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BIOMONITORING OF AMMONIA DEPOSITION BY MEANS OF HIGHER PLANTS

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By

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DECLARATION

I hereby declare that this doctoral thesis has been completely and independently written by me. In addition, I also confirm that no other sources than those specified in this thesis have been used. I therefore declare that this thesis, in the current or similar format has not been submitted to any other institute in order to obtain a PhD or any other academic degree.

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DEDICATION

This research work is dedicated to Almighty God, for HIS boundless love, mercies and favors all my life especially throughout my academic career. May HIS name be praised and glorified forevermore.

TABLE OF CONTENTS

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LIST OF FIGURES

LIST OF TABLES

LIST OF ABBREVIATIONS

ANOVA:	Analysis of Variance
Ala:	Alanine
Arg:	Arginine
Asn:	Asparagine
Asp:	Aspartic acid
C:	Carbon
CH_4 :	Methane
CO_2:	Carbon dioxide
DFG:	German Research Foundation
EEA:	European Environmental Assessment
EU:	European Union
FAO:	Food and Agricultural Organization
FE:	Fertilizer Europe
GABA:	Gamma-aminobutyric acid
Gln:	Glutamine
Glu:	Glutamic acid
Gly:	Glycine
GS:	Glutamine synthetase
ha:	Hectare
His:	Histidine
ICP:	International Cooperative Programme
Ile:	Isoleucine
IRTG:	Internal Research Training Group
kg:	Kilogram
Leu:	Leucine

Lys:	Lysine
m:	Metre
Met:	Methionine
m^{-3}:	Per cubic metre
N:	Nitrogen
NCP:	North China Plain
NEC:	National Emissions Ceiling
NFA:	Non-filtered air
NFA+:	Non-filtered air fumigated with low NH_3 concentration
NFA++:	Non-filtered air fumigated with high NH_3 concentration
NH_3:	Ammonia
NH_4^+:	Ammonium
NO:	Nitrogen Oxide
NO_3^-:	Nitrate
N_2O:	Nitrous Oxide
NOx:	Nitrogen Oxides
O_3:	Ozone
PAH:	Polycyclic Aromatic Hydrocarbon
Phe:	Phenylalanine
PM:	Particulate Matter
ppb:	Parts per billion
ppm:	Parts per million
Pro:	Proline
r^2 :	Coefficient of determination
Ser:	Serine
SO_2:	Sulphur dioxide
Tg:	Teragram

Thr:	Threonine
Trp:	Tryptophan
Tyr:	Tyrosine
UNECE:	United Nations Economic Commission for Europe
Val:	Valine
yr^{-1}:	Per year
µg:	Microgramm
^{o}C:	Degree Celsius
% :	Percent

SUMMARY

Atmospheric nitrogen deposition emanating from oxidized or reduced nitrogen sources has been influenced immensely by human activities. This is as a result of the need to improve and meet the ever changing demands of an increasing growth in global population. The benefits accrued from such activities however, have not been without some negative effects on several ecosystems, plants, air quality and human health. This is due to the emission of reactive nitrogen species and its contribution to the level of atmospheric nitrogen pollution in the environment as well as nitrogen deposition afterwards. Atmospheric ammonia (NH_3) arguably is an important source of nitrogen deposition. Its major source is from agricultural activities involving various aspects of crop production including, fertilizer and manure applications among others and also importantly from livestock management. It is pertinent therefore, to conduct continuous monitoring studies in order to ascertain the prevailing ambient NH_3 concentration in an area, so as to identify periods when threshold values are exceeded and also to determine how certain plants would respond when exposed to NH_3 pollution.

This necessitated the need to investigate in this thesis, through active biomonitoring, the interaction of NH_3 pollution on selected indicator species namely, Italian ryegrass (*Lolium multiflorum* L.), barnyard grass (*Echinochloa crus-galli* L.), stinging nettle (*Urtica dioica* L.) and common lambsquarters (*Chenopodium album* L.). The influence of nitrogen deposition, arising from NH_3 pollution on the selected indicator species were examined by the responses of the free amino acids and above ground biomass accumulation of the various plants studied, as an indicator of nitrogen accumulation. In order to execute plant and atmospheric NH_3 interactions, two different experiments were conducted.

The first experiment was a field study carried out around a livestock farm as a source of NH_3 pollution and nitrogen deposition. Plant materials were exposed alongside passive diffusion tube samplers at three selected distances from the stable along a transect of 804m. The three different sites were selected with increasing distance from the stable, in order to enable a comparison between the plants exposed in close proximity to the source NH_3 emission and those further away. The ambient NH_3 concentration at each site was measured with the passive diffusion tube samplers exposed at each location. This measurement was conducted with a view to determine the ambient NH_3

concentration exposed to the plant materials at each site and also to observe the influence of increasing distance on NH_3 pollution and its exposure on the plants, from a point source of NH_3 pollution. Furthermore, two fumigation experiments were conducted under controlled greenhouse conditions. In the fumigation study, only *Lolium multiflorum* and *Echinochloa crus-galli*, plants were used for this experiment and exposed to three treatment levels of gaseous NH_3 fumigations in different growth chambers. The plants were exposed to the following treatments, non-filtered air (NFA), non-filtered air with low NH_3 concentration (NFA+) and non-filtered air with high NH_3 concentration (NFA++) in both fumigation experiments.

In the field experiment, the ambient NH_3 concentration measured at each location from the stable, decreased with increasing distance from the point of NH_3 emission. This decrease in concentration of NH_3 clearly demonstrates the impact of livestock management as a source of NH_3 pollution. The free amino acid concentrations and compositions investigated in the various plants studied in the field experiments showed a significant response to NH_3 exposure. Several fold increases in the free amino acid concentrations and changes in composition were observed in plant materials exposed to increasing NH_3 concentrations at closer proximity to the stable. Observations made from this study showed that an increase in NH_3 concentration with closer distance to the source of NH_3 pollution influenced remarkably, the percentage increases of low carbon to nitrogen compounds such as Glutamine (Gln) in *Lolium multiflorum* and *Chenopodium album*, Asparagine (Asn) in *Echinochloa crus-galli* and Arginine (Arg) in *Urtica dioica*. The increases and alterations observed in the free amino acid compositions of the plants studied, demonstrates the uptake and sensitivity of the various plants to NH_3 pollution and nitrogen deposition by inducing changes in its free amino acid metabolism. The effects of nitrogen deposition on the above ground biomass of the plants in the field study, indicated a significant effect of the ambient NH_3 concentrations on *Lolium multiflorum*, *Echinochloa crus-galli* and *Urtica dioica*. These findings demonstrate a positive influence of NH_3 pollution as a nitrogen source on growth and biomass accumulation in the plants.

In the controlled fumigation experiments, the exposure of gaseous NH_3 had a positive influence on above ground parameters in *Lolium multiflorum* and *Echinochloa crus-galli.* These effects however, were not statistically significant except in leaves of *Lolium multiflorum* in the first set of the fumigation experiment. However, changes in biomass

compositions observed under the various gaseous NH_3 treatments, is an indication of the plants response to atmospheric NH_3 uptake and its ability to metabolize and incorporate nitrogen from atmospheric NH_3 for growth. Results obtained from the fumigation experiments also showed the influence of gaseous NH_3 on free amino acid metabolites in *Lolium multiflorum* and *Echinochloa crus-galli* across the NFA, NFA+ and NFA++ treatments. The gaseous NH_3 concentrations induced some significant effects and influenced increases in amino acids and accumulation in low C/N ratio compounds such as Asn, Gln and Alanine (Ala), but also Glutamic acid (Glu).

Considering the results obtained in this study based on the responses of the plants to atmospheric NH_3 pollution in the field and in the fumigation studies, it is obvious NH_3 had an influence over the growth and metabolism of the plants studied. This influence indicates the plants were able to detect changes in the ambient NH_3 concentrations in the environment and responded by exhibiting changes in biomass production and alterations in free amino acid compositions, thus indicating they have good potentials as biomonitors of ammonia deposition.

ZUSAMMENFASSUNG

Die Stickstoffdeposition aus der Atmosphäre hat sich durch menschliches Handeln deutlich erhöht, dies betrifft sowohl oxidierte als auch reduzierte Stickstoffverbindungen. Grund für den Anstieg des globalen Stickstoffumsatzes ist die steigende Weltbevölkerung und deren Nachfrage nach Nahrung, industriellen Produkten und Transportleistungen. Die Emission und Luftbelastung mit reaktiven Stickstoffverbindungen hat negative Auswirkungen auf verschiedene Ökosysteme, die Vegetation und auf die Luftqualität im Hinblick auf die menschliche Gesundheit. Ammoniak (NH_3) in der Atmosphäre stellt eine bedeutende Quelle für die Stickstoffdeposition dar. Hauptquelle für NH_3-Emissionen ist die Landwirtschaft mit den wichtigsten Sektoren Viehhaltung, Gülle-Ausbringung und mineralischer Düngung. Im Gegensatz zu anderen Luftschadstoffen existiert für NH_3 kein flächendeckendes Immissionsmessnetz. Gleichwohl ist es wichtig, die NH_3-Belastung zu erfassen, Grenzwertüberschreitungen zu erkennen und zu untersuchen, wie die Vegetation auf NH_3-Deposition reagiert.

Ziel dieser Arbeit war, die Reaktion ausgewählter Pflanzenarten auf Ammoniak zu untersuchen und zu testen, ob diese Arten als Bioindikatoren für NH_3-Deposition geeignet sind. Als Testarten wurden Italienisches Weidelgras (*Lolium multiflorum* Lema), Hühnerhirse (*Echinochloa crus-galli* L.), Brennessel (*Urtica dioica* L.) und Weißer Gänsefuß (*Chenopodium album* L.) ausgewählt. Als Wirkungskriterien für die Ammoniakdeposition wurden das Wachstum und der Gehalt an freien Aminosäuren herangezogen. Die mögliche Eignung der Testarten wurde in zwei unterschiedlichen Ansätzen untersucht.

Beim ersten Ansatz handelte es sich um eine Freilandstudie entlang eines NH_3-Gradienten mit einem Tierstall als Punktemittenten. Testpflanzen und Passivsammler zur NH_3-Konzentrationsmessung wurden entlang einesTransekts von 804 m Länge exponiert und nachfolgend die Beziehung zwischen NH_3-Exposition und Pflanzenreaktion ermittelt. In einem weiteren Ansatz wurden Expositionskammern in einem Gewächshaus errichtet, in denen Atmosphären mit verschiedenen NH_3-Konzentrationen hergestellt werden konnten. In diesen Gewächshauskammern fanden zwei Versuchsreihen statt, in denen zwei der Testarten aus dem Freiland, nämlich *Lolium multiflorum* und *Echinochloa crus-galli*, in ungefilterter Luft (NF), ungefilterter

Luft mit geringer NH_3-Konzentration (NF+) und ungefilteter Luft mit hoher NH_3-Konzentration (NF++) angezogen wurden.

Im Freilandexperiment sanken die NH_3-Konzentrationen erwartungsgemäß mit zunehmendem Abstand vom Tierstall. Die ausgebrachten Testpflanzen reagierten deutlich auf die unterschiedlichen NH_3-Konzentrationen. Die Gehalte einiger freier Aminosäuren stiegen in Stallnähe auf ein Mehrfaches der Kontrollwerte an; ebenso veränderte sich das anteilige Spektrum der Aminosäurezusammensetzung. Die Gehalte an Aminosäuren mit engem Verhältnis von Kohlenstoff zu Stickstoff (C/N) stiegen stärker an als die Gehalte freier Aminosären allgemein; im Speziellen betraf dies Glutamin (Gln) in *Lolium multiflorum* und *Chenopodium album*, Asparagin (Asn) in *Echinochloa crus-galli* und Arginin (Arg) in *Urtica dioica*. Dieser Befund belegt eindeutig die Aufnahme von luftbürtigem Ammoniak durch die exponierten Testpflanzen und die dadurch hervorgerufenen Veränderungen im Aminosäure-Metabolismus. Die Wuchsleistung, gemessen als oberirdische Biomasse, stieg in den Testarten mit zunehmender NH_3-Konzentration ebenfalls an.

In den kontrollierten Begasungsversuchen in den Gewächshauskammern gab es ebenfalls positive Effekte auf die oberirdische Biomasse der beiden Testarten *Lolium multiflorum* und *Echinochloa crus-galli*, die allerdings nur hinsichtlich der Blattmasse von Lolium multiflorum in dererstenVersuchsreihe statistisch absicherbar waren. Hinsichtlich der Effekte auf die Gehalte an freien Aminosäuren waren die Befunde aus der Freilandexposition nachzuvollziehen: auch im Gewächshausexperiment stiegen die Gehalte an freien Aminosäuren, insbesondere an solchen mit niedrigem C/N-Verhältnis wie Asn, Gln und Alanin (Ala), aber auch Glutaminsäure (Glu).

Die Ergebnisse zeigen, dass eine Ammoniak-Aufnahme aus der Atmosphäre stattfand und Wuchsverhalten und Aminosäure-Metabolismus signifikant beeinflusste. Mit den gewählten Messgrößen, insbesondere dem Gehalt und der Zusammensetzung freier Aminosäuren, besteht ein großes Potenzial, pflanzliche Reaktionen als Bioindikationsmethode für Ammoniak-Deposition zu verwenden.

INTRODUCTION

Nitrogen is arguably a very vital nutrient for plant growth. Its vast proportion and abundance in the atmosphere has little or no harmful effects on the environment due to its unreactive state as dinitrogen gas but this however, has to be converted to its beneficial forms (Galloway and Cowling, 2002). The conversion process has been mainly, through natural nitrogen fixation but since the production of synthetic nitrogen fertilizers, anthropogenic nitrogen fixation has led to perturbations in the global nitrogen cycle and increased nitrogen fixation levels (Galloway *et al*., 2004). This has enhanced the pollution of reactive nitrogen in the environment as well as other anthropogenic sources of atmospheric reactive nitrogen such transportation, combustion processes, industrial activities among others (Erisman *et al*., 2008). The consequences of these on biodiversity, ecosystem functioning, plant species composition and plant growth have been demonstrated in several studies and reviews (Stevens *et al*., 2011; Bobbink *et al*., 2010; Butchart, 2010; Fangmeier *et al*., 1994). It is estimated that by 2030 about 40% of the world protected areas would have to contend with atmospheric nitrogen deposition of over 10 kg N ha^{-1} yr^{-1} (Bleeker *et al*., 2011).

Before now attempts have been made such as the Gothenburg protocol in 1999 by developed nations especially in Europe, Canada and USA in order to curtail the rising rate of atmospheric nitrogen deposition and its consequences on the environment (Goodale *et al*., 2011). These were done by policy measures and setting up limit values in order to reduce four major pollutants which also includes NO_x and NH_3 emissions by 41 % and 17 %, respectively, in relation to 1990 emissions by 2010 (UNECE, 1999). At state levels, various countries have also set up modalities in order to curtail increases in atmospheric nitrogen pollution from various emission sources thereby militating against its effects on air quality and the environment in general (van Hove *et al*., 2002). Among the various control measures in curtailing the rise in atmospheric NH_3 emission was the proposition and adoption of threshold values in the late eighties for the expressions known as the critical level for NH_3 concentration in the atmosphere and the critical load for nitrogen deposition and this has been reviewed by different studies since inception (Fangmeier *et al*., 1994). At the moment threshold values for the critical level were set for hourly, daily, monthly and yearly concentrations of 3.300, 270, 23 and 8 µg m^{-3} NH_3 concentrations in the atmosphere respectively, of which below it no harmful effects

were expected (Cape *et al*., 2009). However, recent studies have proposed a revision of the annual average from 8 to 1 µg m^{-3} NH_3 concentrations especially for nitrogen sensitive species such as lichens and bryophytes (Cape *et al*., 2009).

In the EU several measures have been taken in order to reduce emissions from nitrogen oxides (NO_2) such as defining National Emission Ceilings that must be met by EU member states since 2010 and setting the annual average concentration target for NO_2 as 40µg/m^{-3} (EEA, 2011). In the US a primary and secondary standard of 53ppb was set by the US Environmental Protection Agency (EPA) (http://www.epa.gov/air/criteria.html). The NH_3 and NO_x emissions within the EU-27 for the year 2009 were 3.774 Tg and 9.373 Tg respectively, with France, Germany and Italy accounting for 19.7 %, 15.8 % and 10.4 % of the total NH_3 emissions, whereas in terms of NO_x emissions, Germany, France, United Kingdom and Spain recorded the most emissions with 14.6 %, 11.9 %, 11.6 % and 11.3 % accordingly (Eurostat, 2011). According to Eurostat (2011), NO_x and NH_3 emissions between 1990 and 2009 in the EU-27, declined by 45.5 % and 26.4 % respectively. However, for the NO_x emissions, the threshold values for 2010, set by the National Emissions Ceiling Directive (NEC Directive) for member states was exceeded by 12 % by total NO_x emissions in 2009 (EEA, 2011). In 2009, NH_3 and NO_x emissions in the United States was 3.698 Tg and 12.439 Tg respectively, compared to 3.918 Tg and 23.161 Tg emitted in 1990 (http://www.ceip.at/emission-data-webdab/). However notwithstanding, it is quite important to note the positive impacts made by various policy measures and directives aimed at reducing emissions of reactive nitrogen species such as NO_x and NH_3 based on the percentage reductions reported above, when compared to emissions over the last decade and even more (Eurostat, 2011) compared to the Asian continent, where there is a great need to control the potential rise in reactive nitrogen production (Galloway and Cowling, 2002).

The utilization of well established physical and chemical methods, for instance the use of passive diffusion tube samplers in assessing the level of various atmospheric pollutants, has been a useful means but however, has also gotten its own limits as well (Manes *et al*., 2003). Hence the use of bioindicator organisms in carrying out a biomonitoring assessment of atmospheric pollutants is considered as a different approach in evaluating the level of these pollutants in the environment have proven to be a viable method over the years and a typical example is a cultivar Bel-W3 of *Nicotiana tabacum* L. used extensively for several years in biomonitoring of

tropospheric ozone (Klumpp *et al*., 2006; Nali *et al*., 2006; Manes *et al*., 2003). The sensitivity of a proposed indicator organism to a particular pollutant is considered a fundamental requirement of a biomonitoring organism either by exhibiting visible injuries or as an accumulator. Among other requirements include an easy quantification of the said pollutant in the plant as well as an easy culture and propagation of the indicator organism coupled with its ability to withstand certain environmental conditions (Nali *et al*., 2006). The suitability and prospects of certain plants as bioindicators of certain atmospheric pollutants have been demonstrated on a large scale in Europe under the European Biomonitoring Network and the International Cooperative Programme (ICP-Vegetation) of the United Nations Economic Commission for Europe (Manes *et al*., 2003; Klumpp *et al*., 2002). These studies, have therefore led to the development of a generally acceptable guideline for investigating certain atmospheric pollutants using several biomonitoring techniques (ICP Vegetation 2012, Klumpp *et al*., 2002). Arguably, plants have the ability of detecting quite a number of atmospheric pollutants such as ozone (Furlan *et al*., 2008; Nali *et al*., 2004), polycyclic aromatic hydrocarbons (PAH) (Rodriguez *et al*., 2012; Srogi, 2007; Howsam *et al*., 2000), fluoride (Franzaring *et al*., 2007; Rey-Asensio and Carballeira, 2007), heavy metals (Serbula *et al*., 2012, Dmuchowski *et al*., 2011 and Sawidis *et al*., 2011).

In spite of the ability of using physical and chemical measurements in monitoring atmospheric pollution, previous studies where biomonitoring has been successfully applied as reported above, clearly demonstrate why it is imperative to use biomonitoring techniques as a research tool in complementing other methods in the monitoring of atmospheric pollution. For instance, in a study on the pollution of atmospheric polycyclic aromatic hydrocarbon (PAH), Tomashuk *et al*. (2012) conducted physical measurements with high volume samplers and analyzed PAH compounds from particulate matter. This was complemented with passive biomonitoring, by the analysis of pine needles collected from the area of study. They were able to observe PAH compounds with 2, 3 and 4 rings in the needles of *Pinus nigra* and *Pinus strobus* whereas in the particulate matter samples only PAH compounds with 4, 5 and 6 rings were observed. Interestingly, results obtained from the above study, demonstrate how useful biomonitoring can be in the monitoring of atmospheric pollutants and also in effectively augmenting well known standardized techniques.

A general literature review on nitrogen and its various reactive forms in relation to atmospheric pollution has been carried out and included in the second chapter of this PhD thesis. This chapter also comprises of reported studies on the sensitivities of various indicator organisms as effective tools in various biomonitoring studies for detecting the availability of various atmospheric pollutants including NH_3 and NO_x. In addition, an overview of reactive nitrogen species originating from major sources of atmospheric nitrogen pollution and their consequences on the ecosystem as demonstrated by various studies have also been highlighted.

This PhD thesis consists of results obtained during the investigation of the research objectives of this study. The main objective of this present study was to assess the ability of the selected indicator plants consisting of grasses and herbs as biomonitors of atmospheric nitrogen. The responses of these bioindicator plants to sources of NH_3 pollution would be able to indicate the ability of the bioindicator plants to detect atmospheric NH_3 and nitrogen deposition. Therefore in order to assess this response it was important to investigate and test the following hypothesis:

1. Investigate nitrogen accumulation in the selected bioindicator plants around a hotspot of NH_3 pollution and nitrogen deposition.
   Hypothesis:
   (i) Atmospheric NH_3 will cause increase in aboveground biomass accumulation with increasing ambient NH_3 concentration.
   (ii) The interaction of atmospheric NH_3 with the selected bioindicator plants will lead to alterations and increases in the free amino acid concentrations in the various plants as the ambient NH_3 concentration increases.
   (iii) The selected bioindicators are sensitive to NH_3 pollution and could be used in detecting NH_3 emission and nitrogen deposition.
2. Investigate through fumigation study the responses of the selected indicator plants to the exposure of gaseous NH_3 under controlled conditions.
   Hypothesis:
   (i) The effect of gaseous NH_3 induced responses will lead to a possible increase in additional above ground biomass accumulation and increases

in free amino acid concentrations and composition in the selected indicator plants.

2. NITROGEN DEPOSITION A BRIEF OVERVIEW

2.1Nitrogen

Nitrogen constitutes about three-quarters of all elements present in the atmosphere and despite its large abundance in the atmosphere, its dominant form as an inert dinitrogen gas N_2 has no harmful effect on the environment compared to its reactive species (Galloway and Cowling, 2002). However, this can be converted into other nitrogen forms such as nitrogen oxides either naturally or anthropogenically of which lightening and soil microbial activities constitute natural sources of nitrogen oxide formation (Pearson and Stewart, 1993). Similarly, the anthropogenic formation of nitrogen oxide could be through high temperature combustion processes of fossil fuels and the oxidation of molecular nitrogen (N_2) (Erisman *et al*., 2008). Amongst the various forms of nitrogen oxides are nitric oxide (NO), nitrogen dioxide (NO_2), nitrogen oxides (NO_x) which is a combination of nitric oxide and nitrogen dioxide, nitrates (NO_3), nitrites (NO_2) and nitrous oxide (N_2O) (Asman *et al*., 1998). These oxidized nitrogen forms could have severe effects on both human health and the environment at high concentrations. Furthermore, other nitrogen forms include the reduced nitrogen species NH_x a combination of NH_3 and NH_4^+. These are formed mostly from agricultural activities as a result of the emission and volatilization of ammonia due to large scale fertilizer application and livestock management (Fangmeier *et al*., 1994). Additionally, these oxidized and reduced nitrogen forms such as NO_x and NH_x could have beneficial effects by serving as nutrients and can also have harmful effects on the environment as atmospheric pollutants (Asman *et al*., 1998).

2.2 Nitrogen Deposition

The emission of oxidized and reduced forms of reactive nitrogen compounds has increased several folds in the past decades due to large scale anthropogenic activities (Erisman *et al*., 2008; Galloway *et al*., 2008). The rise in production and application of synthetic nitrogen fertilizer, as well as increases in fossil fuel combustion have facilitated the increase in emitted reactive nitrogen species and nitrogen deposition on ecosystems compared to pre-industrial times (Erisman *et al*., 2008; Galloway *et al*., 2004). These reactive nitrogen species consist of both organic and inorganic nitrogen compounds (Cornell, 2011). However, there are only few reports regarding the impact

and contribution of organic nitrogen in nitrogen deposition. It is pertinent to note organic nitrogen constitutes a large fraction in droplets and air borne particulate matter (Saxena and Hildemann, 1996) of the total nitrogen component in atmospheric nitrogen deposition and also as a source of nutrients in aquatic and terrestrial ecosystems (Cornell, 2011). This input of atmospheric nitrogen loads on certain ecosystems adapted to limited nitrogen conditions, have imposed serious consequences such as increased nitrogen availabilities, toxicities arising from nitrogen compounds in aerosol and soil acidification on nitrogen sensitive ecosystems (Bobbink *et al*., 2010; Pearson and Stewart, 1993). Atmospheric nitrogen deposition of NH_x can broadly be classified into two parts namely wet and dry deposition. Dry deposition of gaseous NH_3 occurs in close proximity to the point source of emission, while wet deposition dominates further away (Asman *et al*., 1998). The deposition of NH_3 around a rural area as well as within the surrounding of a livestock establishment is most likely while HNO_3 and NOx are expected to dominate around urban and industrial settings (Shen *et al*., 2011; Fangmeier *et al*., 1994). Furthermore, the dominance in deposited reactive nitrogen species either as reduced or oxidized nitrogen could vary on a continental level, with either of the two exceeding the other in total depositions (Holland *et al*., 2005). In the United States, the annual concentration of nitrogen deposition is mostly in its oxidized nitrogen forms and this accounts for over 50 % in the overall total of deposited nitrogen estimated at 3.7 - 4.5 Tg, whereas in Western Europe out of a total of 8.4-10.8 Tg N deposited yearly, reduced nitrogen accounts for between 4.3 to 6.3 Tg of the deposited nitrogen budget (Holland *et al*., 2005). In a study conducted in China, one of the largest consumers of synthetic nitrogen fertilizer worldwide (He *et al*., 2007), atmospheric nitrogen deposition investigated across several sites within the North China Plain (NCP) showed an average of 43.5 kg N $ha^{-1}yr^{-1}$ in both wet and dry deposition of NH_x (Shen *et al*., 2011). In other words, the influence of activities culminating in the emissions of reactive nitrogen in an area plays an important role in determining the predominant form of deposited nitrogen within a location and this can also be influenced by different climatic conditions (Stevens *et al*., 2011; Bytnerowicz and Fenn 1996).

