maize based on the prevailing micro-environment. More so it could be useful to plant breeders deciding on breeding for more crown roots which are also stem or shoot borne as the brace roots.

6.3. Summary of discussion in relation to research aims:

6.3.1. To determine the spatio-temporal dynamics of nitrogen forms in soil during maize growth:

Nitrogen in soil varies with space and time. This variation involves chemical changes associated with the interaction of soil microbes and the plant roots in the soil environment(Hawkesford and Whalley, 2021; Jackson et al., 2008) (Jackson et al., 2008) In such instances, ammonium or nitrate fluxes may be undetected in soil when plant uptake and microbial nitrogen transformation is rapid due to rapid and multiple turnovers of N pools per day (Jackson et al., 2008). The monitoring of real time fluxes of the applied soil nitrogen (Chapter 2) showed that nitrate and ammonium initially were available at the background soil and adjacent to roots but depleted variably depending on the concentration of nitrogen sprayed and initial nitrogen background. Fastest depletion of ammonium occurred during low soil nitrogen conditions both in the background soil and adjacent to plant roots. While the depletion rate of nitrate was fastest in the background during high soil nitrogen condition and varied with root type. The rapid depletion of ammonium can be partly attributed to plant uptake (as substantiated by the N uptake experiment) and partly by possible soil microbial interaction and/or ammonium volatilisation. This finding could mirror previous studies that demonstrated capture of more ammonium by microbes and crops as compared to nitrate (Jackson et al., 2008). However, my experimental settings (Klassman silver sand:compost 1:1 soil mix, of low nitrogen in rhizobox in the glasshouse) was different from theirs (annual grassland) which create a need to research into the suitability of the rhizobox in nutrient management related studies. Also, because the nitrogen solution

was surface applied it is prone to the risk of volatilisation (Fageria, 2014; Marschner and Rengel, 2012) This can reduce the available nitrogen for root uptake.

6.3.2. To measure nitrogen uptake efficiency of maize root types during suboptimal and adequate nitrogen availability:

The rate of nitrogen uptake by maize root types differed with the nitrogen source and growth condition. The maize crown roots took up more nitrogen than seminal roots during low nitrogen condition and the most preferred N source during such times was ammonium. While the seminal root took more nitrate than crown root during high soil nitrogen availability. This outcome supported previous findings about the maize crown root as being the principal nitrogen and water supplier (Dechorgnat et al., 2018; Saengwilai et al., 2014; Steffens and Rasmussen, 2016) and a viable trait for genetic improvement for low nitrogen soils (Saengwilai et al., 2014; York et al., 2013; Yu et al., 2014b). Whether this nitrogen uptake ability of the crown roots especially during low nitrogen supply, is connected to the nitrogen demand from young developing leaves is yet to be studied. Also, it is important to investigate into the transporter density of the plasma membranes of crown root and seminal roots and their individual laterals during different nitrogen and water regimes for the purpose of advancing the field of maize root research for improved nitrogen use efficiency and global food security.

6.3.3. To determine short-and long-term shoot response to soil nitrogen fluxes after nutrient resupply:

It is generally accepted that low nitrogen conditions reduce the leaf area, leaf area duration, leaf nitrogen content and chlorophyll as well as photosynthesis per unit of leaf area in crops (Cormier et al., 2016; Fageria, 2014). In this study (Chapter 3), plant responses relating to photosynthetic efficiency showed that plants on low nitrogen background did not stop or reduce their photosynthetic capacity even under low nitrogen supply. This was evident from the lack of difference between treatment on the rate of electron exchange between the photosystem I and photosystem II as well as the quantum yield of the photosystem II. However, low nitrogen conditions subjected the plants to give up excess heat and quenched some of the captured solar radiation as safety mechanism to avoid damage to the photosystems. This finding supports the report that mild nitrogen deficiency affects radiation use efficiency (RUE) more than the intercepted photosynthetically active radiation (PAR) in sunflower, sorghum, and maize (Gastal. et al., 2015; Hirel et al., 2007). More so, the significant increase of chlorophyll level by low N plant after 24 hours of receiving high nitrogen solution is novel. This buttresses how fast molecular activities takes place to meet the plant immediate needs.