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2.3 Biomonitoring

The approach of biomonitoring is arguably, quite an old method in detecting and assessing the level of certain environmental pollutants and has been widely used especially in Europe for about a century (De Temmerman *et al*., 2004). Biomonitors could be classified into two basic categories, basically as bioindicators depending on the ability of the organism to exhibit physical changes or characteristics as a result of the prevailing pollutant and are referred to as bioaccumulators as a result of the accumulation of such pollutant, in the absence of any visible effects (De Temmerman *et al*., 2004). Hence the primary role of a biomonitoring study is in the ability to discover any alteration in an environment arising from atmospheric pollution. The ability to measure the concentration of pollutants in plants even at low ambient concentrations (De Temmerman *et al*., 2005) has been described as a useful indicator to determine the level of environmental pollution (Kosior *et al*., 2008). Therefore an organism could be considered a good biomonitor when a good linear relationship between the concentration of a pollutant in the organism and the environment is exhibited (Boquete *et al*., 2011). The response of plants as biomonitors could be a shift in species composition which may occur as a result of several factors such as competition between plant species depending on the ability of the plant to withstand and utilize enhanced N deposition (Zechmeister *et al*., 2008). This process has involved the use of lichens, bryophytes and higher plants as biomonitors, in monitoring various atmospheric pollutants such as ozone, nitrogen deposition, sulphur compounds, hydrocarbons as well as heavy metals (Boquete *et al* ., 2011; Conti *et al*., 2001). Several plants have been reported as important bioaccumulators for certain biomonitoring applications (De Temmerman *et al*., 2005) while some have also been described as useful biomonitors for enhanced N deposition (Pitcairn *et al*., 2003). Using purple moor grass in an active biomonitoring study of traffic related nitrogen oxides, Laffray *et al*. (2010) demonstrated a significant effect of nitrogen oxide pollution from vehicles with respect to the total nitrogen and leaf ^{15}N-abundance of the plant in close proximity to the road. The intensity of road traffic was discovered to have an effect on the overall nitrogen enrichment of plants at very close distance to the road compared to those further away. In a similar study, Gombert *et al*. (2003) used two epiphytic lichens namely a nitrophytic, *Physcia adscendens* and an acidiphytic *Hypogymnia physodes* to investigate the relationship between their nitrogen concentration and traffic in an urban area. The

nitrogen concentration in the nitrophytic lichen *Physcia adscendens,* responded to a source of traffic pollution especially at a close distance to the area of traffic exposure. Also on lichens, Frati *et al*. (2007) investigated the effects of ammonia pollution and nitrogen deposition around a livestock farm on epiphytic lichens *Xanthoria parietina* and *Flavoparmelia caperata.* An accumulation in total nitrogen, with a relationship with the ambient NH_3 concentrations, was, observed in thalli of both species. Apart from responding as indicators of nitrogen deposition through nitrogen accumulation in both species, *Flavoparmelia caperata* exhibited visual injuries arising as a result of exposure to a source of ammonia pollution. In order to observe the responses of terricolous alpine lichens to two different factors made up of, nitrogen concentration and load, Britton and Fisher (2010) evaluated the impact of these treatments on thallus nitrogen content of five species in a simulated nitrogen addition study for 13 weeks. Both treatments induced thallus nitrogen contents however, concentration had a much more profound effect on growth. In another study using mosses as bioindicators of nitrogen deposition, Zechmeister *et al*. (2008) investigated the total nitrogen content and ^{15}N signatures in moss tissues and reported on the suitability in using nitrogen content in moss as indicators of nitrogen deposition, since mosses rely mostly on nitrogen deposition as its source of nitrogen.

2.4 PRIMARY SOURCES OF NITROGEN POLLUTION

2.4.1 Agricultural crop production

The deposition of atmospheric nitrogen originating from agricultural sources are mostly in form of NH_x and this constitutes a dominant form in deposited atmospheric nitrogen especially in some areas in Western Europe with high agricultural activities. On a worldwide scale the Asian continent, primarily India and China, represents a major source of atmospheric nitrogen deposition (van den Berg *et al*., 2008; Galloway and Cowling, 2002). According to Eurostat (2011), agricultural activities constituted the major source of NH_3 emission in the EU by accounting for up to 94 % of the total NH_3 emissions in the region for the year 2009. Zheng *et al*. (2002) studied the state of the situation of anthropogenic reactive nitrogen in Asia and predicted an expected increase up to 105.3 Tg N yr^{-1} by 2030 compared to 67.7 Tg N yr^{-1} in 2000, with agriculture constituting a vital source of nitrogen pollution. In another study, Zhang *et al*. (2010)

investigated NH_3 emissions across different provinces in the North China Plain (NCP), an area known for its vast intensive agricultural activity. They observed that the application of synthetic nitrogen fertilizers and livestock management were major sources of NH_3 emissions within the region investigated, which accounted for 54 % and 46 % in NH_3 emissions respectively. Of the total NH_3 emissions of 3071 kt NH_3-N yr^{-1} produced in the area studied, 1620 kt NH_3-N yr^{-1} originated from fertilizer application, while the rest were emissions predominantly from pigs and other livestock animals. The continuous rise in world population has necessitated the expansion in intensive agricultural production in order to meet rising demand of various agricultural products (Erisman *et al*., 2008; Stewart *et al*., 2005). Therefore the need to apply synthetic nitrogen fertilizer to enhance soil enrichment and increase yield and productivity of agricultural produce have become inevitable based on the positive impacts of commercial fertilizers on crop production (Stewart *et al*., 2005). However, misuse of synthetic nitrogen fertilizer, through excessive application also constitutes in the generation of anthropogenic reactive nitrogen (Zheng et al., 2002). According to Fertilizer Europe (FE, 2011), in EU-27 a total of 135 million hectares of arable land are applied with nitrogen fertilizers and of these, 14 % and 18 % are used for wheat and coarse grain production while fodder crops and oil seeds accounts for 7 % and 6 % respectively. Current statistics showed that nitrogen fertilizer was the most applied nutrient in 2009 within the EU and accounted for 77.4 % of all the nutrients used which includes phosphorus and potassium (Eurostat, 2011). However, the application of nitrogen based fertilizer when compared between 2000 to 2008 showed a slight reduction in most states and this varied among member states but in general, an overall decline not exceeding 4 % per annum was observed (Eurostat, 2011). The Food and Agricultural Organization (FAO) reported a yearly increase of about 2 to 4 percent in food production for the past 10 years in Asia, while Eastern Europe and Latin America and the Caribbean had increases in production, but decreased after 2008 in Eastern Europe, while food production slumped a bit within the region. In 2009, a decline from a yearly increase of 3 to 4 % in food production was observed in Sub Saharan Africa, while in Western Europe an increase in production was observed between 2008 and 2009 (FAO, 2011).

Currently, on a global level the Food and Agricultural Organization (FAO) reported a continuous decrease in arable land per person from 0.38 ha to 0.23 ha between the year 1970 to 2000 and this is expected to decrease further to 0.15 ha by 2050 (http://www.fao.org). As a result of the continuous shrinking in arable land, in order to cope with future constraints and militating challenges, nitrogen fertilizer is an important factor in meeting global food demands under the prevailing decrease in arable land for agricultural production (FE, 2011).

However, despite the benefits of nutrient input on crop yield, this has also influenced losses of nitrogen species in the environment (Erisman *et al*., 2008). The application of manure on fields also constitutes a source of atmospheric nitrogen, due to the volatilization of NH_3 from the soil and this depends on the manure application rate, dry matter content, application method as well as certain meteorological conditions (Sommer *et al*., 2006; Moal *et al*., 1995; Frost, 1994). For instance, under increasing global radiation, NH_3 volatilization increases as a result of increased surface temperature and this influences the volatilization of NH_3 gas released at the surface (Sommer *et al*., 2003). NH_3 losses due to volatilization, from the application of chemical fertilizers and livestock manure in developed countries are estimated at 7 % and 21 % respectively, while in developing countries, NH_3 losses account for 18 % and 26 % (Bouwman *et al*., 2002). Arguably, the use of commercial fertilizers, livestock manure and slurry constitutes sources of nitrogen losses to the environment as a result of NH_3 volatilization. The dominant source of NH_3 volatilization among these sources is dependent on various factors, including, C to N ratio of the manure as well as in the breakdown of the organic matter contents (Petersen and Sommer, 2011).

2.4.2 Livestock Management.

Livestock management constitutes a major source of ammonia emission (Fangmeier *et al*., 1994). Other important trace gases produced as a result of this practice includes methane (CH_4), carbon dioxide (CO_2) and nitrous oxide (N_2O) (Leytem *et al*., 2011; Aneja *et al*., 2008). The number of livestock, type of livestock, the type of feed and its nitrogen content are important parameters in determining the increase of anthropogenic nitrogen emissions from livestock management (Aneja *et al*., 2012; Asman *et al*., 1998). An investigation into the emissions of NH_3 and N_2O was conducted based on the

population of various livestock animals in India and a likewise adoption of emission factors for the respective animals were applied (Aneja *et al*., 2012). NH_3 and N_2O emissions from cattle were the highest compared to other animals by accounting for over 900 Gg yr^{-1} and 90 Gg yr^{-1} in emissions respectively, for both pollutants. This is as a result of the high emission factors for cattles and its large number whereas, when compared with one of the least emitters such as poultry which has the largest livestock population in India, but having a low emission factor, the impact of livestock type on emission is observed. The continuous improvement in various aspects of livestock management including, qualitative feeding and breeding methods, development in medicine and animal health coupled with the increase in demand for various livestock products has influenced intensive livestock production (Thornton, 2010), which in turn has led to a significant rise in NH_3 emissions and nitrogen deposition. In a study conducted in Sydney, Formosa and Singh (2002) observed an increase in the NH_4 and NO_3 concentrations in the soil samples around a poultry farm compared to further distances away from the farm. They evaluated the NH_4^+ and NO_3 concentrations across several depths ranging from 0 and 90 cm and observed higher concentrations of NH_4^+ and NO_3 within a 30 cm depth from the soil surface and this decreased with distance from the farm. Thus, indicating the influence of the poultry farm as well as the existing climatic conditions, on the atmospheric nitrogen concentration within the vicinity of the farm and its subsequent deposition especially at closer distances to the farm (Formosa and Singh, 2002).

Upon emission, NH_3 undergoes series of reactions such as the neutralization of atmospheric oxidation products of NO_x and SO_2 (Asman *et al*., 1998), formation of NH_4^+ aerosols and other NH_4^+ products such as ammonium sulphates and nitrates which constitutes other air pollution sources in form of particulate matter PM2.5 (Leytem *et al*., 2011). Other studies have also demonstrated the contribution of NH_3 emissions from livestock management, in the formation of fine particulate matter (PM2.5) involved in the degradation of air quality (Hristov, 2011; Wu *et al*., 2008). Despite the large scale atmospheric pollution caused by livestock management, several studies have suggested various mitigation strategies in order to combat increases in NH_3 emission. This includes manure handling and housing systems as well as the supply of necessary dietary requirements for livestock production (Petersen and Sommer, 2011). Investigations by Leytem *et al*. (2011), on NH_3 emissions from diary housing and

manure management systems such as open lot, wastewater pond and compost, revealed that 78 % of NH_3 emissions was generated by the open lot areas.

The reduction in manure surface area (Petersen and Sommer, 2011), immediate separation of feces and urine as well as their consistent collection from the surface has been reported to reduce NH_3 volatilization (Carew, 2010). Since the volatility of NH_3 from manure is dependent on certain volatile nitrogen forms such as NH_3 and NH_4^+ which are pH dependent, the separation of feces and urine were observed to mitigate the enzymatic reaction producing NH_4^+ from urine catalyzed by urease enzyme found in feces. The separation procedure reduced NH_3 volatilization by 49 %, while a reduction of 46 % was observed by removing the manure every 2 to 3 days without separation (Ndegwa *et al*., 2008). Petersen *et al*. (2009) showed the ability to eliminate NH_3 volatilization from slurry during storage by creating a surface crust. This involved the mixing of straws with slurry during storage as a result they observed a reduction in NH_3 volatilization within two weeks and a complete elimination in NH_3 volatilization after a period of eight weeks with surface crust. Adjusting the livestock dietary requirement by reducing the crude protein intake by 5% and substituting it with amino acid supplements can reduce nitrogen content in livestock manure thereby significantly minimizing NH_3 emission by 74% in ruminants (Ndegwa *et al*., 2008). Furthermore, Sommer *et al*. (2006) evaluated NH_3 volatilization from a study on the interactions between slurry and soil. NH_3 volatilization was evaluated after the application of untreated cattle and pig slurry and anaerobically digested pig slurry on two soil types namely, sandy and sandy-loam soils. They observed that NH_3 volatilization was determined by the infiltration rate of the slurry into the soil and that was dependent on slurry composition.

2.4.3 Transport

Emission from various means of transportation contributes as sources of atmospheric pollution thereby, constituting in the degradation of air quality and is generally is a major source of NO_x emissions which is also an intermediate gas for the generation of ozone an important atmospheric pollutant (Aneja *et al*., 2008). According to EEA (2011), the transport sector constituted 56 % of the total NO_x emissions in the EEA while the non-transport sector was 44 %. Of all the various compositions within the

transport sector, emissions from road transport exhaust and international shipping accounted for the highest NO_x emissions with 31 % and 15 % respectively (EEA, 2011). The emission of NO_x from vehicular transport constitutes a major source of atmospheric nitrogen, compared to NH_3 which is emitted mostly due to the installation of catalytic converters in vehicle exhausts (Cape *et al*., 2004). In the aviation sector, air transport constitutes a source of NO_x pollution especially from aircrafts operating with large engines (Mazaheri *et al*., 2011). According to the EEA, the past twenty years have witnessed a rise in NO_x emissions from international aviation services and a 40% increase in NO_x and NMVOCs arising from international shipping (EEA, 2011). Mazaheri *et al*., (2011) conducted a study on gaseous emissions from large aircraft engine operations. They observed that 97% of total emissions including NO_x occurred mostly during land and taking off cycles of the aircraft and emissions were greater during takeoff as a result of increased aircraft emission rate during this period. In most European countries, various traffic management strategies have been enforced in order to ensure reduction in vehicle emissions such as implementation of congestion charge, camera surveillance and enforcement of various speed limits (Keuken *et al*., 2010; Dijkema *et al*., 2008). Keuken *et al*. (2010) in a study on speed management in the Netherland reported a reduction of 5-30% in NO_x emissions. They observed that, the effect of speed limit impulsion was evident as unfavorable for NO_x emission, which is a by-product of high temperature combustion process. Traffic congestions have been reported to increase exhaust emissions compared to a free flowing traffic as a result of continuous acceleration and deceleration of vehicles (Keuken *et al*., 2010; Smit *et al*., 2008). The introduction of several policies and European standard limits for road transport has played a key role in curtailing NO_x emissions in Europe. A decrease in emission from road transport was observed between 1990 and 2000, while between 2000 and 2005 NO_x emissions from road transport dominated the overall anthropogenic NO_x sources by 40% due to economic growth in Eastern Europe (Vestreng *et al*., 2009). In the EU-27, road transport constituted the dominant source of NO_x emissions in 2009 by 42.2 % (Eurostat, 2011). However, current report from the European Environment Agency (EEA) showed that across EEA- 32 countries, a 25 % decline in NO_x emissions were observed between 1990 and 2009, while NH_3 emissions rose until 2000 as a result of the reduction of nitrogen monoxide (NO) by hydrogen in vehicles with catalytic converters (EEA, 2011). The economic growth within the EU between 1999 and 2009 coupled with free flow of goods among member states induced a 79 % rise in road

haulage in 2009, while a 12 % rise in passenger transport was observed within the same period in EEA-32 countries due to ease, speed and efficiency of the various transport systems (EEA, 2011).

2.5 CONSEQUENCES OF ATMOSPHERIC NITROGEN DEPOSITION

2.5.1 Effects on species richness.

The continuous rise in atmospheric nitrogen deposition has been proven by studies to have profound effects on plant communities (Bobbink *et al*., 2010). Studies involving the application of various nitrogen concentrations have been carried out on different grassland communities in order to investigate potential effects of increasing nitrogen availability on plant biodiversity and species richness. Song *et al*. (2011) investigated the effects of nitrogen enrichment on temperate steppe ecosystem in a field experiment with six nitrogen treatments of 0, 30, 60, 120, 240 and 480 kg N $ha^{-1}yr^{-1}$on Chinese grasslands. A decrease in species richness of grasses and forbs were observed under increasing nitrogen availability. Furthermore, Wilson and Tilman (2002) evaluated the impact of disturbance and four nitrogen levels on species richness. They observed a decrease in species richness as nitrogen addition increased at all various levels of disturbance. After a period of one year plots dominated by native grasses *Schizachyrium* disappeared in plots with high nitrogen treatments and were replaced by *Poa* and *Agropyron* but remained in plots with low nitrogen treatments.

In another study, Seastedt and Vaccaro (2001) reported a similar decline in species richness on alpine tundra communities as a result of increased nitrogen conditions under the highest nitrogen application treatment. Stevens and Tilman (2010) conducted a study on prairie grassland around a livestock farm. The impact of nitrogen deposition on prairie grassland around the 500m transect close to a source of NH_3 emission showed no significant effect on species richness with NH_3 deposition with close proximity to the farm. However, a significant relationship in above ground biomass was observed with nitrogen deposition.

Apart from other factors such as soil types, climatic conditions, as well as tree species, atmospheric nitrogen deposition was reported to have significant effect on understorey

forest vegetation and this has resulted in a shift to nitrophytic species in European forests especially under high nitrogen deposition (van Dobben and de Vries, 2010). In order to detect possible effects of atmospheric nitrogen deposition on conservation sites, Stevens *et al*. (2009) investigated several indicators on acid grasslands. Amongst the indicators studied are species richness, the availability of certain plant species and the unavailability of certain plant species as well as cover of some vital plant groups. Most of the indicators showed positive responses as indicators of atmospheric nitrogen deposition. However, a graminoid to forb ratio was considered as the most dependable and simplest method in assessing the impact of atmospheric nitrogen deposition in a monitoring study on grassland ecosystems (Stevens *et al*., 2009). Further studies on the vegetation effects of nitrogen loads on acidic and calcareous grassland plots in a six years study were reported by Carroll *et al*. (2003). Increasing nitrogen concentrations were added to these plots consisting of both higher plants and bryophytes within this period. The initial four years showed no significant effect on higher plants in both grassland plots, except in bryophytes which responded with a decrease in cover under high nitrogen concentrations in the acid grassland plots. The fifth and sixth years however, showed a remarkable response in grasses and higher plants exhibiting significant decrease in total touches of grasses and higher plants in both acid and calcareous grasslands exposed to high nitrogen conditions.

In order to determine possible responses to interactive effects of pollutants, Payne *et al*. (2011) conducted a study and investigated if there were responses of a combined effect of both nitrogen and ozone pollutants. There was no identification of a particular species as an indicator exhibiting sensitivities to both nitrogen and ozone pollution. However responses were based on sensitivity to a particular pollutant and none to the other and this was the observation, reported of *Dicranum scoparium* which is susceptible to nitrogen pollution while it remains unsusceptible to ozone. Earlier studies by Dueck *et al*. (1987) evaluated the ability of grasses to adapt to different air pollutants in the Netherlands, using different populations of the species *Agrostis capilaris* L., *Nardus stricta* L., and *Lolium perenne* L., respectively. The above populations were obtained from various areas with varying concentrations of air pollutants, and then grown under controlled growth conditions in the greenhouse and subsequently transplanted prior to fumigation with SO_2, O_3, NO_2 and NH_3 respectively. The various air pollutants did not impede the growth of *Lolium perenne* either individually or

collectively rather the mixture of all pollutants, under high concentrations induced a significant increase in biomass of *Lolium perenne*. However, it was a different scenario for *Nardus strica* having been induced in growth after fumigation with a combination of both SO_2 and NH_3, but this occurred only in populations obtained from highly polluted areas during sampling. The fumigation of *Agrostis capilaris* showed an overall positive response except under high concentrations in individual fumigations with O_3 and SO_2 or in their combinations with NO_2.

2.5.2 Eutrophying effects of nitrogen enrichment.

Over the years, anthropogenic activities resulting in the increased levels of reactive nitrogen species, have triggered the enrichment of coastal ecosystems (Gooday *et al*., 2009; Smith *et al*., 1999; Vitousek *et al*., 1997). The eutrophication of surface waters such as in coastal marine ecosystem is mostly determined by increase in nitrogen nutrients, while in lakes phosphorus is the most important nutrient enriching element (Vitousek *et al*., 1997). The inflow of water bodies into estuarine and coastal ecosystems as well as deposition of atmospheric nitrogen poses a risk of nitrogen enrichment to these sensitive ecosystems. However, several parameters including its morphological and biogeochemical attributes are important factors that could also determine the level of sensitivity of these ecosystems to nitrogen enrichment (Paerl *et al*., 2002). The rise in human induced nitrogen fixation arising as a result of increases in fertilizer production and application, nitrogen mobilization through biomass burning, fossil fuel combustion among others, have created an alteration in the normal nitrogen cycle processes thereby increasing nitrogen availability in surface waters (Vitousek *et al*., 1997). Nitrogenous pollution from agricultural sources such as livestock management, surface runoff from agricultural fields constitutes a significant proportion of nutrient enrichment on surface waters (Howarth, 2008). Whitall *et al*. (2003) studied the influence of nitrogen enrichment from atmospheric nitrogen deposition on Neuse River. They reported that half of the total additional nitrogen input into the Neuse River estuary was as a result of wet deposition from organic and inorganic nitrogen inform of NH_4^+ and NO_3^-. Investigations by Howarth *et al*. (1996) on the impact of anthropogenic and natural influences on riverine nitrogen and phosphorus fluxes across different areas, into the North Atlantic Ocean revealed a strong relationship in the correlation between

total nitrogen of river fluxes and inputs of various anthropogenic nitrogen sources in the temperate regions. The input of anthropogenic nitrogen includes application of synthetic nitrogen fertilizers, and deposition of reactive nitrogen species mostly from anthropogenic sources among others. Although the eutrophication of aquatic ecosystems could have a beneficial effect in the growth of aquatic plants however certain negative effects such as hypoxic conditions of the water bodies, generation of algal blooms as well as harmful effects on human health cannot be over emphasized (Smith, 1999). In a study conducted in the United States, Dodd *et al.* (2009) revealed that over 90% of rivers studied, exceeded the reference limits for total nitrogen and phosphorus concentrations.

The enrichment of terrestrial ecosystem has not also been spared as a result of the consequences of atmospheric nitrogen deposition. However, such effects have been relative based on certain parameters, which includes the plant species and habitat type (Rowe *et al*., 2012), the nature and type of soil, nitrogen nutrient availability among others, also constitute important factors in determining the influence of nitrogen enrichment arising from nitrogen deposition (Diwold *et al*., 2010). Rowe *et al.* (2012), in their study, compared mineralisable nitrogen stocks in both intensively and extensively managed habitats composed of different plants, climatic conditions and exposure to nitrogen deposition. They observed positive significant effects in the influence of increasing nitrogen deposition on nitrate proportions and a correlation on mineralisable nitrogen stocks in extensively managed habitats contrary to intensively managed habitats. In a different study, Ceulemans *et al.* (2011) investigated the influence of nitrogen availability on the losses of grassland species in soils exposed to various doses of atmospheric nitrogen. In this study 132 locations were selected around North-west Europe and a total of 61 grassland species were evaluated. Although, only five plant species were affected as a result of increased nitrogen availability in the soil, but this also demonstrated the influence of nitrogen nutrient enrichment in sensitive ecosystems and on plant diversity (Ceulemans *et al*., 2011). Nitrogen enrichment could also have different effects on certain ecosystem depending on the nitrogen form, for instance in nitrogen sensitive ecosystems such as in heathlands where reduced nitrogen has a more profound effect on plants and soil compared to the oxidized nitrogen forms (Jones and Power, 2012; van den Berg et al., 2008).