6.3.4. To determine the effect of nitrogen resupply to adequately fed and sub-optimally fed maize over time:

The quantity of photosynthetic active radiation intercepted by crops during its life cycle relates to the biomass. Biomass allocation patterns resulting from nutrients limitation are the strongest of allocation responses with higher allocation to the roots at the expense of the stem and particularly of leaf biomass (Poorter et al., 2012). However, a pulse of high nitrogen enabled structural adjustment in favour of the stem as compared to the leaves and the roots like the plants growing on adequate nitrogen availability (Chapter 4). This adjustment in functional equilibrium in allometric relationship as influenced by nitrogen supply gives a clue about the most important need of the plants. By and large, the priority given to the stem may mean a demand for more recycling of photo assimilate and more translocation of water and nutrients from the roots. This is because the stem houses the vascular tissues involved in remobilization of nutrients from older leaves to young leaves (phloem) through the so-called long-distance travel and transporting nutrients and water to the leaves (xylem). The partitioning of harvested plants organs control yields (Engels. et al., 2012).

6.4. Comparison of the performance of the two maize lines used in the study

This study used the KWS recombinant inbreed lines for the nitrogen uptake and utilization studies (Chapter 2, 3, 4), while the B73 recombinant inbred lines were used for the hormone studies (Chapter 5). The reason a different line was used to contribute to a bigger collaboration with the Sparks lab in the US. I successfully developed a method for the application of hormones to maize and was able to change the number of emerged braces roots.

While I didn't measure the uptake of nitrogen in B73, Dechorgnat and colleagues showed that ammonium was preferred over nitrate (Dechorgnat et al., 2018) in B73 lines, which is similar to my work with KWS lines. My study investigated these patterns in multiple soil conditions and found this affinity for ammonium as compared to nitrate increased under continuous low nitrogen condition in the experimental KWS lines. It is unclear if that is also true about the B73 recombinant inbred lines, highlighting the need for more studies to ascertain if the two inbred lines share similar genetic root trait and root system architecture which can modify the selectivity of nitrogen form.

On the other hand, I measured detailed shoot parameters for both lines during all my experiments and observed a marked contrast in the shoot response between the two lines to nitrogen rates. Low nitrogen significantly reduced the nitrogen balance index and chlorophyll of KWS (Chapter 3), while no visible or significant difference was seen in B73 (Chapter 5). The same was observed for plant height and leaf area during the vegetative stages of both inbred lines. The contrasting observation was

surprising because the nutrient solution used for all the experiment were the same concentration per treatment type (Low and High N), grown in the rhizobox in the glasshouse. This shows differences in nitrogen assimilation and metabolism between these genotypes.

Phenotypic variation between genotypes is important for identification of genes associated with traits of interest to the breeder. As a crop physiologist, I identified some of those hidden traits and made it known for further studies and applications. Here, I suggest the profiling and characterization of dominant genes associated with nitrogen metabolism in B73 and KWS lines. This should be done under low nitrogen condition for more understanding of the process that contributes to nitrogen use efficiency.

6.5. *Contribution to knowledge:*

My research investigated real-time flux rates of nitrate and ammonium in soils adjacent to or far from maize root types. This was made possible using microdialysis which provides real time information about the concentration of nutrients ion at *in situ* with minimal soil disturbance. No one has ever worked in this area before. Some nitrogen uptake research in maize used hydroponics (Dechorgnat et al., 2018; Garnett et al., 2013), while others in sort of soils (Yu et al., 2014a). Microdialysis was used to sample nitrogen ions from sugarcane farm (Brackin et al., 2015), maize (Brackin et al., 2017), multilevel soil sampling (Buckley et al., 2017). But no one was able to monitor the depletion in flux rate at real time sequentially. Also, during this real time measurement of nutrient fluxes, the plants response to these nutrient dynamics was also monitored giving real time understanding of whole plant response. This is new in nutrient uptake research.