The influence of nitrogen deposition on forest ecosystems is quite revealing. Early studies by van Breemen and van Dijk (1988) in the Netherlands, reported the severe deterioration of forest ecosystems as a result of atmospheric nitrogen deposition due to the prevailing rate of NH_3 emissions especially from livestock management and dry deposition of both gaseous NH_3 and NH_4^+ inorganic nitrogen compounds. Among the consequences of these additional nitrogen enrichment on forest ecosystems includes, increase in parasitic infection of trees by fungi, substantial depletion in number of needles, alterations in tree colour and nutritional constituents of foliage coupled with injury and changes in carbon and nitrogen mineralization rates (Månsson and Falkengren-Grerup, 2003; Fenn and Poth, 2001; Fangmeier *et al*., 1994; van Breemen and van Dijk, 1988; van Dijk and Roelofs, 1988). In the same process, the growth and biomass of sensitive understorey plants such as bryophytes could also be declined under conditions of increased nitrogen additions (Mäkipää, 1998). A decline of 60 % and 78 % was reported in *Pleurozium schreberi* (Mitt) and *Dicranum polysetum* (Sw) sequel to a nitrogen and sulphur addition study in a (*Picea abies* Karst.) stand at the rate of 25 kg N ha^{-1} and 30 kg S ha^{-1} for 4 years (Mäkipää, 1998). In soils the effect of nitrogen saturation, could vary depending on the soil type and its leaching tendency, for instance in acidic soils enriched with both ammonium and nitrates, an additional supply in ammonium would facilitate nitrate production and losses, whereas in forests stands saturated with nitrates, ammonium is absorbed at the expense of nitrate (Emmett *et al*., 1995). Hagedorn *et al*. (2001) conducted a study on gleysols in forest stands and investigated the influence of nitrogen deposition on the possible absorption or leaching of ammonium and nitrates. They found an increase in the leaching of nitrates under conditions of increased nitrogen deposition whereas, losses of ammonium were not observed. Nitrogen deposition on the other hand could be very beneficial for tree growth, but could also have detrimental effects on trees (Bytnerowicz and Fenn, 1996) and this could vary between trees for instance, the responses of spruce trees and pine trees to acidification differ especially with pine trees which are well adapted to acidified soils thereby demonstrating less susceptibility to conditions of increased acidification levels in soils (Solberg and Tørseth, 1997). However, certain parameters such as the foliar nitrogen composition, nitrogen to phosphorus ratio and the induced growth of foliage are some positive signals of nitrogen enrichment in forest trees (Bytnerowicz and Fenn, 1996). In a study conducted in a Norway spruce stand in Denmark, Gundersen (1998) reported an increase in the nitrogen contents of needles from litter

fall and also a rise in uptake of nitrogen from 32 % to 43 % by the various tree parts exposed to an ambient nitrogen deposition between 15 to 20 kg N ha^{-1} yr^{-1} and supplemented with a nitrogen addition of 35 kg N $ha^{-1}yr^{-1}$. Similarly, Brumme *et al*. (1992) reported an increase in the uptake of nitrogen by above ground parts of *Fagus silvatica* L. trees when exposed and augmented with ^{15}N additions at different concentrations.

2.6 Atmospheric Ammonia and its Effects on Plant Metabolism

Ammonia is an important atmospheric pollutant originating mostly from intensive agricultural activities (Fangmeier *et al*., 1994). Amongst other reactive nitrogen species from agricultural production such as nitrogen oxide (NO), nitrogen dioxide (NO_2) and nitrous oxide (N_2O), NH_3 emission constitutes a major source of atmospheric reactive nitrogen (Aneja *et al*., 2008). This phenomenon has been greatly influenced since the initial production, commercialization and application of synthetic nitrogen fertilizers for agricultural production, thereby resulting in nitrogen losses to the environment (Erisman *et al*., 2008; Galloway *et al*., 2008). The beneficial effect of synthetic nitrogen in agricultural production cannot be overemphasized, and this is evidenced from the growth and yield of agricultural products which is important in meeting the demands in global food requirement for a rising global population over the years (Aneja *et al*., 2008, Galloway *et al*., 2008). However, the benefits accrued from these, is not without some severe consequences on the environment such as an increased production of atmospheric reactive nitrogen species, its effects on vegetation and plant communities and an alteration in the global nitrogen cycle among others (Galloway *et al*., 2008; Fangmeier *et al*., 1994).

Sequel to NH_3 emission, atmospheric NH_3, could be deposited dry on vegetation in form of particulate ammonium (NH_4^+), gaseous ammonia (NH_3) or wet in form of NH_4^+ (Pitcairn *et al*., 2003; Asman *et al*., 1998). Early studies have reported the ability of plants to take up NH_3 mostly through the stomata (van Hove *et al*., 1987) and the capability of plants as possible means of NH_3 emission or avenues of uptake of atmospheric NH_3 (Fangmeier *et al*., 1994). In a study on three grass species exposed to solutions of two different nitrogen treatments and fumigated with 0 and 70 nmol mol^{-1}

air concentrations of gaseous NH_3, over a period of 24 days, Hanstein *et al*., (1999) reported that NH_3 uptake through the stomata was the primary point of uptake in the plants studied. Their findings were established by the strong level of correlation observed between the stomatal conductance of the plants and the total NH_3 conductance. However, this process is determined by the NH_3 compensation point and is the difference between the atmospheric NH_3 concentration and that above the mesophyllic layer of the plant; therefore, NH_3 emission would occur when the concentration in the plant is higher compared to the atmospheric NH_3 concentration, while under a reverse situation, the plant acts as sink and takes up atmospheric NH_3 (van Hove *et al*., 2002; Schjoerring *et al*., 2000; Fangmeier *et al*., 1994). Porter *et al*. (1972) observed an uptake in NH_3, after a 24 hours exposure of maize plants to various concentrations of 1, 10 and 20 parts per million of labeled NH_3 in air. At the lowest NH_3 concentration of 1 ppm the plants were able to take up to 40% of NH_3 while about 30% were taken up at concentrations of 10 and 20 ppm respectively. In a similar study, van Hove *et al*. (1987) observed an uptake of NH_3 in leaves of *Phaseolus vulgaris* L. after 9 hours fumigation with various NH_3 concentrations and revealed the influence of light intensity in enhancing NH_3 uptake. Furthermore, with increasing NH_3 concentration, the flux density of NH_3 is also elevated especially under increasing light intensity. Gessler *et al*. (2002) in their study revealed the ability of spruce tree *Picea abies* to serve as either sources or sinks of NH_3 depending on the prevailing atmospheric concentration. The NH_3 flux and increase in ammonia deposition on the canopy was dependent on increasing NH_3 concentration as well as stomatal conductance. The effects of six simulated treatments of nitrogen and sulphur mists were evaluated by Cape *et al*. (2001) on Sitka spruce plantation. Nitrogen was supplied as ammonium nitrate (NH_4NO_3) and sulphur as disodium sulphate (Na_2SO_4). They reported the impact of cation fluxes in the retention of 20 to 40 % nitrogen on the canopy, mostly in form of NH_4^+. Similarly, Wilson and Tiley (1998) studied the nitrogen uptake of Norway spruce from simulated mists containing NH_4^+ -N and NO_3-N. Stable ^{15}N isotope was used to distinguish the source of nitrogen uptake i.e either as ammonium (NH_4^+) or as nitrate (NO_3). However, nitrogen uptake was higher in trees exposed ammonium treatment compared to those treated with nitrate mists. This study, showed the ability of foliar absorption and preference of ammonium (NH_4^+) as a nitrogen source for canopy uptake.

Although, the stomatal compensation point of the plant has been reported to determine atmospheric NH_3 uptake or emission from plants, but these can also be influenced by certain prevailing conditions. Husted and Schjoerring (1996) have shown the effects of temperature and light intensities on exchanges between NH_3 flux and the atmosphere in *Brassica napus.* An increase in light intensity up to 600µmol m^{-2} s^{-1} increased the NH_3 flux as well as the leaf conductance of the plant while, a rise in temperature up to 35^oC resulted in a change of the plant to a source of NH_3 emission. Similar results obtained by van Hove *et al*. (2002) in a study conducted around a pasture composed of *Lolium perenne* L. plants, reported the effect of temperature increases on stomatal compensation point and its impact on dissolved NH_3 concentration in the leaf apoplast. Therefore, increased temperature conditions led to alterations in the apoplastic NH_3 concentration and NH_3 emission from the plant.

Foliar uptake of atmospheric NH_3 by plants could be assimilated and stored into other nitrogen compounds such as the free amino acids but however, the compensation point plays a vital role in determining the NHy concentration in the apoplastic film after the conversion of NH_3 to NH_4^+ sequel to the dissolution of NH_3 in the apoplast (Stulen *et al*., 1998). At this point NH_4 is further transferred to the cytoplasm and assimilated by both glutamine synthetase (GS) and glutamate synthase (GOGAT) systems for glutamine and glutamic acid synthesis or could rather be stored in the plant vacuoles (Stulen *et al*., 1998; Lea and Miflin, 1974). The initial reaction involving the formation of 2-oxoglutarate after the incorporation of NH_3 on gluthamic acid is catalyzed by glutamine synthetase which exists in two isoforms namely, GS1 and GS2 with the latter located in the chloroplast and the former in cytosol (Stulen *et al*., 1998). However, under increased NH_4 concentration, GS activity is enhanced (Horchani *et al*., 2010). Horchani *et al*., (2010) investigated the impact of nitrogen assimilation on tomatoes when increasing concentrations of NH_4^+ and NO_3^- were applied as sources of nitrogen nutrients. Under increased NH_4^+ concentrations, NH_4^+ increased in the roots, compared to those in leaves and also influenced an increase in the activities of root glutamine synthetase (GS) in the plant. Therefore NH_4^+ could influence an enhanced GS activity in plants and its ability to withstand NH_4^+ availability (Cruz *et al*., 2006). Similarly, Pearson and Soares (1998) evaluated the impact of misting with NH_4^+ or fumigation of gaseous NH_3 on GS activity in five different plant species. In the fumigated treated plants, they were fumigated with high NH_3 concentrations of 3mg m^{-3} for 1 hour while

the misting treated plants were misted with an NH_4Cl solution of 3mM and sampled a day later. Results obtained from this study showed an increase in GS activity in all plant species studied when misted with an NH_4^+ solution. However, GS activity increased in *P. vulgaris* due to NH_3 fumigation in the fumigated treated plants as well as in the other misting treatment with NH_4^+.

The accumulation of atmospheric NH_3 into free amino acids have been observed to induce increases primarily, in free amino acid compounds with a low carbon to nitrogen ratio, such as arginine, asparagine and glutamine, compared to other compounds based on the minimal carbon required for additional nitrogen storage in low carbon to nitrogen compounds (Nordin *et al*., 1998; Fangmeier *et al*., 1994; Näsholm *et al*., 1994). As a result of this accumulation, changes in the free amino acid concentrations and compositions of individual free amino acids are observed in plants exposed to an additional source of nitrogen supply, for instance atmospheric NH_3 due to a resultant change in the plant metabolism (Fangmeier *et al*., 1994; Näsholm *et al*., 1994). Huhn and Schulz (1996) investigated the free amino acid compositions in needles of *Pinus sylvestris* in various locations exposed to different rates of nitrogen deposition. Needles with higher nitrogen contents, demonstrated several folds increases in free amino acid compositions, especially in glutamine and arginine compounds. Pitcairn *et al*. (2003) also evaluated the free amino acid compositions of *Rhytidiadelphus triquetrus*, *Brachythecium rutabulum* and *Pseudocleropodium purum* along a 276 m long transect around a poultry farm. The free amino acid composition in all the species studied demonstrated a positive relationship with the NH_3 concentrations at each site with arginine accounting for the most dominant amino acid and has been reported as an important signal of nitrogen deposition in mosses (Pitcairn *et al*., 2003), *Pinus sylvestris* (van Dijk and Roelofs, 1988; Edfast *et al*., 1996; Hunn and Schulz, 1996), *Picea engelmannii* (Calani *et al*., 1999), *Picea abies* (Zedler *et al*., 1986). Similarly, a shift from an initial accumulation in glutamine to arginine was observed in some forest boreal plants exposed to nitrogen additions in form of NH_4^+ and NO_3^- nutrients (Ohlson *et al*., 1995).

The sensitivity of certain plants to NH_3 uptake, by demonstrating changes in the plant biomass composition and allocation, have also been confirmed as the ability of these plants, to utilize this nitrogen compound, as a source of nitrogen nutrient (Castro *et al*., 2008; Perez-Soba and van der Eerden, 1993). Zhang *et al*. (2011) conducted a

fumigation study and evaluated the effects of atmospheric NH_3 on maize cultivars exposed to two nutrient levels. At the end of the fumigation period, plants grown with limited nitrogen nutrients were able to incorporate atmospheric NH_3 for growth by inducing 14 and 18 % increases respectively, in the overall dry matter production of both cultivars exposed to two treatment levels of atmospheric NH_3. This study indicates the beneficial effects of atmospheric NH_3 uptake through foliar absorption and its ability to compensate for limiting nitrogen supply to roots (Zhang *et al*., 2011).

In forest ecosystem where a lot of studies have been conducted in the past, atmospheric nitrogen induced effect is well established and the effects of this can be observed as an example, based on the biomass accumulation of forest ecosystems in response to the uptake of readily available atmospheric nitrogen (Dueck *et al*., 1998). In an open chamber experiment, (Dueck *et al*., 1998) reported that, NH_3 induced increases in the needle biomass and also influenced a significant effect on root biomass composition of *Pinus sylvestris* trees after exposure to NH_3 fumigation at concentrations of 16, 55 and 110 ppb daily for a period of 15 months. A similar observation on the accumulation of needle biomass on *Pinus sylvestris*, was reported by Perez-Soba and van der Eerden (1993) when plants were fumigated with 53 and 105 µg m^{-3} concentrations of gaseous NH_3 over a 12 months period. In other studies, Baba *et al*. (2001) reported a reduction of up to 50 % of nitrogen in bulk precipitation to forest canopies as a result of throughfall input thereby, enhancing the growth of the forest ecosystems.

3. MATERIALS AND METHODS

3.1 Species selected and their cultivation

The influence of atmospheric NH_3 on the following selected indicator plants, Italian rye grass (*Lolium multiflorum* Lema.), banyard grass (*Echinochloa crus-galli* L.), common nettle (*Urtica dioica* L.) and common lambsquarters (*Chenopodium album* L.) and their ability to detect and respond to NH_3 availability were determined by two different experiments. The four plant species were chosen for this study since they are fast growing and easily to be cultivated, coupled with their high demand for nitrogen according to Ellenbergs nitrogen indicator values. Furthermore, the selected indicator plants comprised of both annual and perennial, consisting of competitive and ruderal species (Franzaring pers. communication.). Prior to field exposures, *Lolium multiflorum* seeds supplied by NPZ-Lembke, Germany, *and Echinochloa cruss-galli*, *Chenopodium album* and *Urtica dioica* supplied by Appels Wilde Samen Darmstadt, Germany, were sown and grown on neutral peat in the greenhouse of Hohenheim University, Germany under controlled conditions. *Lolium multiflorum and Echinochloa cruss-galli* seeds were sown at a rate of 0.6g per pot, while *Chenopodium album* and *Urtica dioica* had 5 plants per pot (volume 1.5 litres) fitted with two fibre glass wicks, passed through the pots across each other, which enabled a self watering system VDI (2003) and watering was done with deionized water so as to avoid additional nitrogen supply during the experiment. The plants were fertilised in three doses to achieve an application of nitrogen nutrition at a rate of 50 kg N ha^{-1} in grasses and 75 kg N ha^{-1} in herbs and an additional 20mL solution of nitrogen free nutrients thrice composed of modified Hoaglands solution. Growth within the greenhouse lasted for a period of 4 to 6 weeks before the plants were taken out to the field. Shortly before field exposure *Echinochloa crus-galli* and *Lolium multiflorum* plants were clipped to a height of 4 cm. Plants were grown at 13/11h day/night inside the greenhouse and were rotated every five days within this period, to ensure uniformity in positions and plant condition in order to minimize bias.

Table 1: Details of growing periods and 4 weeks exposure of respective plants used in the field and fumigation studies until destructive harvest conducted in 2010 and 2011.

	Date		
	Sowing	Beginning of field exposure / fumigation	Harvest
Field study, 2010			
Lolium multiflorum		3rd Aug	31st Aug
Echinochloa crus-galli		20th Jul	17th Aug
	23rd Jun		
Urtica dioica		17th Aug	14th Sept
Chenopodium album		3rd Aug	31st Aug
Fumigation study, 2011			
First Set			
Lolium multiflorum			
Echinochloa crus-galli	19th Apr	17th May	14th Jun
Second Set			
Lolium multiflorum	11th Jun	8th Jul	5th Aug
Echinochloa crus-galli			22nd Jul

3.2 Field study and site description

3.2.1 Site description.

The first experiment was a field study whereby, the plants were exposed outside in the field in close proximity to a livestock farm located at Blaustein - Wippingen, Germany. At this site, the mean annual temperature for the year during this study was 7.2°C while the annual precipitation was 811 mm.

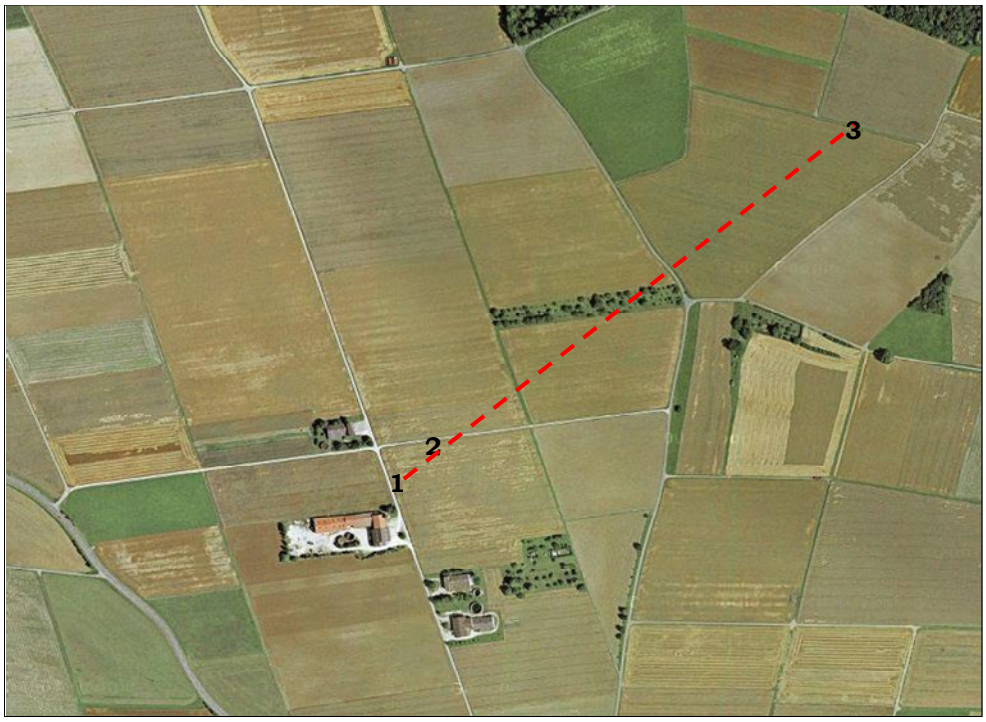

Fig 1: The location of three different sites around a livestock farm across a gradient of NH_3 emissions and nitrogen deposition.

The livestock farm served as a source of NH_3 pollution and three monitoring sites with distances of 67 m, 149 m and 804 m from the stable were selected. The sites were selected along a gradient in the North East direction of the farm which is in line with the predominant wind direction. At each site along the selected distances, three replicates of each plant species were mounted about 2 m aboveground alongside with passive diffusion tube samplers (Radiello®) used in measuring the ambient NH_3 concentrations at each site. The passive diffusion tube samplers consisted of a cartridge inserted and housed into a cylindrical tube with diffusive capabilities. The cartridge has the ability of absorbing NH_3 as NH_4^+ ion and at the end of each exposure period, NH_3 analysis were carried-out strictly according to the manufacturers instruction manual. The passive diffusion tube samplers were changed bi-weekly and the plants were exposed for a period of 4 weeks before destructive harvest. The plants were mounted in a water reservoir, which supplied deionised water to the plants through fibre wicks for the entire period.

Fig 2: Exposure of bioindicator plants and passive diffusion tube samplers at a site in the field.

3.2.2 Climatic conditions during the field exposure periods

The meteorological data obtained throughout the field study shows the climatic condition of the various sites throughout the exposure period. Data used in this study was from the closest weather station located in Gerstetten (http://www.wetter-bw.de). The daily ambient air temperature was mostly between 11 and 18^{o}C while higher precipitation events were observed within the first one month of field exposure. The exposure period was under relatively humid conditions with relative humidity measurements in the range of 80 to 100 %. Wind speed data shows that wind speed was mostly between 0.2 and 0.9 m/s for the entire period.

Table 2: Mean values of air temperature and relative humidity during field study of the various plant species.

Variable	Air Temperature (^{o}C)	Relative Humidity (%)
Field study		
Echinochloa crus-galli	15.9	87.6
Urtica dioica	13.9	87.7
Lolium multiflorum	15.6	87.6
Chenopodium album	15.6	87.6

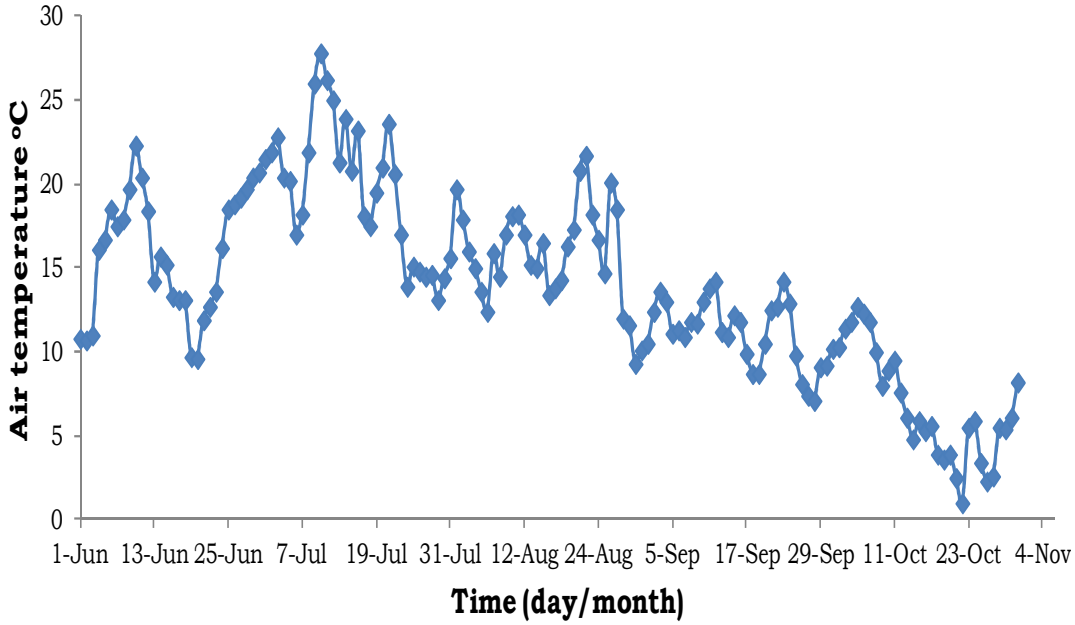

Fig 3A: Mean air temperature during the exposure period of plants and passive sampler

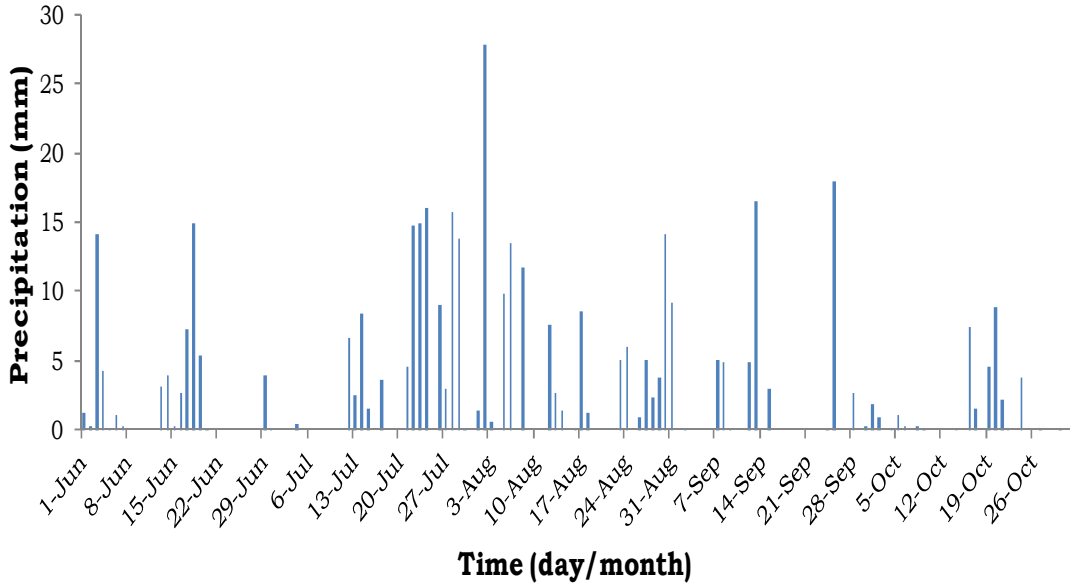

Fig 3B: Total precipitation (mm) during the exposure period of plants and passive samplers

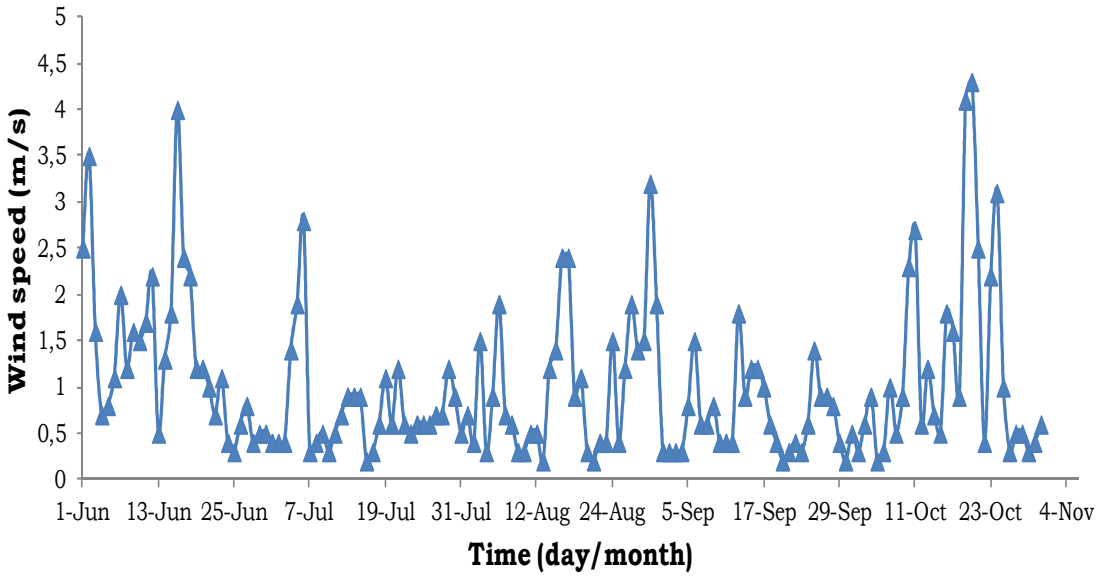

Fig 3C: Mean wind speed during the exposure period of plants and passive samplers

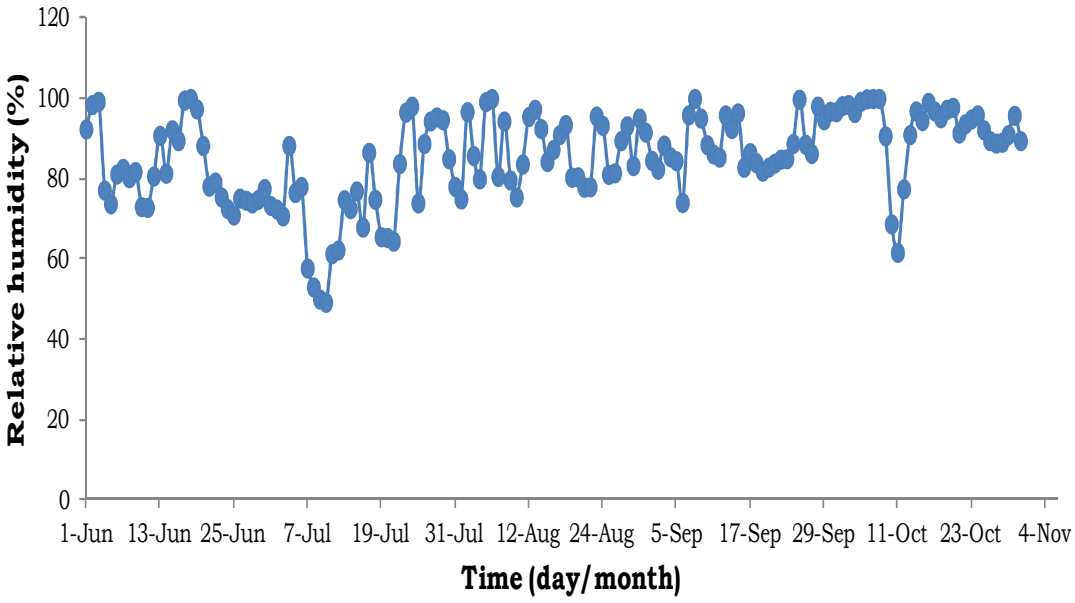

Fig 3D. Relative humidity during the exposure period of plants and passive samplers

3.3 Fumigation study

3.3.1 Plant culture

The second experiment was a fumigation study conducted in fumigation chambers inside the greenhouse. Two different sets of fumigation studies were executed based on different soil types. In the first set of experiments in this study, seeds of *Lolium multiflorum* and *Echinochloa crus-galli* were sown in 1.5 litres pot containing neutral peat soil. Immediately after emergence, seedlings of *Echinochloa crus-galli* were thinned to 60 plants per pot in all the pots of *Echinochloa crus-galli* plants in this study. Fertiliser was applied in three stages as follows: pre emergence, after emergence and post emergence (i.e one week after emergence) to give a total nitrogen application at the rate of 50 kg N ha^{-1} per pot in form of NH_4NO_3. In addition other nutrients were supplied as above. At the end of four weeks, plants were transferred into fumigation chambers.

In the second set of experiment in the fumigation study, seeds of *Lolium multiflorum* and *Echinochloa crus-galli* were sown in 1.5 litres pot containing fertilised soil (LD 80). The LD 80 soil in each 1.5 litres pot already contained 225 mg N per pot. Similarly, in this batch of the fumigation study, seedlings of *Echinochloa crus-galli* were also thinned to 60 plants per pot after emergence. No other nutrient was applied in this experiment from the moment of sowing till destructive harvest. Plants were allowed to grow for four weeks and then transferred into fumigation chambers.

3.3.2 Fumigation chambers

The three chambers used in this fumigation study, were constructed and designed in a cylindrical structure in order to enable effective circulation and flow of gaseous NH_3 within the chambers. The dimensions of the each chamber were fabricated to a height of 105 cm and 86 cm in diameter with a total volume capacity of 610 L big enough to accommodate the growth of the selected plant species throughout the fumigation period. The chambers were constructed with wire gauze as its frame and mounted on a wooden bottom plate of 1 x 1 m. The wire gauze frames were large enough to enable the transfer of light into the chambers and to the plants. A UV greenhouse plastic film, with the capacity of an excellent light transmission and a high capability to enable the transfer of

photosynthetic active radiation to the plants from both daylight and series of flora lamps placed above each chamber, was used to enclose the top and sides of the frames. Afterwards, the plastic films were properly sealed unto the frames to minimize the losses of gaseous NH_3 from the chambers during fumigation, while the flora lamps were switched on and off in a 12hours sequence. Each chamber was equipped with a ventilator (140x140x25mm) producing an air flow at the rate of $60m^3/h$ which ensured uniform distribution as well as efficient mixing and circulation of NH_3 inside the chambers, by passing the air stream containing gaseous NH_3 through the fan. The chambers were also fitted with data loggers which measured the temperature and relative humidity inside the chamber every 30 minutes. Throughout the entire fumigation period, the chambers were continuously supplied with an inlet air from the base that produced an air change at the rate of 3.7 air changes per hour in each chamber. Independent connection of pipes were made, which supplied gaseous NH_3 or non filtered air from the bottom of each chamber and the exhaust in likewise manner was through the exhaust duct located at the top of the chambers. Non filtered air was supplied through pipes and measured with already calibrated independent gas meters for each chamber. For the generation of gaseous NH_3, an automatic peristaltic pump (Watson Marlow) was used to transfer equal drops of sodium hydroxide (NaOH) into an NH_3 generation chamber containing a solution of ammonium chloride (NH_4Cl) for 12 hours daily. The concentration of the salts, were based on the required gaseous NH_3 concentrations for either low or high treatments. The continuous supply of the alkaline salt resulted in a chemical reaction of both salts that culminated in the continuous generation and volatilization of gaseous NH_3 inside the NH_3 generation chamber. Ambient air was supplied into the NH_3 generation chamber, where it was properly mixed with NH_3 gas and subsequently pumped out of the NH_3 generation chamber with an air pump and the air stream already mixed with NH_3 was supplied in the air flow direction of the ventilator located inside the chambers.

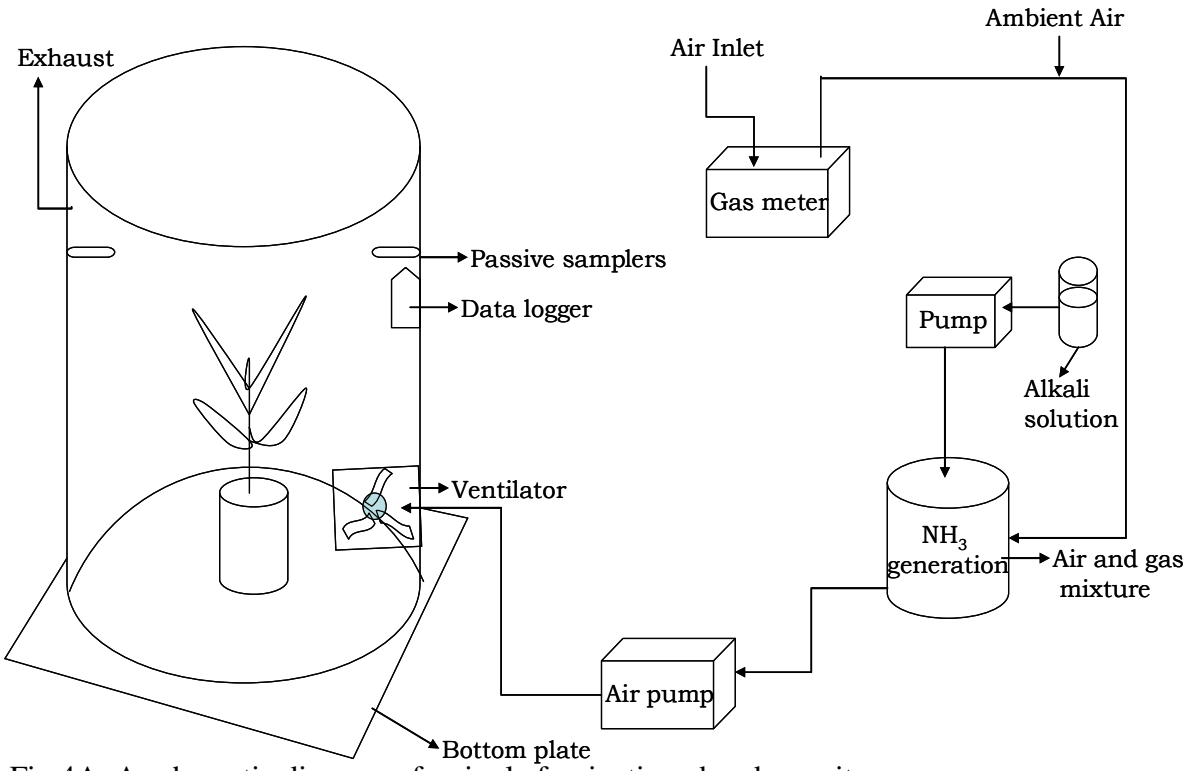

Fig 4A. A schematic diagram of a single fumigation chamber unit.

3.3.3 NH_3 fumigation treatments

In this study, three different treatments were applied as follows, non-filtered air (NFA), non-filtered air with low NH_3 concentration (NFA+) and non-filtered air with high NH_3 concentration (NFA++). Plants with non-filtered air treatments were not supplied with NH_3 but only ambient air. Four replicates of each plant, *Lolium multiflorum* and *Echinochloa crus-galli*, were placed in each chamber and were fumigated for a period of 12 hours between 8:00 and 20:00 daily for 4 weeks, with gaseous NH_3 in an air stream supplied into the chambers. Each pot was supplied with water reservoirs which enabled an independent watering system through fibre glass wicks inserted in each pot. The various treatments as well as plants in each chamber were rotated weekly to minimize experimental error due position effects. At the end of the entire fumigation period, plants were immediately subjected to destructive harvest for further analysis.

3.3.4 Climatic conditions in the fumigation chambers

The automatic data loggers recorded air temperatures and relative humidity inside the greenhouse and fumigation every 30 minutes and in the first set of the fumigation study conducted, the average air temperature inside the chamber was 27.5^{o}C during the day and 19.7^{o}C at night, while greenhouse accounted for 26.7/18.9^{o}C day and night temperatures respectively.

Fig 4B: Fumigations chambers used for this study within the green house facility of the University of Hohenheim.

The relative humidity inside the chambers was mostly between 80-100 %, whereas inside the greenhouse was generally within 30-65 %. In the second set of experiment, the average air temperature inside the chambers was also higher compared to the greenhouse and accounted for day and night temperatures of 28.1^{o}C in the chambers and 20^{o}C inside the greenhouse. The chambers also recorded higher relative humidity

compared to the greenhouse. In the chambers the relative humidity was generally between 90-100 %, while in the greenhouse it was mostly within 35-80 %.

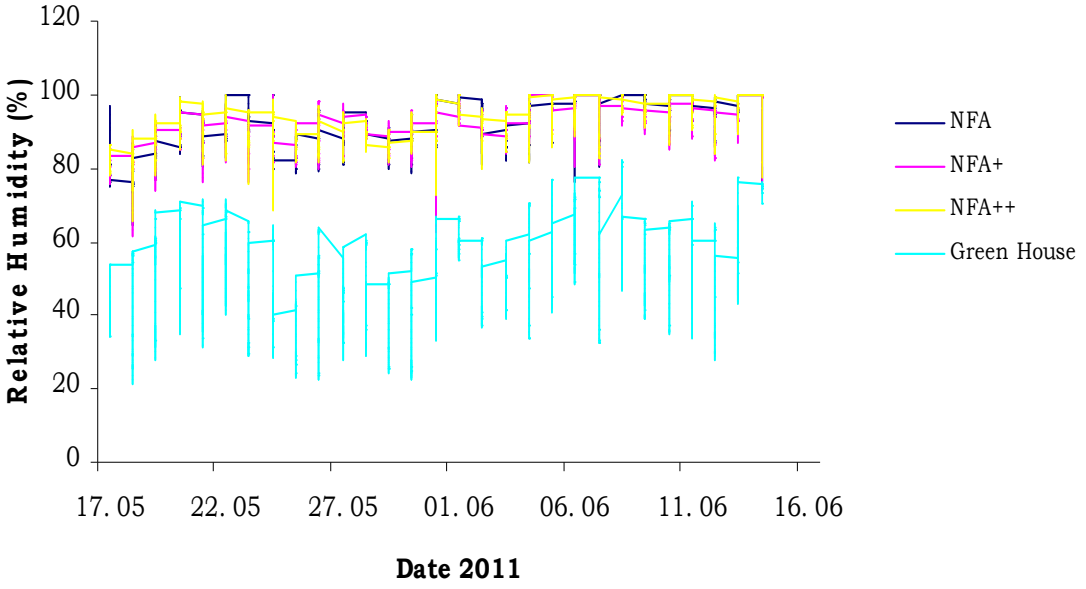

Fig 5A: Relative humidity measured every 30 minutes in the green house and each chamber treated with NFA, NFA+ and NFA++ treatments respectively in the first set of fumigation experiment.

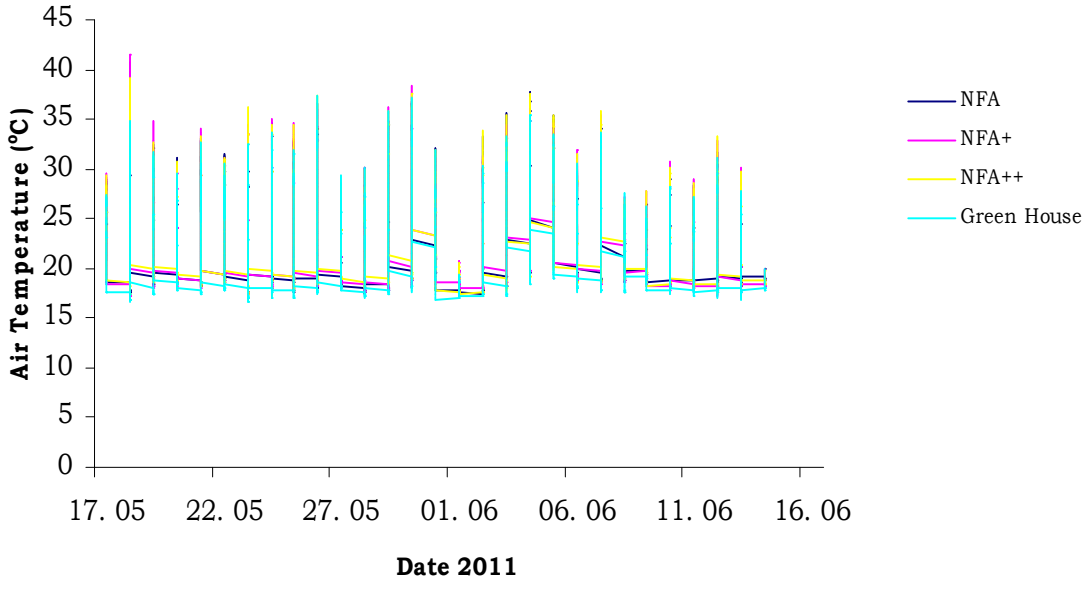

Fig 5B: Air temperature measured every 30 minutes in the green house and each chamber treated with NFA, NFA+ and NFA++ treatments respectively in the first set of fumigation experiment.

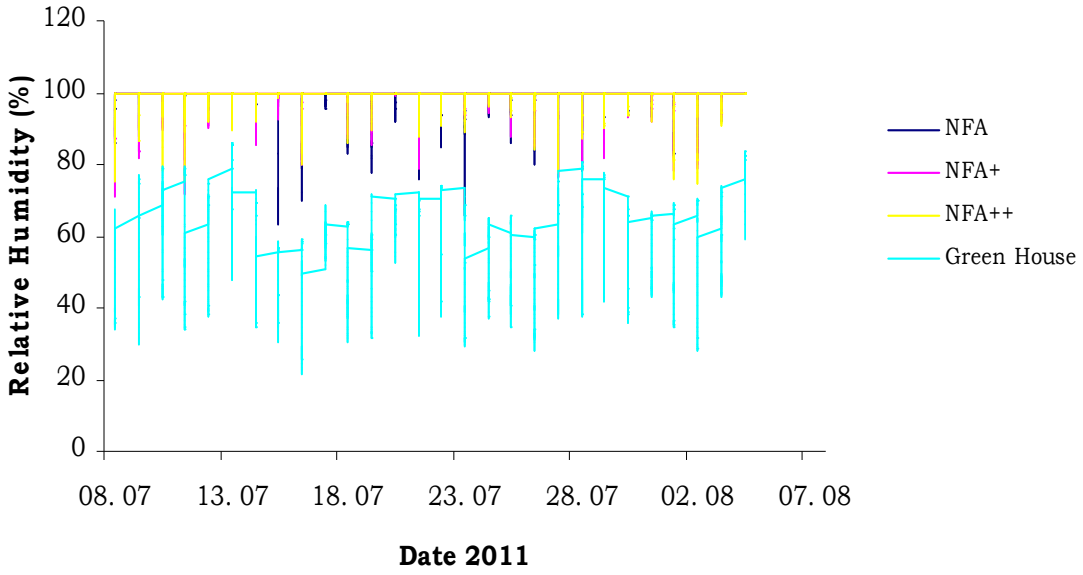

Fig 6A: Relative humidity measured every 30 minutes in the green house and each chamber treated with NFA, NFA+ and NFA++ treatments respectively in the second set of fumigation experiment.

Fig 6B: Air temperature measured every 30 minutes in the green house and each chamber treated with NFA, NFA+ and NFA++ treatments respectively in the second set of fumigation experiment.

Table 3: Mean values of air temperature and relative humidity in fumigation chambers during the fumigation of the plant species.

Variable	Air Temperature (^{o}C)	Relative Humidity (%)
Fumigation study		
First Set		
NFA	23.7	91.5
NFA+	23.8	90.9
NFA++	23.8	91.9
Second Set		
NFA	24	97.2
NFA+	24.1	97.9
NFA++	24	98.4

NFA, NFA+ and NFA++ values represent non filtered air, non filtered air with low NH_3 concentrations and non filtered air with high NH_3 concentration respectively.

3.4 Plant sampling and amino acid analyses.

Fresh leaves from each pot of all the plant species studied in the field and in the fumigation study were collected and weighed separately. Sequel to the determination of fresh weights the samples were immediately stored in -80^{o}C prior to lyophilisation of the plant materials. After freeze drying, the leaf samples were milled (Retsch MM 301 GMBH) in order to homogenize the plant material before amino acid analysis. However, in very few cases due to the small biomass fractions of leaves in some of the field exposed plants, a small part of the stem fractions were added in order to obtain sufficient plant materials for amino acid analysis. Amino acids were analysed by UPLC (Waters, Milford. MA, USA) enabled by a fluorescence detector. The methodology has been described in detail by Thiel *et al*. (2009). In brief, the derivatives were obtained based on the guideline manual of the device according to AccQ®Tag method (Waters, http://waters.com), while column separation was done by an AccQ®Tag Ultra column (2.1 x 100mm). Amino acids from finely milled dried plant tissues, were extracted in 440ul of (80%, v/v) ethanol for 45mins. This extraction was carried-out twice from samples of each plant species at 80^{o}C. The extracts were vacuumed and the concentrated samples subsequently re-dissolved in 40ul of water and an added internal standard of 5 mM norvaline at the rate of 1 ul to each sample. Supernatants obtained after centrifugation at 4^{o}C for 5 min were stored in -20^{o}C. Various gradients were used at different time (min) and in AccQ®Tag Ultra Eluent A (%) and AccQ®Tag Ultra

Eluent B (%) while separations were carried out at 60^{o}C at a flow rate of 0.7ml min^{-1}. The excitation and emission wavelength used were 266 nm and 473 nm at a sampling rate of 20 points s^{-1}. In total, 20 free amino acids were detected and the WATERS EMPOWER software was used in calculating and quantifying the various amino acid compounds.

3.5. Statistical analysis

The data obtained from both the field study and fumigation experiment, were evaluated separately. The effects of ambient NH_3 concentrations on growth parameters were processed by one-way analysis of variance (ANOVA) and the significance of effects was tested at ($p \leq 0.05$) level. Linear regression analysis was used to test the relationship between above ground biomass, free amino acid concentrations and total free amino acid concentrations of the plants with selected distances around the farm and also with the ambient NH_3 concentration measured along the gradient of NH_3 emission and nitrogen deposition.

4. RESULTS

4.1 Ambient NH_3 concentrations across different sites

The ambient NH_3 concentrations measured with passive diffusion tube samplers across the three selected sites, showed variability in NH_3 concentrations for the different exposure periods. The ambient NH_3 concentrations across these sites were measured continuously in a total of nine series between the periods of 7th June to 12th October 2010 (Figure 7). However, the plants investigated in this study were exposed in the field between July and September, before they were conveyed back to the laboratory for destructive harvest, measurements and free amino acid analysis. Results of the ambient NH_3 measurement obtained across the entire nine series showed that the passive samplers deployed in close proximity to the stable at 67 m had the highest NH_3 concentration, followed by those at 149 m and 804 m respectively.

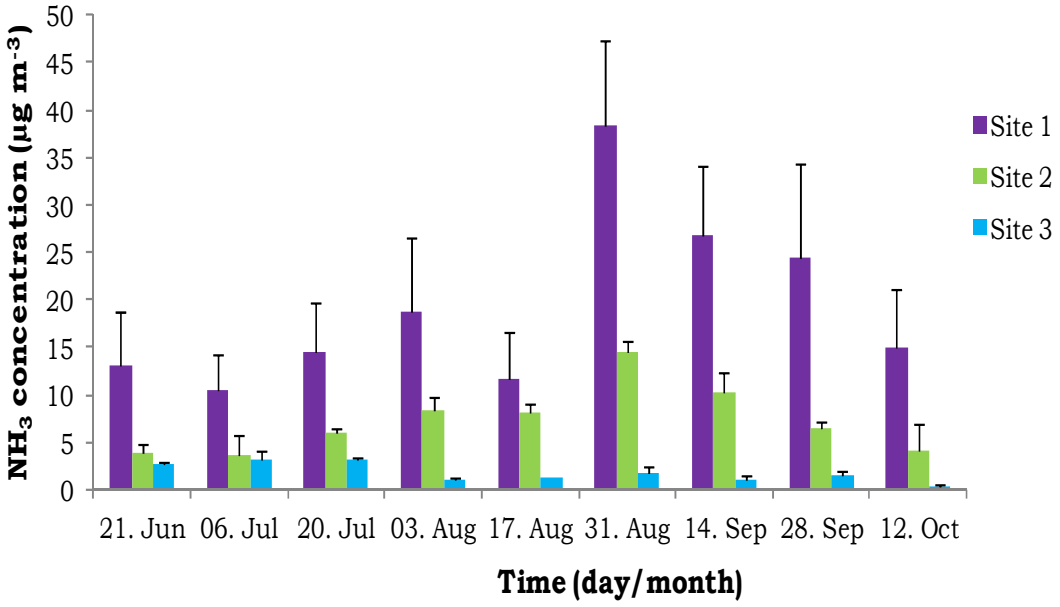

Fig 7: Mean ambient NH_3 concentrations measured bi-weekly across sites for the exposure period between (Jun – Oct) 2010.