I have also explored the effective method of administering hormone in adult maize plants growing in soil using gibberellins. The most effective method was applying hormone dissolved in water to the scratched lower stem area of maize plant. I used the method in applying auxin (IAA) to score the emergence of maize brace root and was successful. I also monitored the shoot response before hormone application and weeks after application.

6.6. Wider implication of the study:

This study showed that ammonium rapidly depleted variably on the soil surface during low and high soil nitrogen condition. This implies that the type of nitrogen fertiliser that will be used for maize growing on specific soil condition is important for improved N uptake. Therefore, the ratio of ammonium to nitrate in a combined fertiliser should be done according to the prevailing micro-environment. For instance, soil tests should be carried out and climatic data of the farm location should be studied for the purpose of fertilizer recommendation. This will enable fertiliser producers

make combinations that is appropriate for each geographical locations were maize is grown. This is important because higher proportion of ammonium in a fertilizer should not be used in farmlands where the soil is sandy especially in the dry, semi-dry tropical areas, if the goal is to maximise yield at minimal cost. Even though maize is predominantly grown in the tropics and ammonium is the preferred N as demonstrated in this present study, preferential uptake of N sources may differ with different varieties or lines. For instance, Dechorgnant and colleagues found the inbred lines B73 having more affinity for ammonium and F 44 lines for nitrate. This differences in the preference of N sources were traced to their root morphology and root system architecture (Dechorgnat et al., 2018).

The ability of the low N plants to continue to effectively photosynthesise, as revealed by the similarities in linear electron transport rate and quantum yield of the photosystem II among treatment here is a pointer to the activities of genes and phytohormones associated with nitrogen metabolism. Management practices relating to fertiliser application regime and quantity can be adopted to improve yield of maize. For instance, maize can be grown on low nitrogen and subsequently provided with a supplementary high dose of nitrogen fertiliser to boost yield and minimise cost of production. In this study, high pulse of nitrogen solution increased the nitrogen balance index and chlorophyll levels of maize growing on low nitrogen. This is because supplementary application of high nitrogen will trigger the biosynthesis of cytokinin (Gu et al., 2018; Pavlů et al., 2018; Vega et al., 2019). Cytokinin will trigger the production of PEPC and PPDK which are key enzymes of photosynthesis that are affected by low nitrogen condition as RuBisco is not affected by nitrogen rates (Urban et al., 2021) . This will collectively help to increase yield since plant biomass and yield directly relates to the photosynthesis of plants.

6.7. Future work:

To advance in our knowledge of nitrogen nutrition in maize, more work is required in other to improve our understanding of nitrogen uptake, assimilation and utilization in maize and they are as follows:

6.7.1. Soil nitrogen dynamics:

This study employed the microdialysis techniques on rhizobox soils containing growing maize plants to determine the real time fluxes of nitrate and ammonium over sampling period of 44 hours. Similar studies with control experiment without live plants in the rhizobox soil for the sampling duration is needed. This will give a clue about the plants influence on the nitrogen dynamics. This can also be carried out with different soil types differing in texture and pH value. Additional study on the interaction between water and nitrogen on the soil nitrogen dynamics is required to ascertain the influence of soil moisture on the uptake of nitrate and ammonium. This may mean employing both homogenous and heterogenous nitrogen supply methods.

6.7.2. Microbial interaction:

The interaction of microbes, arbuscular mycorrhizal with the different root types during low and adequate nitrogen can be studied in a soil-plant system, both in the field and the glasshouse to understand the contribution of soil biodiversity on nitrogen availability to plants. Water can be introduced as factorial design and the shoot response should also be measured. Microbial biomass in terms of carbon and nitrogen ratio in soil-plant system should be investigated.