During the exposure period of the *Echinochloa crus-galli,* plants in the field, between 20th July and 18th August, the ambient NH_3 concentration measured by the passive diffusion tube samplers was 15 µg m^{-3}, 8 µg m^{-3} and 1 µg m^{-3} respectively across distances of 67 m, 149 m and 804 m (Figure 8A). The ambient NH_3 concentration at

time of the exposure period of *Lolium multiflorum* and *Chenopodium album* plants from the 3rd to 30th August, was 25 µg m^{-3}, 11 µg m^{-3} and 1.5 µg m^{-3} respectively, across the selected transect (Figure 8B).

Furthermore, the highest NH_3 concentration was observed during the exposure period of *Urtica dioica* plants between 17th August to 14th September accounting for NH_3 concentrations of 33 µg m^{-3}, 12 µg m^{-3} and 1.5 µg m^{-3} across the three sites (Figure 8C).

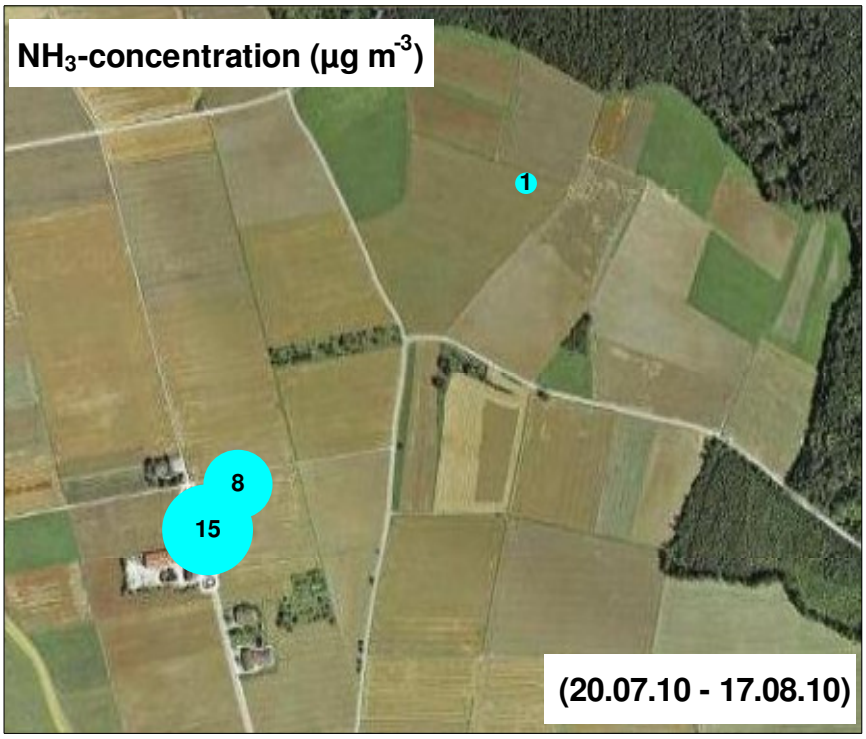

Fig 8A: Mean ambient NH_3 concentration across sites in the livestock farm for the exposure period between 20th July and 17th August.

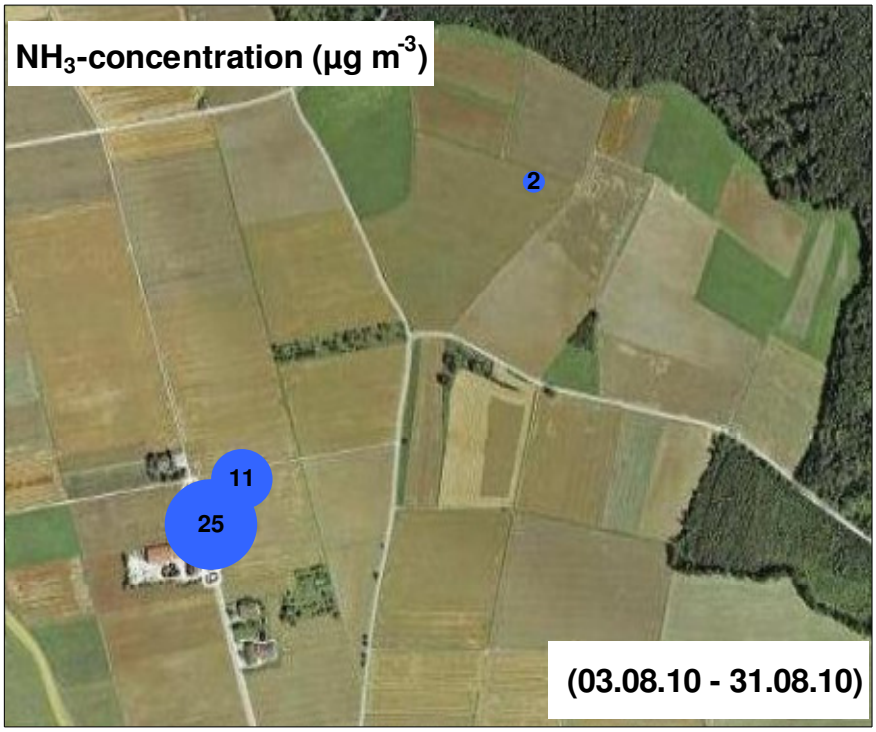

Fig 8B: Mean ambient NH_3 concentration across sites in the livestock farm for the exposure period between 3rd and 31st August

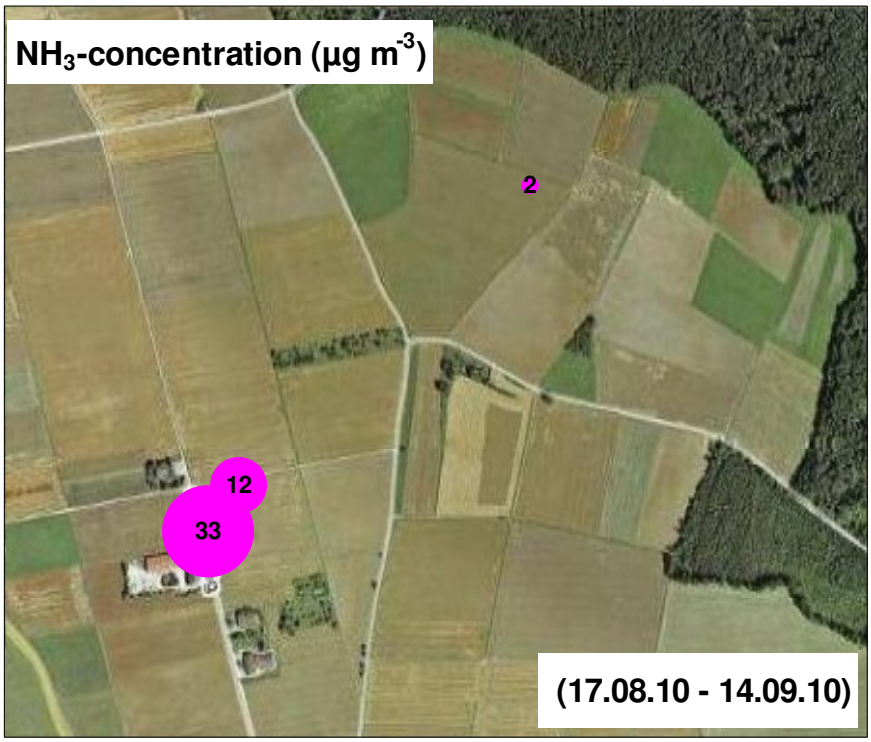

Fig 8C: Mean ambient NH_3 concentration across sites in the livestock farm for the exposure period between 17th August and 14th September.

4.2. The effects of ambient NH_3 concentrations on free amino acid concentrations and compositions in *Lolium multiflorum*, *Chenopodium album*, *Echinochloa crus-galli* and *Urtica dioica*

The ambient NH_3 concentration measured across several distances around the livestock farm induced changes in cellular metabolites such as the free amino acids in the selected plants studied. All the free amino acids concentrations in *Lolium multiflorum* increased with increasing proximity to the livestock farm except in tryptophan. Glutamine (Gln), Alanine (Ala) and Asparagine (Asn) dominated the amino acid pool in *Lolium multiflorum* with concentrations ranging from 1.9 - 8 µmol g^{-1}, 2.9 - 7.6 µmol g^{-1} and 0.2 – 6 µmol g^{-1} respectively, across the distances investigated with increasing ambient NH_3 concentration (Figure 9A). The percentage composition of each individual amino acid to the total amino acid concentration in *Lolium multiflorum* is shown in (Figure 10A). Gln was the predominant amino acid in *Lolium multiflorum* located 67 m away from the stable and constituted 19.1% of the total free amino acids. This is followed by Ala and Asn which constituted of 18.2% and 14.2% respectively of the total free amino acids (Figure 10A).

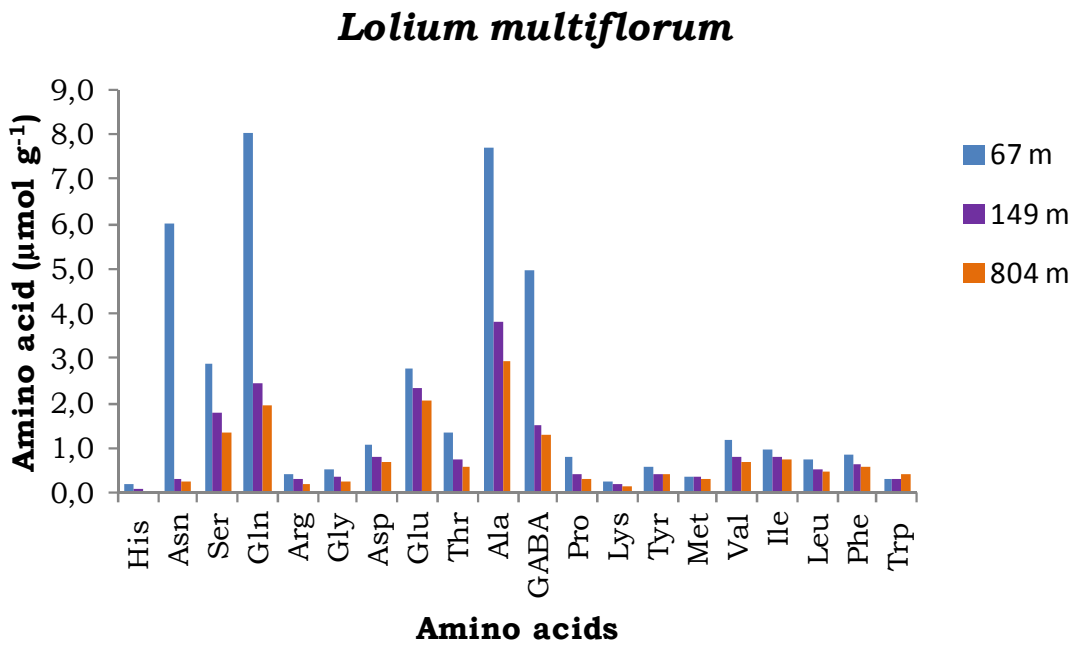

Fig 9A: Relationship between free amino acids in *Lolium multiflorum* with distance from the farm. Means (n=3) for each amino acid at distances of 67 m, 149 m and 804 m from the stable.

Ala, Gln and Glu dominated the amino acid in *Lolium multiflorum* species at 149 m and 804 m from the point source. The percentage composition of Ala was 20%, Gln 12.8% and Glu 12.1% of the total free amino acids at a distance of 149 m and 18.7%, 12.2% and 13.1%, of the total free amino acids at 804 m respectively from the stable. The contents of all other amino acids were less than 5% in *Lolium multiflorum* across all distances studied except in Serine (Ser) and GABA (Figure 10A).

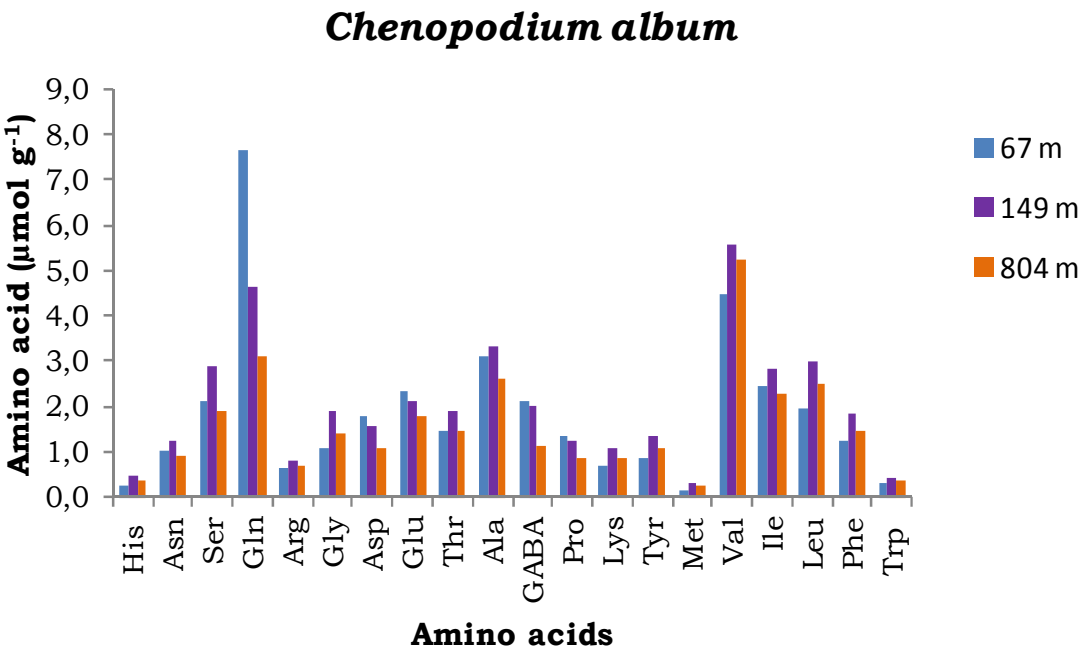

Fig 9B: Relationship between free amino acids in *Chenopodium album* with distance from the farm. Means (*n*=3) for each amino acid at distances of 67 m, 149 m and 804 m from the stable.

Gln was the most prominent amino acid in *Chenopodium album* at the closest distance from the stable. High Gln concentrations were also observed at further distances, but lower than Val concentration which dominated the amino acid pool at 149 m and 804 m respectively (Figure 9B). Gln accounted for 20.5% of the total amino acids at 67 m while Val was 13.7% and 16.6% at 149 m and 804 m away from the stable (Figure 10B). Ala was the most dominant amino acid in all the biochemically analysed *Echinochloa crus-galli* samples. The Ala concentration in *Echinochloa crus-galli* was

11.1 µmol g^{-1},9.7 µmol g^{-1} and 7.1µmol g^{-1} across the gradient of 67 m, 149 m and 804 m respectively (Figure 9C). Hence this accounted for 25.5%, 28.5% and 25.7% of the entire amino acid composition in the plant with increasing distance from the stable (Figure 10C). The other major amino acids which dominated the amino acid concentrations and percentage composition, at all the distances studied were Ser, Gln, GABA and Glu respectively (Figures 9C and 10C).

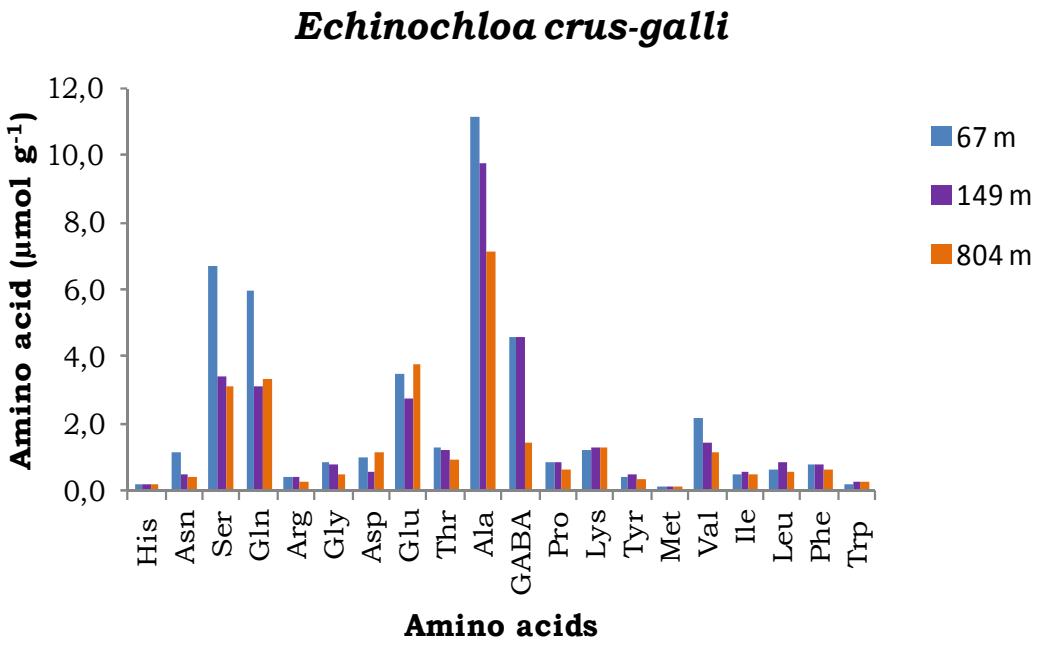

Fig 9C: Relationship between free amino acids in *Echinochloa crus-galli* with distance from the farm. Means (*n*=3) for each amino acid at distances of 67 m, 149 m and 804 m from the stable.

Results obtained from *Urtica dioica* showed Lys dominance across all distances of field exposure. Lys concentration increased with closer proximity to the stable constituting 17.6 µmol g^{-1}, 13.1 µmol g^{-1} and 5.7 µmol g^{-1} of the total amino acid concentration and accounting for 29.2%, 30.3% and 17.4% of the total amino acid composition across distances of 67 m, 149 m, and 804 m (Figures 9D and 10D). At the highest NH_3 concentration, Ala, Asn, Gln and Glu also made up a major proportion of the total amino acids in *Urtica dioica.*

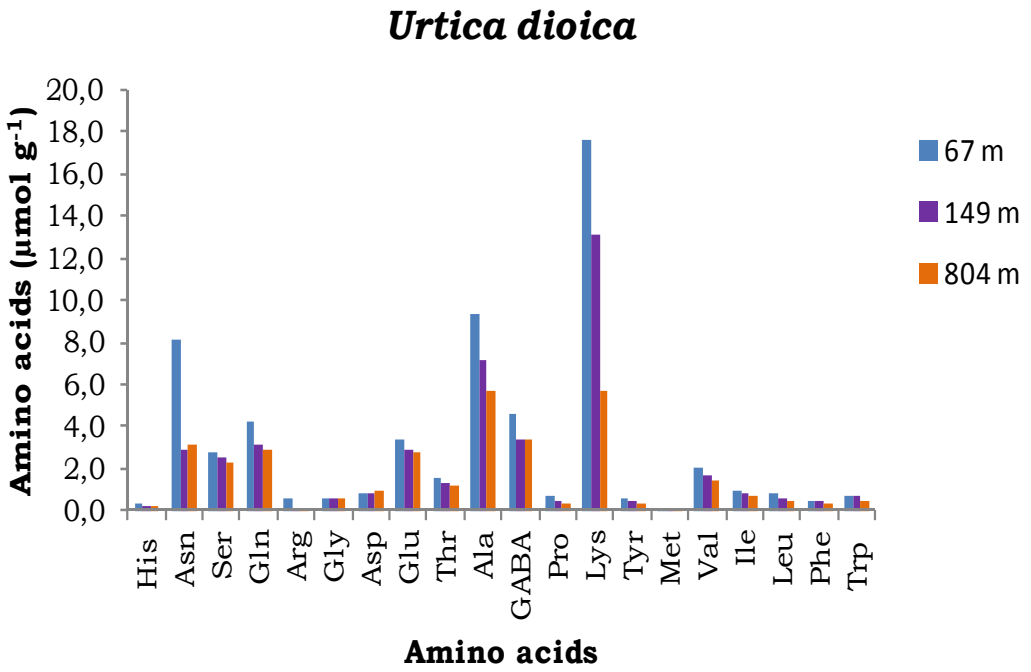

Fig 9D: Relationship between free amino acids in *Urtica dioica* with distance from the farm. Means (*n*=3) for each amino acid at distances of 67 m, 149 m and 804 m from the stable.

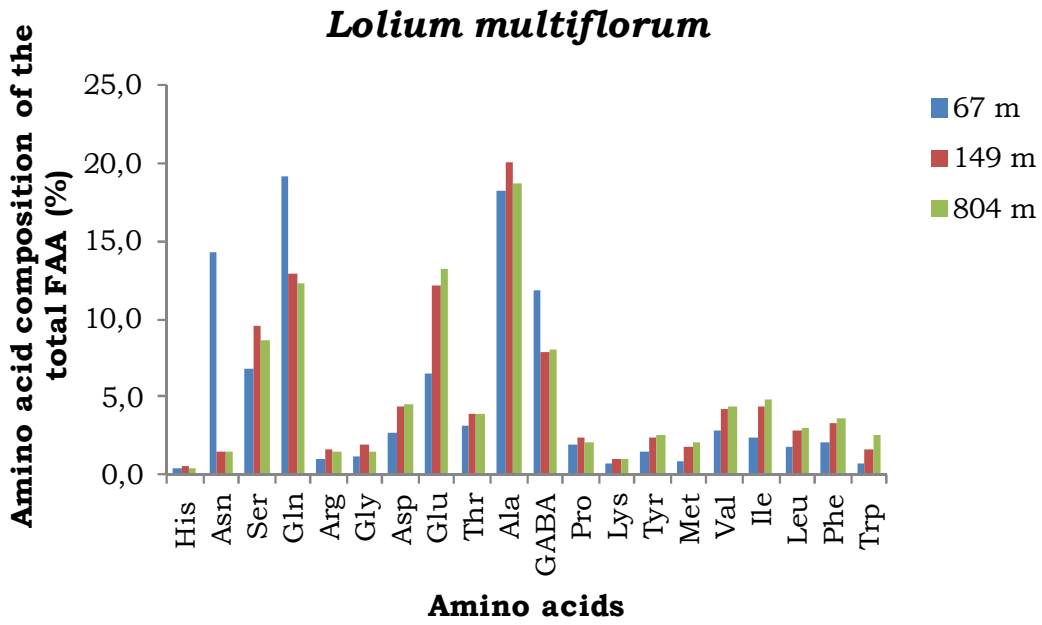

Fig 10A: Percentage composition of individual amino acids of the total free amino acids (FAA) in *Lolium multiflorum*. Means (*n*=3) for each amino acid at distances of 67 m, 149 m and 804 m from the stable.

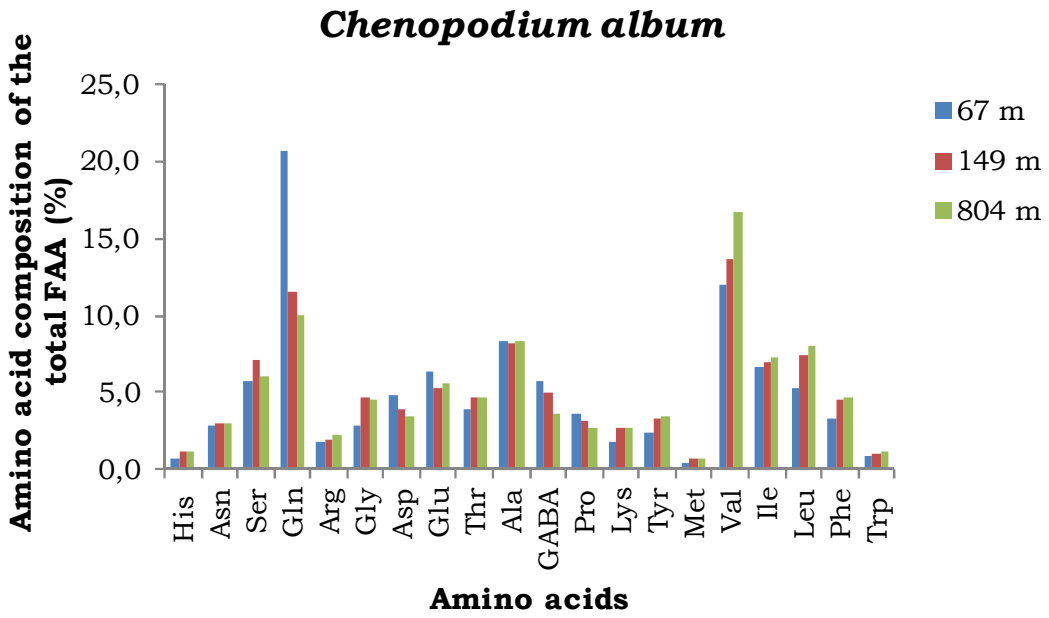

Fig 10B: Percentage composition of individual amino acids of the total free amino acids (FAA) in *Chenopodium album*. Means (*n*=3) for each amino acid at distances of 67 m, 149 m and 804 m from the stable.

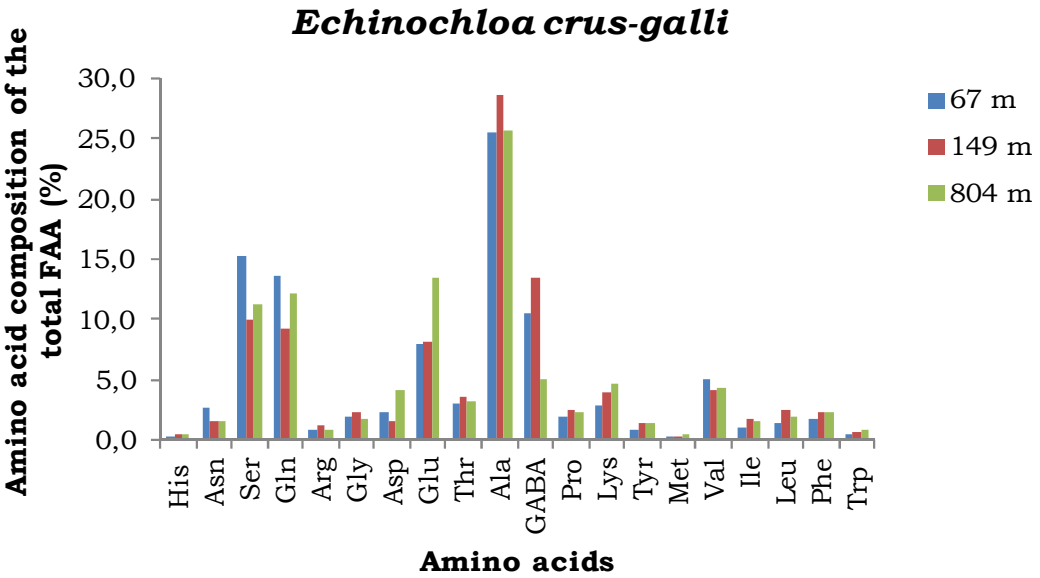

Fig 10C: Percentage composition of individual amino acids of the total free amino acids (FAA) in *Echinochloa crus-galli* Means (*n*=3) for each amino acid at distances of 67 m, 149 m and 804 m from the stable.

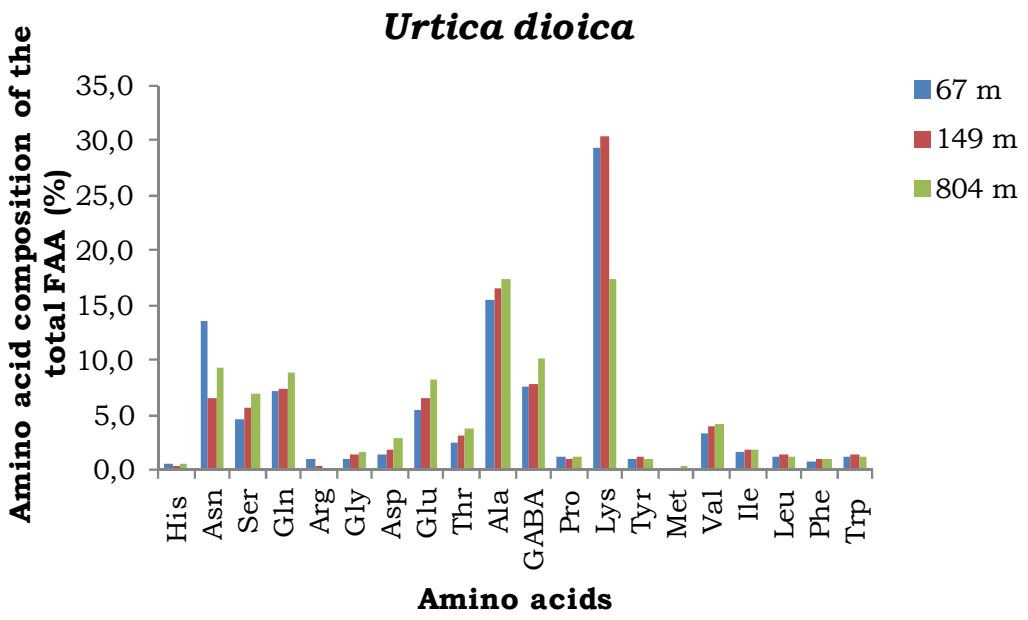

Fig 10D: Percentage composition of individual amino acids of the total free amino acids (FAA) in *Urtica dioica*. Means (*n*=3) for each amino acid at distances of 67 m, 149 m and 804 m from the stable.

4.3 The relationship between total free amino acid concentrations and increasing ambient NH_3 concentrations in *Lolium multiflorum*, *Chenopodium album*, *Echinochloa crus-galli* and *Urtica dioica*

A relationship between the total free amino acids in all the plant species studied with increasing ambient NH_3 concentrations is shown in (Figures 11A-D). *Lolium multiflorum* showed a linear increase (R^2 = 0.9035) in total amino acids with increasing NH_3 concentration. The total amino acids increased from 15.8 to 42 µmol g^{-1} under increasing NH_3 concentration of 1.5 and 25.1µg m^{-3} (Figure 11A). A similar trend was also observed in *Echinochloa crus – galli* and *Urtica dioica*. They both showed linear increases (R^2 = 0.9858 and R^2 =0.9987) with increase in NH_3 concentration. NH_3 concentrations between 1.1 and 15µg m^{-3} in *Echinochloa crus- galli* and 1.45 to 32.6 µg m^{-3} in *Urtica dioica* induced increases in total amino acid concentrations in both plants to 43.5 and 60.3 µmol g^{-1} respectively (Figures 11C and 11D). However, in *Chenopodium album*, a weak relationship (R^2 = 0.2832) between total amino acids and increasing NH_3 concentration was observed (Figure 11B).

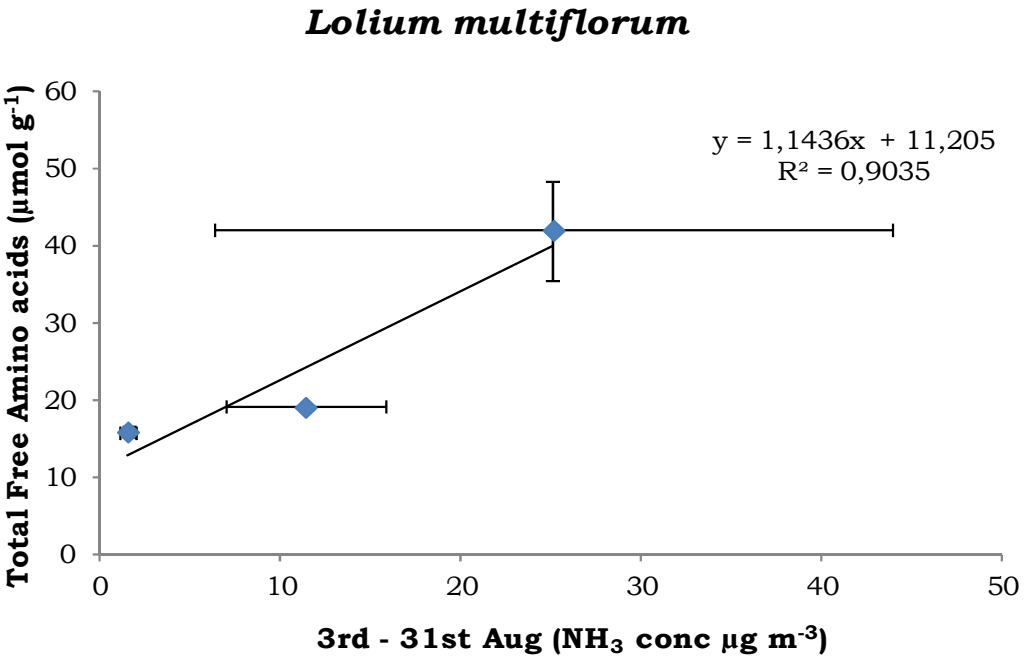

Fig 11A: A linear increase in total free amino acids in *Lolium multiflorum* with increasing ambient NH_3 concentrations. Means (n=3) ± SD for total free amino acids and NH_3 concentrations respectively.

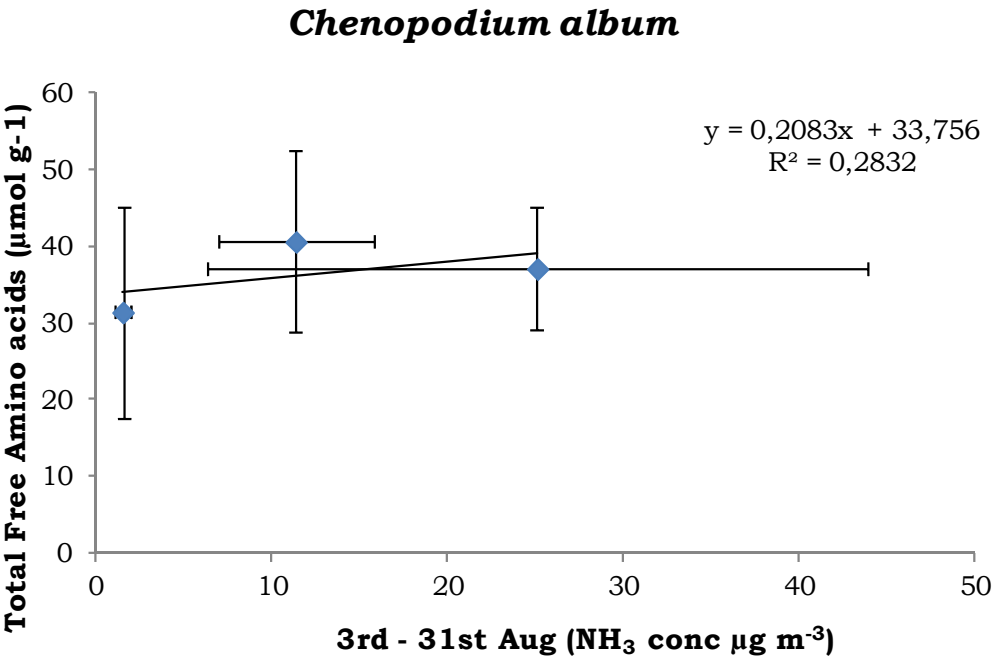

Fig 11B: A linear increase in total free amino acids in *Chenopodium album* with increasing ambient NH_3 concentrations. Means (n=3) ± SD for total free amino acids and NH_3 concentrations respectively.

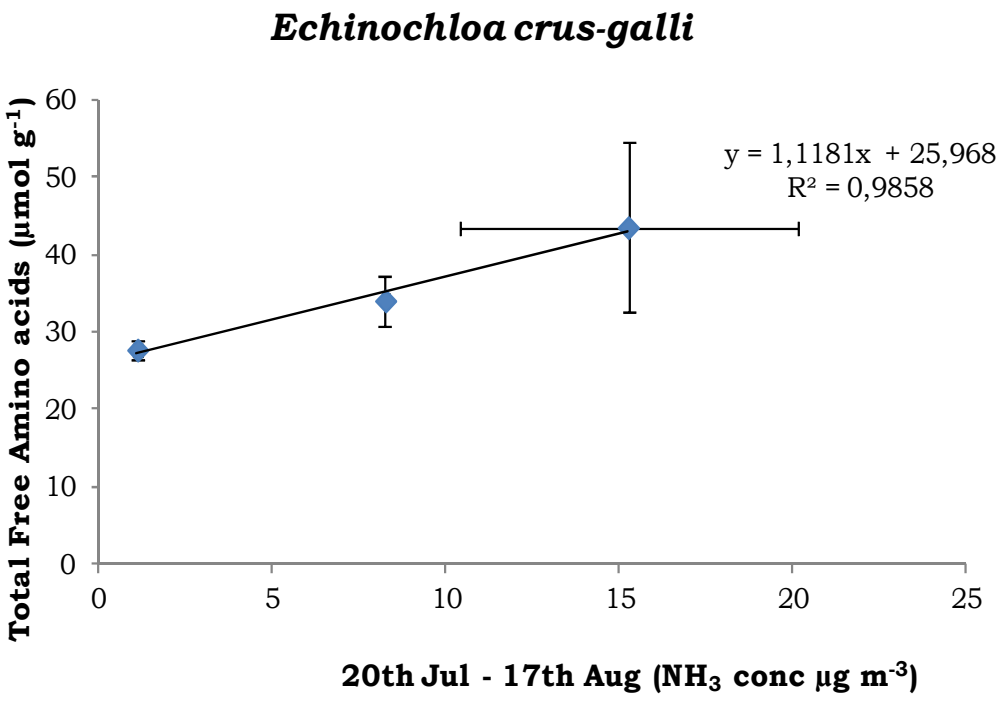

Fig 11C: A linear increase in total free amino acids in *Echinochloa crus - galli* with increasing ambient NH_3 concentrations. Means (n=3) ± SD for total free amino acids and NH_3 concentrations respectively.

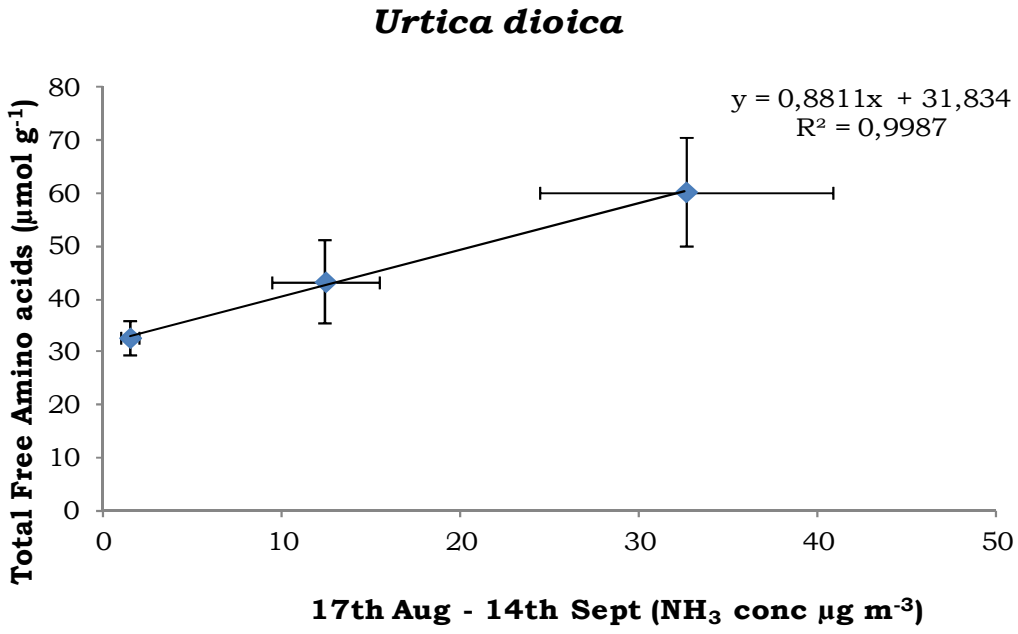

Fig 11D: A linear increase in total free amino acids in *Urtica dioica* with increasing ambient NH_3 concentrations. Means (n=3) ± SD for total free amino acids and NH_3 concentrations respectively.

A summary of the regression analysis of all the twenty free amino acid concentrations with increasing NH_3 concentration of each plant species is given in (Table 4). The percentage increases of each amino acid with increasing NH_3 concentrations have also been highlighted. Significant relationships were observed in the following amino acids Arg, Glu, Gly and His in *Lolium multiflorum* with increase in NH_3 concentration. All the amino acids also increased with various percentages ranging from 80- 400%. Gln and Asn accounted for the highest percentage increase of all the amino acids in *Lolium multiflorum* with 415 and 412% increase respectively (Table 4). There was not a statistically significant effect in the relationship between amino acids in *Echinochloa crus-galli* and increasing NH_3 concentration. Percentage increase in amino acids showed a lower range from 88 to 286% with Asn accounting for the highest percentage increase in amino acid with increasing NH_3 concentration. Similarly, *Chenopodium album* showed no significant relationship with increasing NH_3 concentration. The percentage increase of all the amino acids was between 80-190%. Gln accounted for the highest percentage increase in *Chenopodium album*. The amino acid concentrations in *Urtica dioica* showed a strong relationship with increasing NH_3 concentrations. Of all the amino acids analysed in *Urtica dioica*, Ala, Pro and Thr showed the only statistically significant relationship with increasing NH_3 concentration. Arg accounted for the highest percentage increase in amino acid concentration with 1438% under increasing NH_3concentration. Group classification of free amino acids in the biochemically analysed plants showed mostly similar response pattern and trends, with few exceptions (Figures 12A-D). In *Lolium multiflorum* and *Urtica dioica* amino acids belonging to the following groups Asn, Asp Thr, Met, Lys; Gln, Arg, Glu, Pro; Ala,Val, Leu and His, Tyr, Phe increased with increasing ammonia concentration. However, this was different for *Echinochloa crus-galli* and *Chenopodium album.* All the amino acid groups in *Chenopodium album* decreased at the highest ammonia concentration except the group consisting of Gln, Arg, Glu and Pro.

Table 4: Percentage increase in free amino acids between 1.5µg m^{-3} to 15µg m^{-3} NH_3 concentration in all plant species and effects of ambient NH_3 concentration on free amino acids in *Lolium multiflorum, Echinochloa crus-galli, Chenopodium album and Urtica dioica* after a one month exposure period in the field between 20th July – 17th Aug, 3rd - 30th Aug and 17th Aug - 14th Sept (*., $P \leq 0.05$; n.s., not significant). The percentage increases in free amino acids were derived using linear regression equations.

Amino acid	*Lolium multiflorum*			*Echinochloa crus-galli*			*Chenopodium album*			*Urtica dioica*		
	% Increase	R^2	Sig	% Increase	R^2	Sig	% Increase	R^2	Sig	% Increase	R^2	Sig
Alanine	210	0.936	*ns*	151	0.972	*ns*	109	0.365	*ns*	127	0.998	*
Arginine	151	1.000	*	142	0.501	*ns*	96	0.133	*ns*	1439	0.917	*ns*
Asparagine	413	0.834	*ns*	287	0.839	*ns*	105	0.076	*ns*	205	0.855	*ns*
Aspartic acid	134	0.982	*ns*	86	0.046	*ns*	135	0.892	*ns*	97	0.158	*ns*
Glutamic acid	119	0.997	*	92	0.087	*ns*	117	0.931	*ns*	110	0.983	*ns*
Glutamine	416	0.881	*ns*	183	0.685	*ns*	187	0.992	*ns*	121	0.966	*ns*
Glycine	169	0.998	*	156	0.822	*ns*	86	0.236	*ns*	102	0.768	*ns*
Histidine	226	0.997	*	104	0.031	*ns*	86	0.211	*ns*	137	0.947	*ns*
Isoleucine	117	0.984	*ns*	100	0.000	*ns*	103	0.054	*ns*	121	0.977	*ns*
Leucine	137	0.961	*ns*	116	0.096	*ns*	88	0.356	*ns*	138	0.991	*ns*
Lysine	153	0.958	*ns*	95	0.648	*ns*	88	0.253	*ns*	169	0.907	*ns*
Methionine	107	0.964	*ns*	107	0.521	*ns*	81	0.385	*ns*	105	0.813	*ns*
Phenylalanine	130	0.947	*ns*	114	0.435	*ns*	90	0.231	*ns*	111	0.679	*ns*
Proline	196	0.964	*ns*	134	0.807	*ns*	130	0.809	*ns*	141	0.999	*
Serine	168	0.981	*ns*	224	0.800	*ns*	103	0.013	*ns*	109	0.993	*ns*
Threonine	178	0.935	*ns*	143	0.947	*ns*	98	0.013	*ns*	109	0.998	*
Tryptophan	84	0.902	*ns*	89	0.745	*ns*	86	0.506	*ns*	132	0.880	*ns*
Tyrosine	131	0.935	*ns*	108	0.072	*ns*	88	0.266	*ns*	120	0.788	*ns*
Valine	142	0.952	*ns*	186	0.923	*ns*	91	0.565	*ns*	120	0.972	*ns*
GABA	385	0.868	*ns*	248	0.749	*ns*	138	0.721	*ns*	119	0.917	*ns*

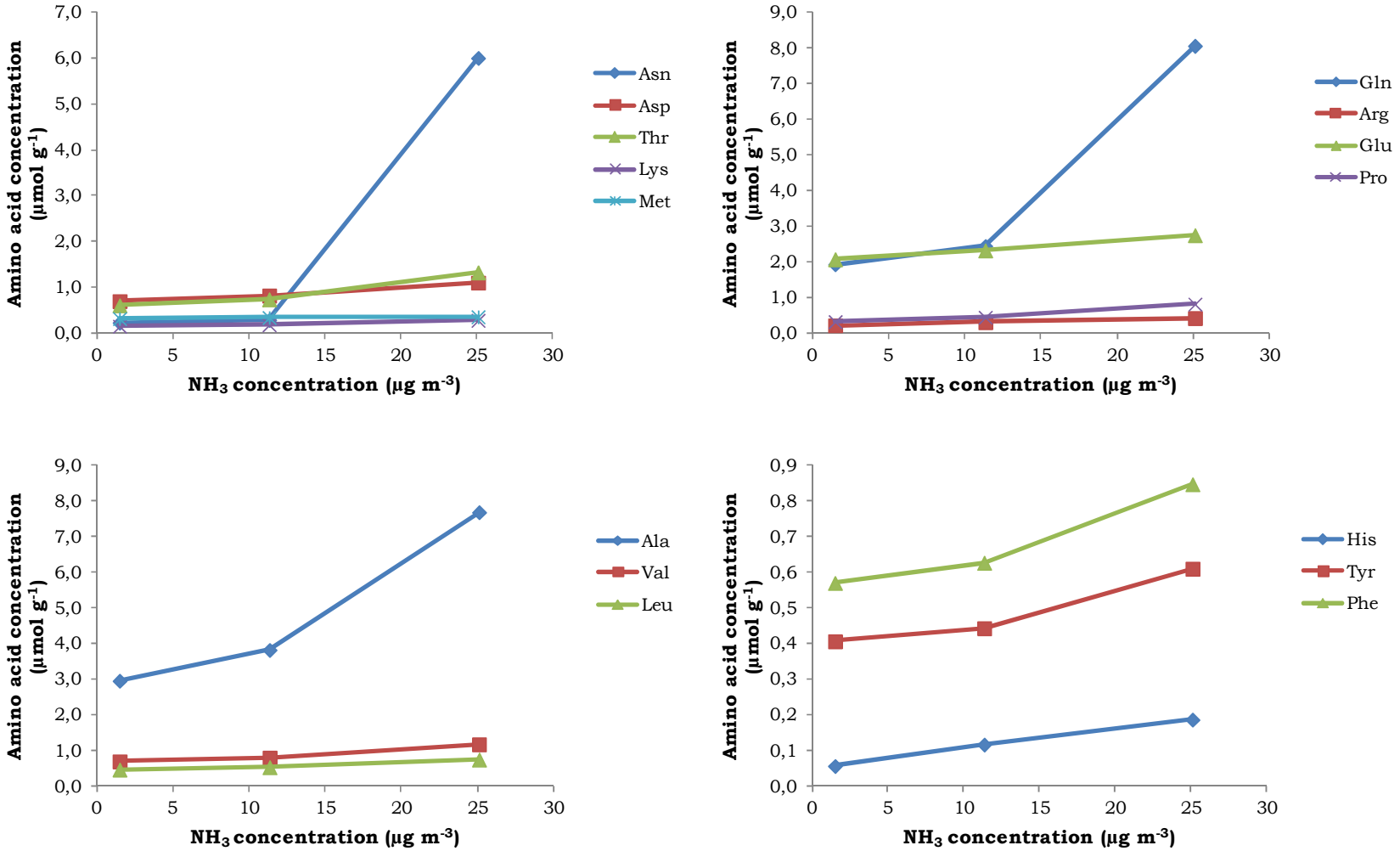

Fig.12A: Response pattern of various amino acids groups to increasing NH_3 concentrations in *Lolium multiflorum*, after one month exposure period close to a source of NH_3 pollution. Values represent means (n=3) for free amino acids and NH_3 concentrations respectively.