6.7.3. Nitrate and ammonium transporter density:

There is need to further study the nitrate and ammonium transporter density at the plasma membrane of maize root types (Brace, crown, seminal and primary) during low and adequate nitrogen condition. This can be extended to incorporate water availability in a factorial design. Simultaneously, the shoot response should equally be monitored to broaden our understanding of the regulatory mechanism controlling the plants nitrogen demand pathway. Further research is also required on the regulation of the nitrate and ammonium low affinity transporters (LATs) throughout the plants developments programmes. Ammonium (LATs) has not been characterized in plants, clearly showing a gap in knowledge of the regulatory processes of ammonium uptake. Furthermore, it will be great to know the direct contribution of ammonium uptake to maize nitrogen economy at molecular and physiological levels (Cormier et al., 2016; Gu et al., 2013). Post transcriptional regulation of N uptake remained to be explored as no miRNA has been identified and characterised for the regulation of the nitrate and ammonium transporters (Plett et al., 2018).

6.7.4. Root system architecture:

The root system architecture is the driving force behind plants survival. A study on the impact of water and nitrogen on root growth angle, root depth, total root length, root hairs, lateral root density and root numbers of each root type among maize genotypes is important. This should be done in the fields having different soil characteristics and in the control systems. Monitoring the impact of the scarce resources on root system architecture should be carried out at both vegetative and reproductive stages to help understand the influence of component traits on nitrogen and water uptake. Direct and indirect selection of maize phenotypes with longer root hairs could usher in the opportunity to maximise nitrogen and water capture.

6.7.5. Nitrogen assimilation during ontogenetic phases:

Full scale analysis of gene expression and localization of GS isoenzymes in developing and senescence leaves and reproductive tissues, from contrasting cultivars during low nitrogen and water availability is required. This was done in wheat (Cormer et al., 2016). This will broaden our understanding on the

regulatory control of nitrogen assimilation for the identification of key genes that can be manipulated to improve nitrogen assimilation after uptake.

6.7.6. Photosynthetic efficiency:

The photosynthetic components of maize plants should be studied under adequate and limited nitrogen and water deficit condition. This will help expose the level of maize tolerance to the scarcity of two major resources. Furthermore, the quality and quantity of photo assimilate during the scarcity of nitrogen and water is worth looking at. Measuring of the stem flow during these conditions this should incorporate the plants ontogenetic phases from seedlings stage up to post-silking stage. Physiological avenues for increasing fractional interception of solar radiation under low nitrogen condition should be explored. This should involve genotypic study of different phenotypes with contrasting leaf angle, leaf area and stay-green leaf characteristics under low nitrogen and water availability. Direct and indirect selection of for phenotypes with lower canopy temperature during limited water and nitrogen will assist in Identifying the molecular players of such characteristics for improved photosynthetic efficiency in maize other cereals.

6.7.7. Exogenous application of phytohormones:

Phytohormones are chemical messengers that signals specific developmental programmes of plants, and so are important aspect to research for improve nitrogen use efficiency. The exogenous application of cytokinin during adequate and low nitrogen condition should be explored in the light of its influence on the reduction or delaying of senescence of older leaves. The role of Abscisic acid (ABA) ethylene and auxin should pe expounded during low nitrogen and water availability. This should involve the identification of key genes and other transcription factors that crosstalk during nitrogen metabolism. This should be extended among genotypes of contrasting performance.

6.7.8. Genome-wide study:

Genome-wide studies on root system architecture, plants nitrogen content, biomass and yield response to nitrate and ammonium required more attention (Kant, 2018). The molecular aspects of brace roots initiation can be studied in order to identify and characterise the genes and other transcription factors involved. This important because previous study has shown evidence of of exceptional capabilities of maize brace roots in terms of resource capture ((Van Deynze et al., 2018; Yu et al., 2016).