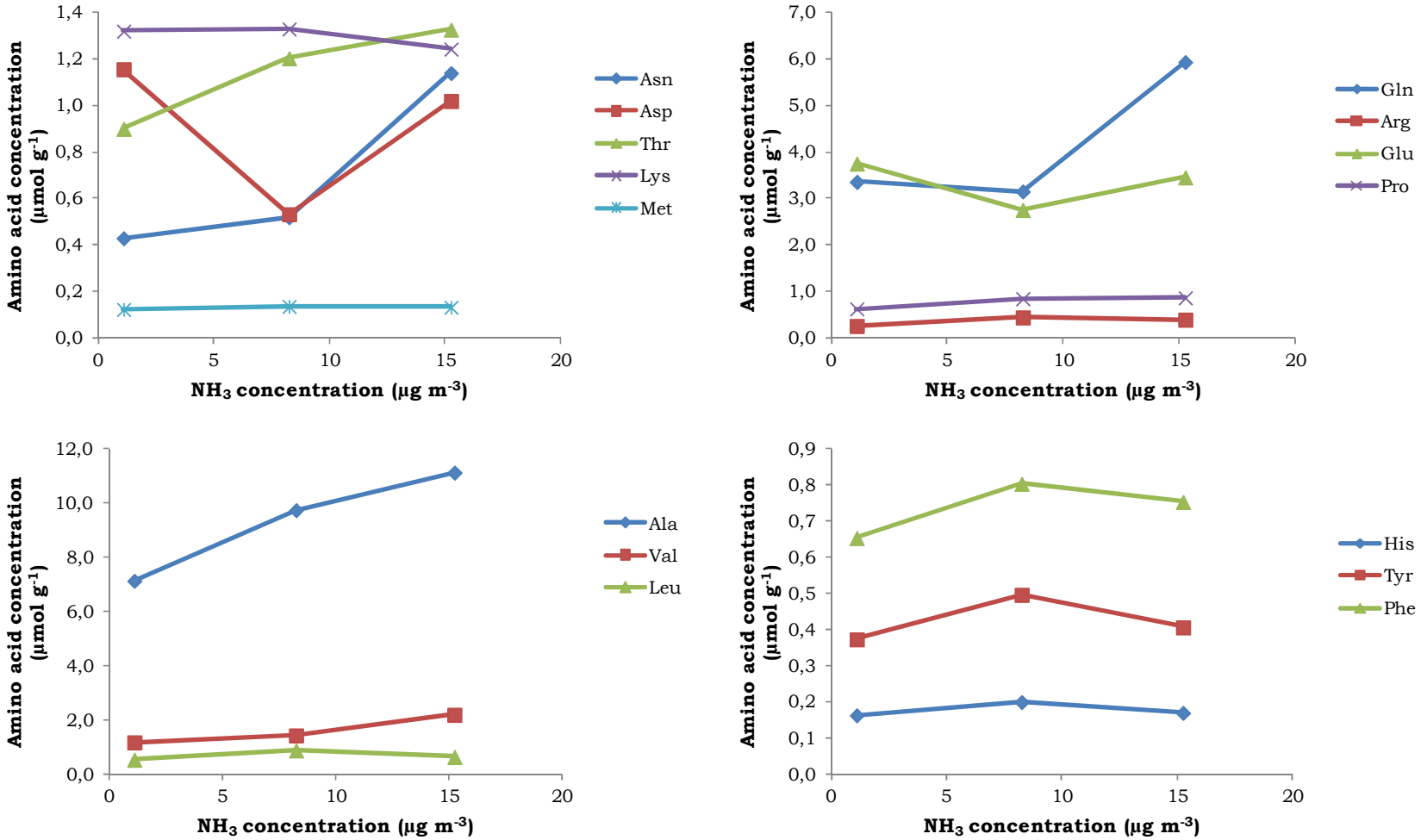

Fig.12B: Response pattern of various amino acids groups to increasing NH_3 concentrations in *Echinochloa crus-galli*, after one month exposure period close to a source of NH_3 pollution. Values represent means (n=3) for free amino acids and NH_3 concentrations respectively.

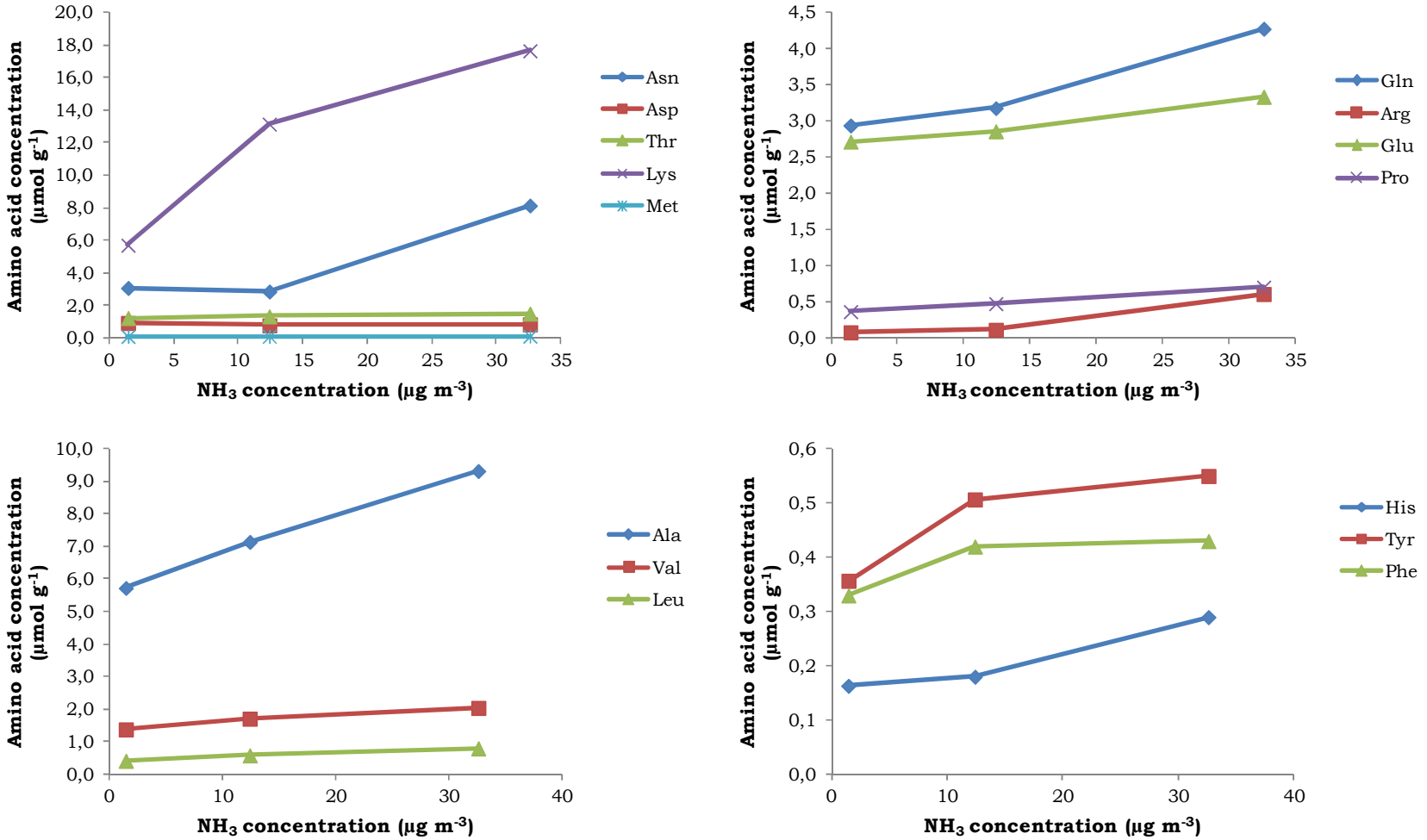

Fig.12C: Response pattern of various amino acids groups to increasing NH_3 concentrations in *Uritica dioica*, after one month exposure period close to a source of NH_3 pollution. Values represent means (n=3) for free amino acids and NH_3 concentrations respectively.

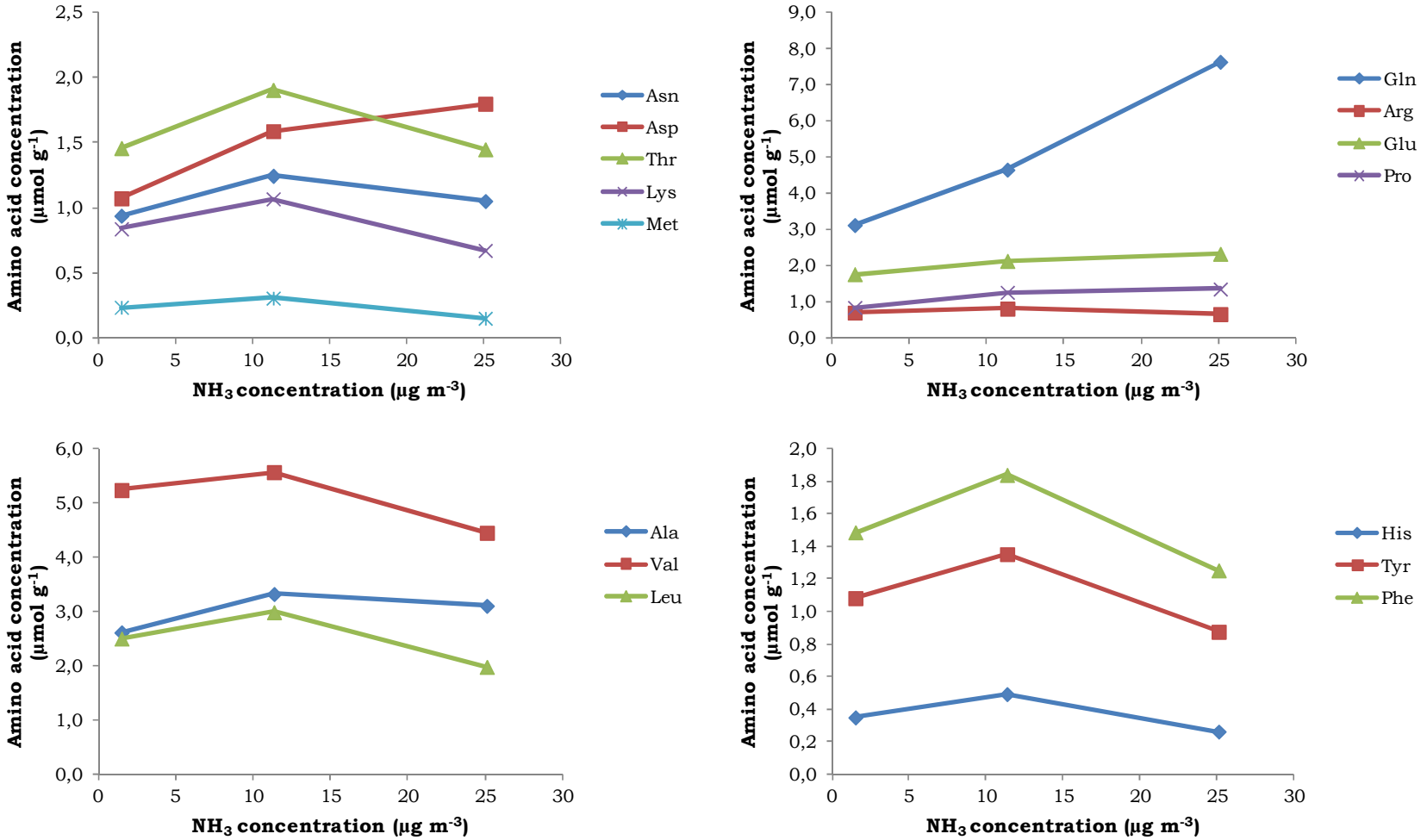

Fig.12D: Response pattern of various amino acids groups to increasing NH_3 concentrations in *Chenopodium album*, after one month exposure period close to a source of NH_3 pollution. Values represent means (n=3) for free amino acids and NH_3 concentrations respectively.

4.4 Effects of the ambient NH_3 concentrations on above ground biomass in *Lolium multiflorum*, *Chenopodium album*, *Echinochloa crus-galli* and *Urtica dioica*

4.4.1. Field study

In this study a strong linear decrease with distance was observed in *Lolium multiflorum* (R^2=0.53), *Echinochloa crus-galli* (R^2 =0.87) and *Urtica dioica* (R^2=0.67) exposed in the field close to a point source of NH_3 emission (Figures 13A and 13C-D). This trend was not observed in *Chenopodium album* as it showed a continuous increase in biomass with increasing distance (Figure 13B). The mean above ground biomass production based on dry weight per pot, across selected distances of 67 m, 149 m and 804 m in *Echinochloa crus-galli* was 2.27 g, 2.08 g and 1.84 g, while *Lolium multiflorum* had 1.57 g, 1.25 g and 1.17 g respectively. Whereas for the herbs, 1.39 g, 1.05 g and 0.89 g was produced by *Urtica dioica* along the same distances, while in *Chenopodium album* the biomass produced was 3.96 g, 4.10 g and 4.33 g. In addition, the effects of ambient NH_3 across each distance on growth parameters of each plant species were evaluated and shown in (Table 5).

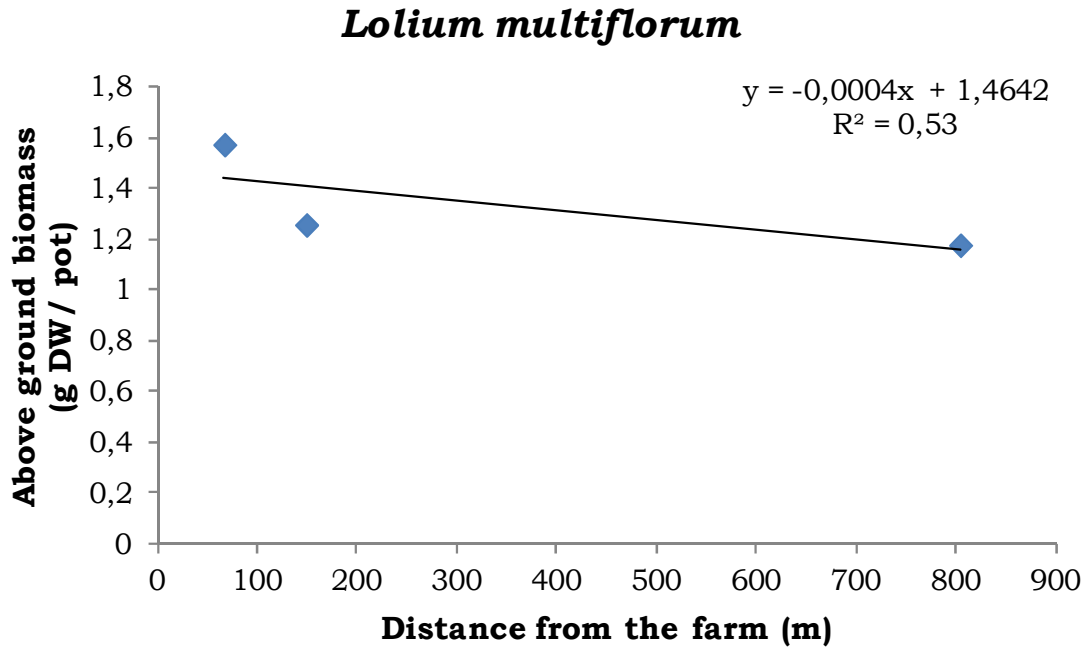

Fig 13A: The relationships between mean above ground biomass and distance from the farm in *Lolium multiflorum* after 4 weeks exposure to a source of NH_3 emissions.

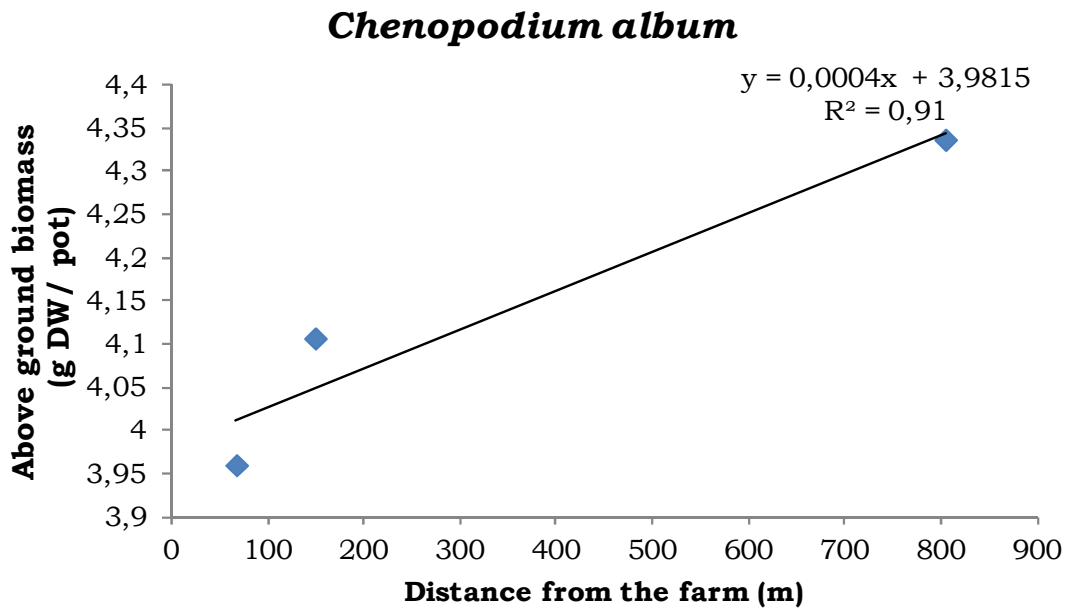

Fig 13B: The relationships between mean above ground biomass and distance from the farm in *Chenopodium album* after 4 weeks exposure to a source of NH_3 emissions.

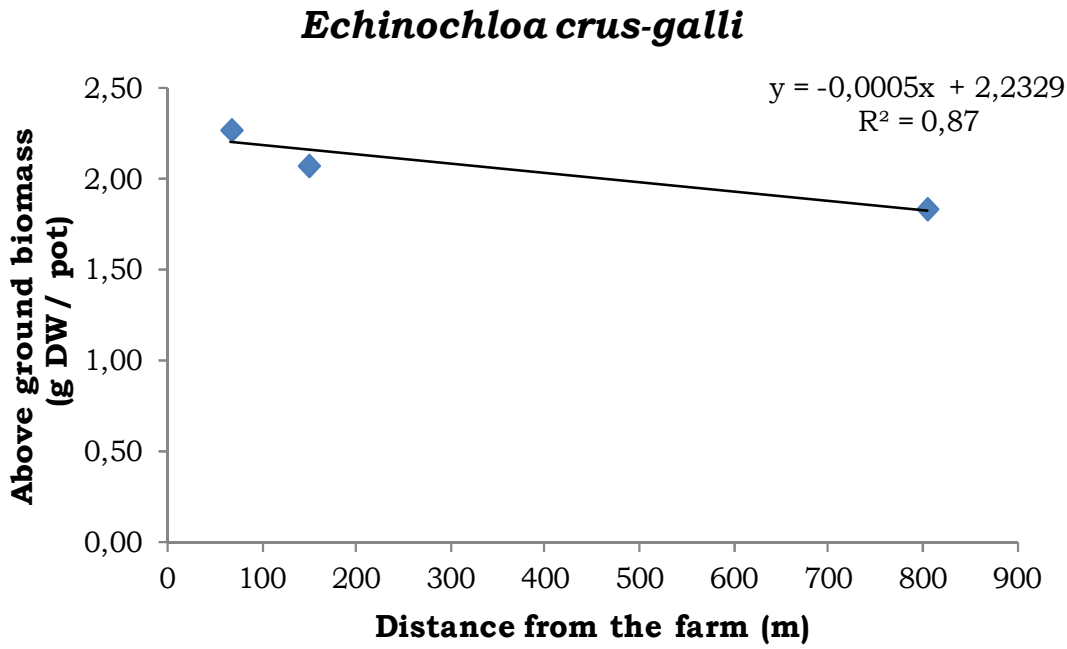

Fig 13C: The relationships between mean above ground biomass and distance from the farm in *Echinochloa crus-galli* after 4 weeks exposure to a source of NH_3 emissions.

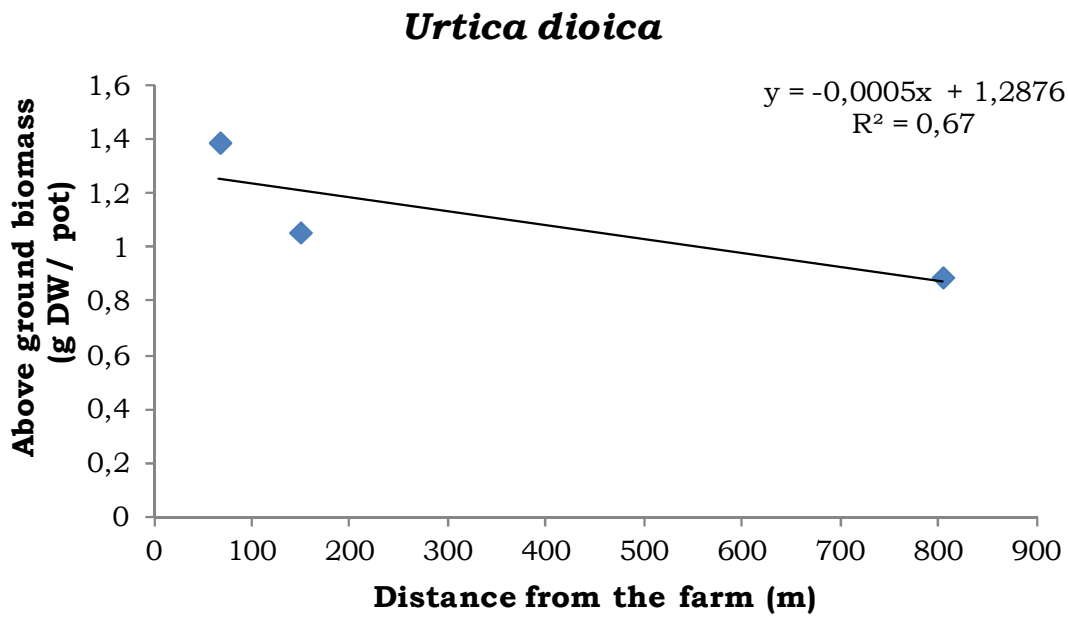

Fig 13D: The relationships between mean above ground biomass and distance from the farm in *Urtica dioica* after 4 weeks exposure to a source of NH_3 emissions.

The interaction of ambient NH_3 concentrations on leaves, stem and seed head demonstrated no significant effect on *Echinochloa crus-galli*, but showed a significant effect in above ground biomass of the plant. This however, demonstrates a positive effect of NH_3 interaction, on the above ground biomass production, regardless of a non-significant effect in the above ground biomass fractions of the plant. A significant effect was observed in leaves and above ground biomass of *Urtica dioica*, while the stems were not significantly affected with the interactions of ambient NH_3 concentrations across the selected transect. *Lolium multiflorum* responded with a positive effect on its above ground biomass, while none of the growth parameters or above ground biomass in *Chenopodium album* showed a positive effect in response to the ambient NH_3 concentrations (Table 5).

Table 5: Effects of ambient NH_3 concentrations on growth parameters of *Echinochloa crus-galli*, *Urtica dioica*, *Lolium multiflorum* and *Chenopodium album* exposed in the field. Results are calculated based on one way ANOVA showing F-values and level of significance.

Plant *spp*	Parameters (g DW/pot)	NH_3 Conc at distances from the farm			F value	Sign
		67 m	149 m	804 m		
Echinochloa crus-galli	Leaf	15.25	8.25	1.1	0.642	ns
	Stem				1.900	ns
	Seed head				0.866	ns
	Above ground biomass				16.209	0.004
Urtica dioica	Leaf	32.6	12.4	1.45	5.338	0.047
	Stem				4.262	ns
	Above ground biomass				6.030	0.037
Lolium multiflorum	Above ground biomass				21.524	0.002
Chenopodium album	Leaf	25.1	11.35	1.5	1.323	ns
	Stem				1.252	ns
	Steed head				0.244	ns
	Above ground biomass				0.386	ns

Level of significance; * $p \leq 0.05$, ns = no significance

With the exception of *Chenopodium album,* the above ground biomass in all plant species exposed along the selected transect of NH_3 emissions demonstrated an increase in above ground biomass with increasing NH_3 concentration (Figures 14A,C and D). This is evidenced from a strong linear increase in above ground biomass of *Lolium multiflorum* (R^2 = 0.94), *Echinochloa crus-galli* (R^2 = 0.99) and *Urtica dioica (*R^2 = 0.99*)*, with the ambient NH_3 concentration at each distance. This increase in above ground biomass is characterized by the plants response to changes in NH_3 concentration at each site which showed an increase with increasing proximity to the stable. However, in *Chenopodium album* a decrease in biomass was observed as the NH_3 concentration decreased (Figure 14B).

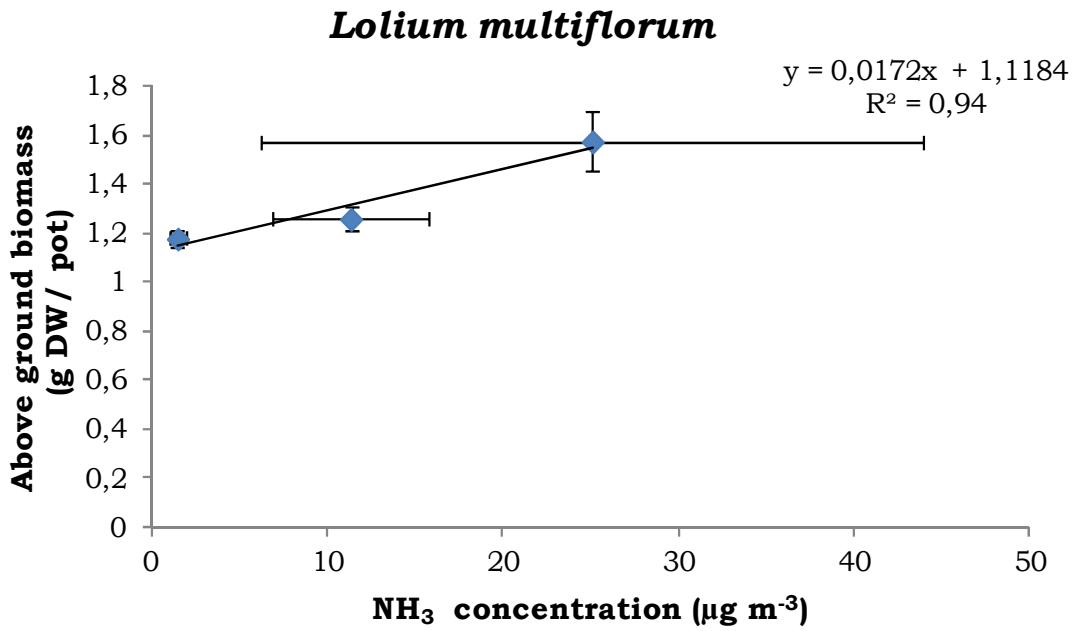

Fig 14A: Relationships between mean above ground biomass in *Lolium multiflorum*, with ambient NH_3 concentrations measured along the selected transect during 4 weeks of field exposure. Values are means (n = 3) ±SD.