6.8. Conclusion:

Soil nitrogen availability varies with space and time. This affects the amount of nitrogen available for crop uptake. The ability of maize plants to take up nitrogen depends on the concentration of nitrogen in the growing medium, the form of nitrogen and the frequency of application which affects transporter expression at the root plasma membrane. These variables influence specific gene expression pattern that regulate root-type uptake pattern in maize. The same variables affect the shoots nitrogen content and budget of the plant (shoot response). Other signals that alter with the root system architecture is the hormone metabolic pathway. Our knowledge of these processes including genotypic differences among phenotypes, will provide avenue for root optimization with the goal of improving uptake and utilization for improved nitrogen use efficiency in maize and other crops.

6.9. References:

Brackin, R., Atkinson, B. S., Sturrock, C. J., and Rasmussen, A. (2017). Roots-eye view: Using microdialysis and microCT to non-destructively map root nutrient depletion and accumulation zones. *Plant, cell and environment* **40**, 3135-3142.

Buckley, S., Brackin, R., Näsholm, T., Schmidt, S., and Jämtgård, S. (2017). Improving in situ recovery of soil nitrogen using the microdialysis technique. *Soil biology & biochemistry* **114**, 93-103.

Cormier, F., Foulkes, J., Hirel, B., Gouache, D., Moënne-Loccoz, Y., and Le Gouis, J. (2016). Breeding for increased nitrogen-use efficiency: a review for wheat (T. aestivum L.). *Plant breeding* **135**, 255-278.

Dechorgnat, J., Francis, K., Dhugga, K. S., Rafalski, J. A., Tyerman, S. D., and Kaiser, B. N. (2018). Root Ideotype Influences Nitrogen Transport and Assimilation in Maize. *Frontiers in Plant Science* **9**, undefined-undefined.

Engels., C., Kirkby., E., and White., P. (2012). Mineral nutrition, Yield and Source-sink relationship. *In* "Marschner's Mineral Nutrition of Higher Plants" (P. Marschner, ed.). Academic Press, United States of America.

Fageria, N. K. (2014). "Nitrogen Management in Crop Production," First edition/Ed. CRC Press, Boca Raton.

Garnett, T., Conn, V., Plett, D., Conn, S., Zanghellini, J., Mackenzie, N., Enju, A., Francis, K., Holtham, L., Roessner, U., Boughton, B., Bacic, A., Shirley, N., Rafalski, A., Dhugga, K., Tester, M., and Kaiser, B. N. (2013). The response of the maize nitrate transport system to nitrogen demand and supply across the lifecycle. *New Phytologist* **198**, 82-94.

Gastal., F., Lemaire., G., Durand., J.-L., and Louarn., G. (2015). Quantifying crop responses to nitrogen and avenues to improve nitrogen-use efficiency. *In* "Crop Physiology

Applications for Genetic Improvement and Agronomy" (V. O. S. a. D. F. Calderini, ed.), pp. 161-206. Academic Press, United Kingdom.

Gu, J., Li, Z., Mao, Y., Struik, P., Zhang, H., Liu, L., Wang, Z., and Yang, J. (2018). Roles of nitrogen and cytokinin signals in root and shoot communications in maximizing of plant productivity and their agronomic applications. *Plant Science* **274**, 320-331.

Gu, R., Duan, F., An, X., Zhang, F., Von Wirén, N., and Yuan, L. (2013). Characterization of AMT-mediated high-affinity ammonium uptake in roots of maize (Zea mays L.). *Plant and cell physiology* **54**, 1515-1524.

Hawkesford, M. J., and Whalley, W. R. (2021). Advances in the understanding of nitrogen uptake by plant roots. *In* "Understanding and improving crop root function" (P. Gregory, ed.), Vol. 1, pp. Pages 303-320. Burleigh dodds, United Kingdom.

Hirel, B., Le Gouis, J., Ney, B., and Gallais, A. (2007). challenge of improving nitrogen use efficiency in crop plants: towards a more central role for genetic variability and quantitative genetics within integrated approaches. *Journal of experimental botany* **58**, 2369-2387.

Jackson, L. E., Burger, M., and Cavagnaro, T. R. (2008). Roots, Nitrogen Transformations, and Ecosystem Services. *Annual review of plant biology* **59**, 341-363.

Kant, S. (2018). Understanding nitrate uptake, signaling and remobilisation for improving plant nitrogen use efficiency. *Seminars in Cell and Developmental Biology* **74**, 89-96.

Marschner, P., and Rengel, Z. (2012). Nutrients availability in soils. *In* "Marschners Mineral Nutrition for Higher Plants" (P. Marschner, ed.), pp. 315-328. Academic Press, United Kingdom.

Pavlů, J., Novák, J., Koukalová, V., Luklová, M., Brzobohatý, B., and Černý, M. (2018). Cytokinin at the Crossroads of Abiotic Stress Signalling Pathways. *International journal of molecular sciences* **19**.

Plett, D. C., Holtham, L. R., Okamoto, M., and Garnett, T. P. (2018). Nitrate uptake and its regulation in relation to improving nitrogen use efficiency in cereals. *Seminars in Cell and Developmental Biology* **74**, 97-104.

Poorter, H., Niklas, K. J., Reich, P. B., Oleksyn, J., Poot, P., and Mommer, L. (2012). Biomass allocation to leaves, stems and roots: meta-analyses of interspecific variation and environmental control. *The New phytologist* **193**, 30-50.

Saengwilai, P., Tian, X., and Lynch, J. P. (2014). Low Crown Root Number Enhances Nitrogen Acquisition from Low-Nitrogen Soils in Maize. *Plant physiology (Bethesda)* **166**, 581-589.

Steffens, B., and Rasmussen, A. (2016). The physiology of adventitious roots. *Plant Physiology* **170**, 603-617.

Urban, A., Rogowski, P., Wasilewska-Dębowska, W., and Romanowska, E. (2021). Understanding Maize Response to Nitrogen Limitation in Different Light Conditions for the Improvement of Photosynthesis. *Plants (Basel)* **10**, 1932.

Van Deynze, A., Zamora, P., Delaux, P.-M., Heitmann, C., Jayaraman, D., Rajasekar, S., Graham, D., Maeda, J., Gibson, D., Schwartz, K. D., Berry, A. M., Bhatnagar, S., Jospin, G., Darling, A., Jeannotte, R., Lopez, J., Weimer, B. C., Eisen, J. A., Shapiro, H.-Y., Ané, J.-M., and Bennett, A. B. (2018). Nitrogen fixation in a landrace of maize is supported by a mucilage-associated diazotrophic microbiota. *PLoS biology* **16**, e2006352-e2006352.

Vega, A., O'Brien, J. A., and Gutiérrez, R. A. (2019). Nitrate and hormonal signaling crosstalk for plant growth and development. *Current opinion in plant biology* **52**, 155-163.

York, L. M., Nord, E. A., and Lynch, J. P. (2013). Integration of root phenes for soil resource acquisition. *Frontiers in plant science* **4**, 355-355.

Yu, P., Baldauf, J. A., Lithio, A., Marcon, C., Nettleton, D., Li, C., and Hochholdinger, F. (2016). Root Type-Specific Reprogramming of Maize Pericycle Transcriptomes by Local High Nitrate Results in Disparate Lateral Root Branching Patterns. *Plant physiology (Bethesda)* **170**, 1783-1798.

Yu, P., Li, X., Yuan, L., and Li, C. (2014a). A novel morphological response of maize (Zea mays) adult roots to heterogeneous nitrate supply revealed by a split-root experiment. *Physiologia plantarum* **150**, 133-144.

Yu, P., White, P., Hochholdinger, F., and Li, C. (2014b). Phenotypic plasticity of the maize root system in response to heterogeneous nitrogen availability. *An International Journal of Plant Biology* **240**, 667-678.