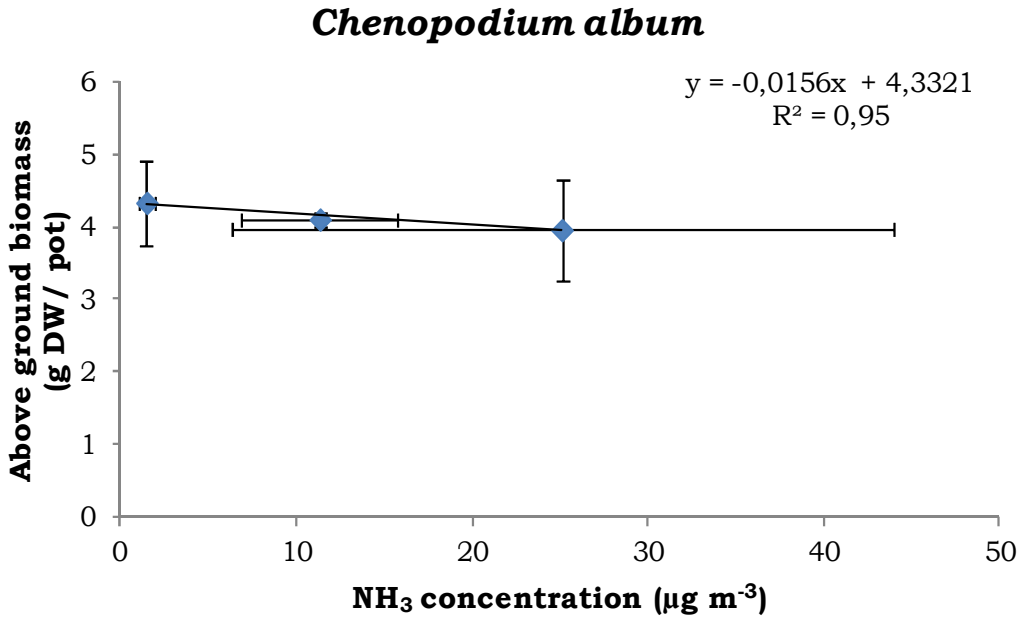

Fig 14B: Relationships between mean above ground biomass in *Chenopodium album* with ambient NH_3 concentrations measured along the selected transect during 4 weeks of field exposure. Values are means (n = 3) ±SD.

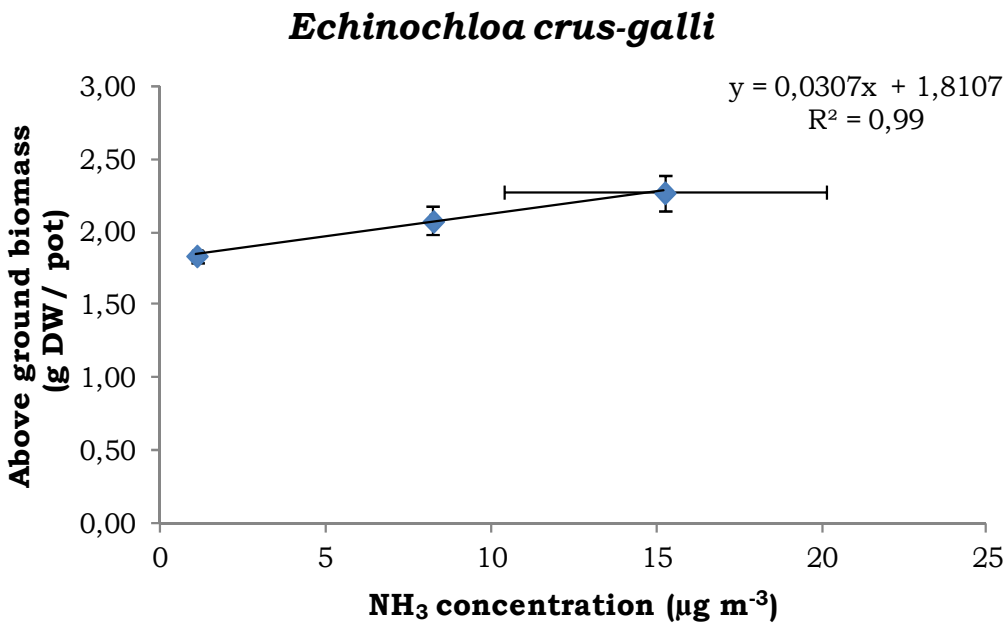

Fig 14C: Relationships between mean above ground biomass in *Echinochloa crus-galli* with ambient NH_3 concentrations measured along the selected transect during 4 weeks of field exposure. Values are means (n = 3) ±SD.

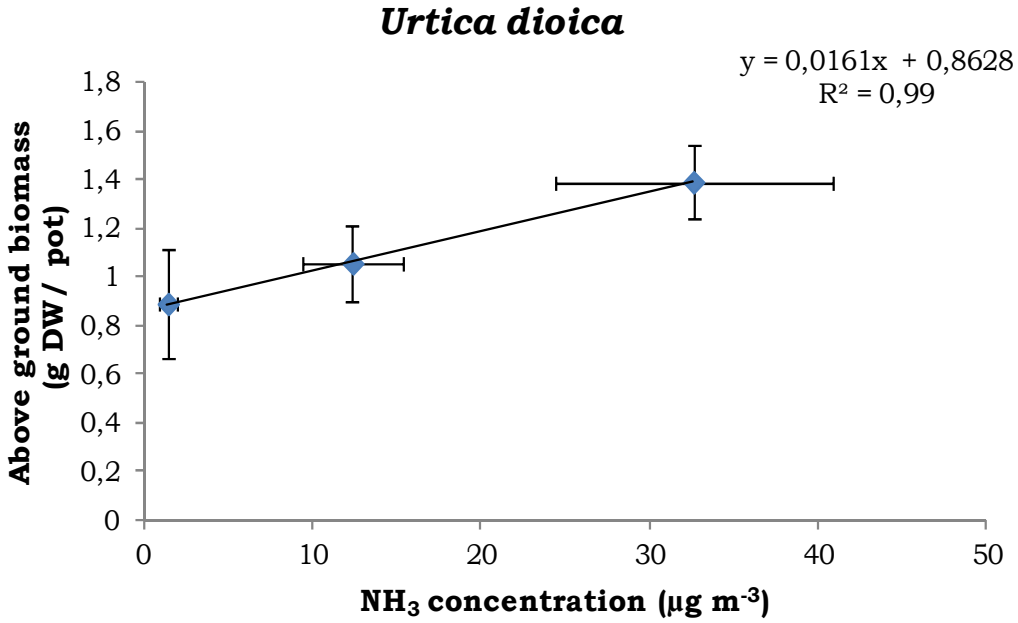

Fig 14D: Relationships between mean above ground biomass in *Urtica dioica* with ambient NH_3 concentrations measured along the selected transect during 4 weeks of field exposure. Values are means (n = 3) ±SD.

4.4.2. Fumigation study

In the first set of fumigation experiments, the interaction of gaseous NH_3 with various plant parameters and above ground biomass of *Lolium multiflorum* and *Echinochloa crus-galli* showed no significant influence of NH_3 except in leaves of *Lolium multiflorum* (Table 6). Although slight increases in above ground biomass was observed in both plants after a four weeks exposure to gaseous NH_3 however, there were no significant differences between the treatments (Figure 15A). The mean above ground biomass on dry weight basis per pot increased from 5.27 g, 5.49 g to 5.81 g in *Lolium multiflorum* across NFA, NFA+ and NFA++ treatments, while increases from 5.31 g, 5.88 g to 6.07 g was observed in *Echinochloa crus-galli.* The percent biomass production of the above biomass fractions in *Lolium multiflorum* and *Echinochloa crus-galli* showed a positive response with NH_3 treatments with regards to changes in composition of the biomass fractions (Figure 15B).

Table 6: Effects of ambient NH_3 concentrations in fumigation chambers on above ground biomass of *Lolium multiflorum* and *Echicochloa crus-galli* in the first set of fumigation experiment. Results shows F values and significance level of plant parameters based on one way analysis of variance (ANOVA).

Plant spp	Parameter (g DW/pot)	NH_3 Conc ($\mu g\ m^{-3}$) in chambers			F value	Sign
		NFA	NFA+	NFA++		
Lolium multiflorum	Leaf	3.65	25	84.3	6.357	0.019
	Stem				0.949	ns
	Above ground biomass				3.200	ns
Echinochloa crus-galli	Leaf				0.749	ns
	Stem				0.482	ns
	Seed head				2.899	ns
	Above ground biomass				0.749	ns

NFA, NFA+ and NFA++ values represent ambient NH_3 concentrations in chambers with Non filtered air, Non filtered air fumigated with low NH_3 concentrations and Non filtered air fumigated with high NH_3 concentration respectively. Level of significance; * $p \leq 0.05$, ns = no significance

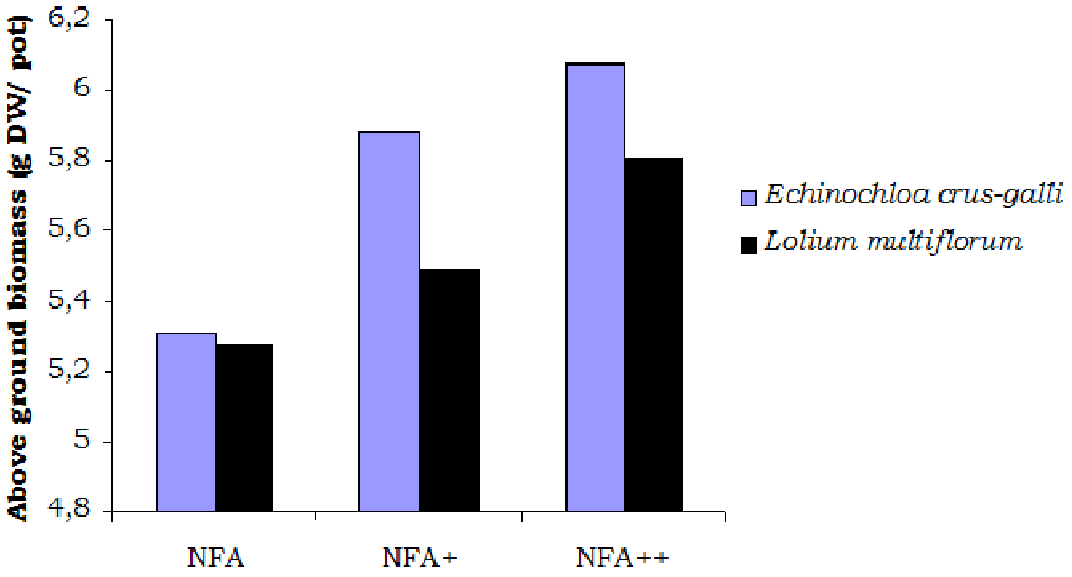

Fig 15A: Total above ground biomass production in *Lolium multiflorum* and *Echinochloa crus-galli* exposed to three different treatments of NH_3 concentrations in the first set of the fumigation experiment. Values are means (n = 4). NFA, NFA+ and NFA++ represent different NH_3 treatments.

In *Lolium multiflorum* an increase in percent biomass composition of the leaves to the total biomass from 62 % at the NFA treatment level to 69 % at NFA++ treatment was observed. This increase in leaf composition simultaneously induced a decrease in the stem composition, from 37 % under NFA treatment conditions to 30 % at NFA++ treatment level (Figure 15B). *Echinochloa crus-galli* demonstrated changes in compositions of leaf, stem and seed head across the three treatment levels. A slight increase from 41 % to 46 % and 45 % in leaf composition to the total above ground biomass was observed across NFA, NFA+ and NFA++ treatment levels. This was accompanied with a subsequent decrease in the stem composition from 30 % to 24 % and an increase in seed head production from 28 % to 31 % of the total biomass, from the least to the highest treatment level (Figure 15B).

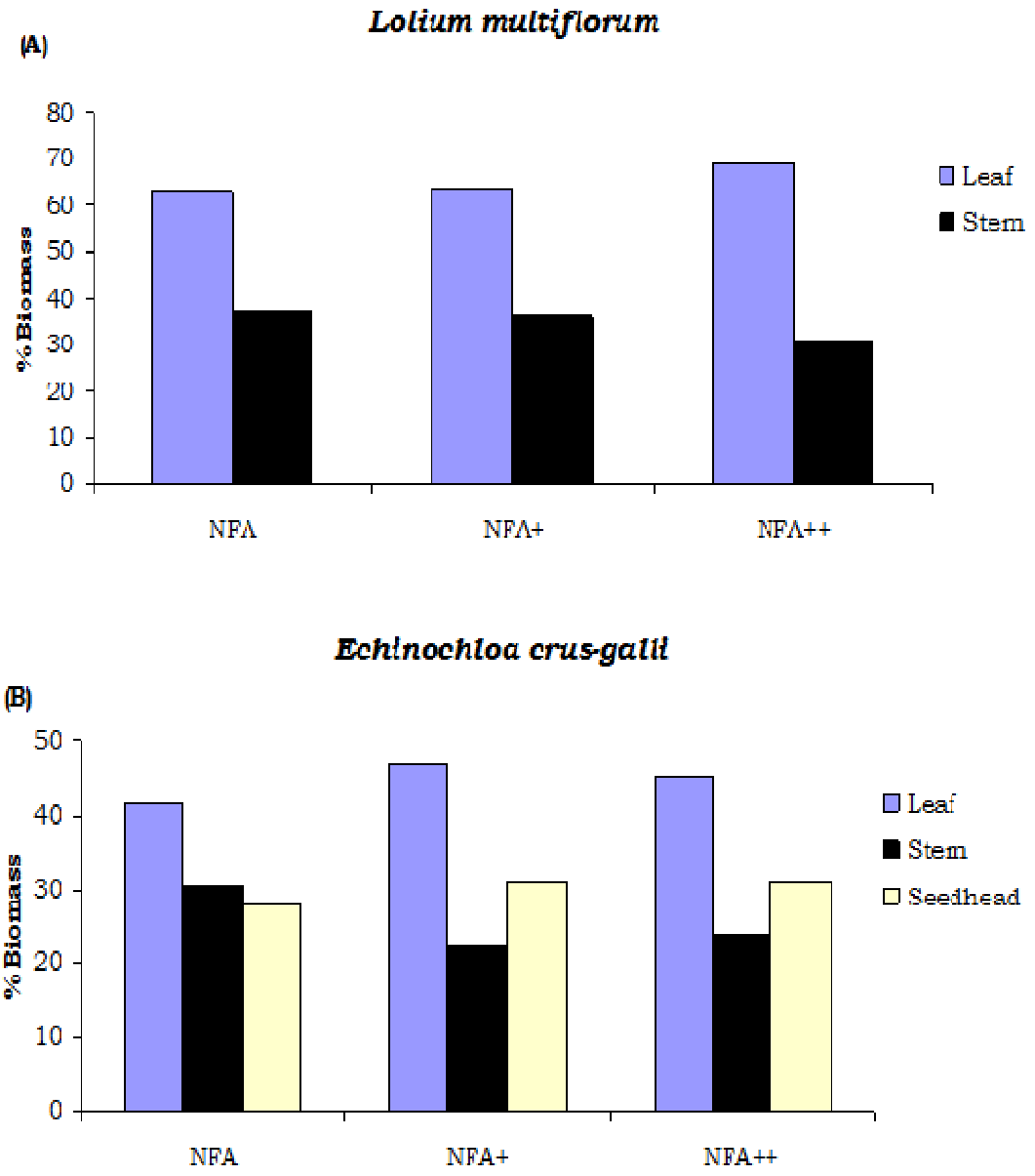

Fig 15B: The % biomass fractions to the total aboveground biomass in (a) *Lolium multiflorum* and (b) *Echinochloa crus-galli* exposed to three different treatments of NH_3 concentrations in the first set of the fumigation experiment. Values are means (n = 4). NFA, NFA+ and NFA++ represent different NH_3 treatments.

In the second set of the fumigation experiment, *Lolium multiflorum* and *Echinochloa crus-galli* were exposed to different concentrations of gaseous NH_3 at NFA, NFA+ and NFA++ treatment levels because *Echinochloa crus-galli* were only fumigated for 2 weeks since the biomass of the plants were almost outgrowing the size of the chambers, while in the case of *Lolium multiflorum,* the plants were exposed to a fumigation period of 4 weeks. However, the interactions of the various concentrations of gaseous NH_3 on

the above ground biomass fractions of both plants yielded no significant effects (Table 7).

Table 7: Effects of ambient NH_3 concentrations in fumigation chambers on above ground biomass of *Lolium multiflorum* and *Echicochloa crus-galli,*in the second set of fumigation experiment. Results shows F values and significance level of plant parameters based on one way analysis of variance (ANOVA).

Plant spp	Parameter (g DW/pot)	NH_3 Conc (µg m^{-3}) in chambers			F value	Sign
		NFA	NFA+	NFA++		
Lolium multiflorum	Leaf	9.9	26.3	119.5	2.287	ns
	Stem				0.058	ns
	Above ground biomass				1.188	ns
Echinochloa crus-galli	Leaf	7.6	16	112.7	1.341	ns
	Stem				0.522	ns
	Seed head				1.254	ns
	Above ground biomass				0.733	ns

NFA, NFA+ and NFA++ values represent ambient NH_3 concentrations in chambers with non filtered air, non filtered air fumigated with low NH_3 concentrations and non filtered air fumigated with high NH_3 concentration respectively. Level of significance; * $p \leq 0.05$, ns = no significance.

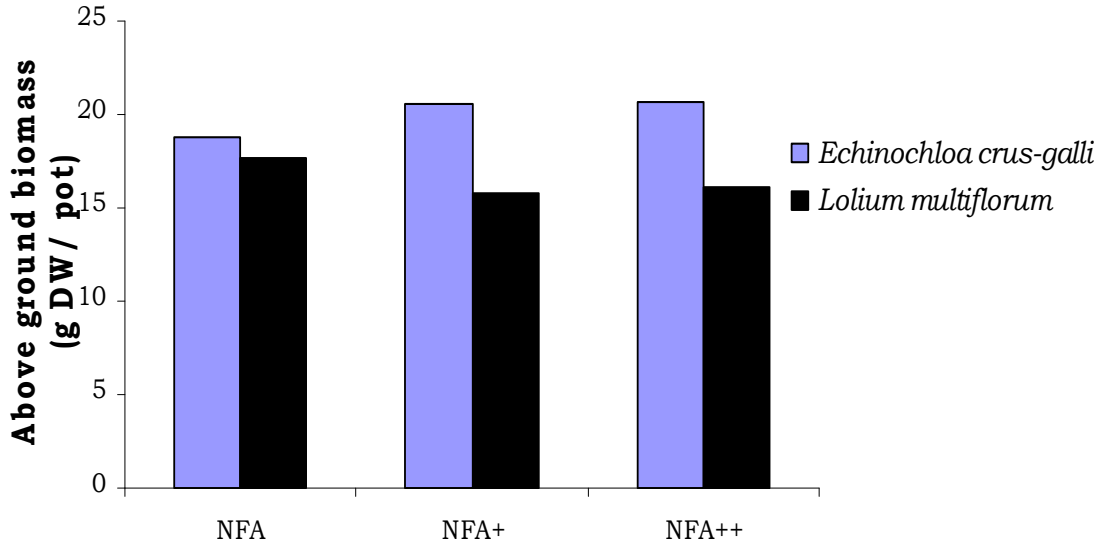

Fig 16A: Total above ground biomass production in *Lolium multiflorum* and *Echinochloa crus-galli* exposed to three different treatments of NH_3 concentrations in the second set of fumigation the experiment. Values are means (n = 4). NFA, NFA+ and NFA++ represent different NH_3 treatments.

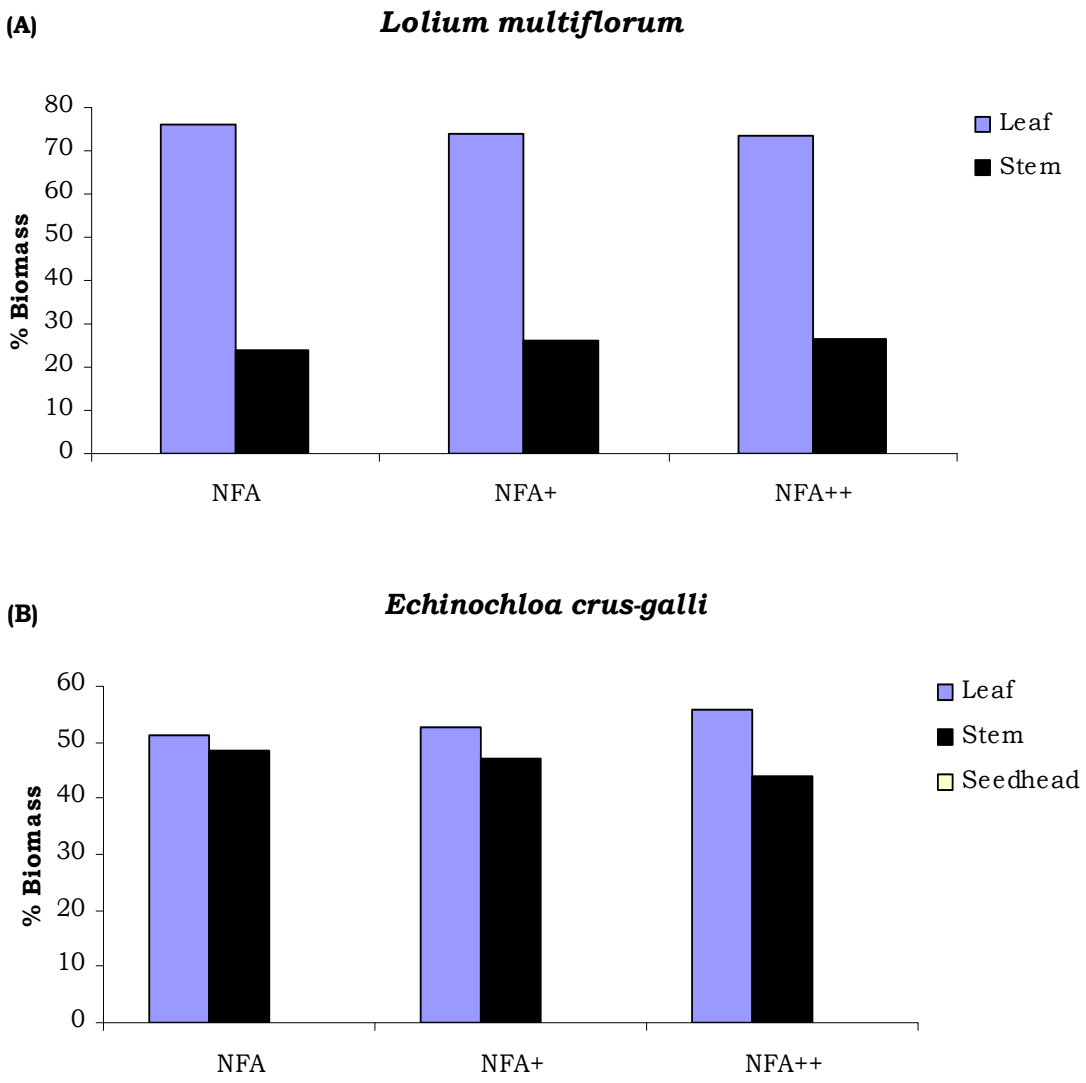

Fig 16B: The % biomass fractions to the total aboveground biomass in (a) *Lolium multiflorum* and (b) *Echinochloa crus-galli* exposed to three different treatments of NH_3 concentrations in the second set of the fumigation experiment. Values are means (n = 4). NFA, NFA+ and NFA++ represent different NH_3 treatments.

The mean above ground biomass of *Echinochloa crus-galli* exposed to the following gaseous NH_3 treatments of NFA, NFA+ and NFA++ was 18.7 g, 20.5 g and 20.6 g dry weight per pot respectively. In the case of *Lolium multiflorum,* the mean above ground biomass produced in NFA treated plants was 17.6 g, while the biomass produced by plants exposed to NFA+ and NFA++ treatments, were 15.7 g and 16.1 g respectively (Figure 16A). In terms of the percent biomass compositions of the above biomass

fractions, leaves dominated the total above ground biomass fractions in *Lolium multiflorum* by accounting for 76 %, 74 % and 73 % respectively in the NFA, NFA+ and NFA++ treatments whereas, the stems recorded the remaining 23.9 %, 25.9 % and 26.6 % accordingly. However, in *Echinochloa crus-galli*, the leaves also had higher biomass compositions than stems, but were similar in biomass fractions and accounted for 51.4 %, 52.6 % and 55.7 % respectively while the stems had 48.4 %, 47.1 % and 44 % with the remaining compositions being accounted for by seed heads in the following treatment order of NFA, NFA+ and NFA++ (Figure 16B).

4.5 The effects of gaseous NH_3 fumigations on free amino acid concentrations and compositions in *Lolium multiflorum* and *Echinochloa crus-galli*.

The gaseous NH_3 concentration in the green house chamber air in the NFA, NFA+ and NFA++ treatments exposed to *Lolium multiflorum* and *Echinochloa crus-galli* plants, in both first and second set of the fumigation experiment induced alterations in free amino acid metabolites in both plants. In the *Lolium multiflorum* plants, all the free amino acids increased in concentration with increasing gaseous NH_3 treatments of NFA, NFA+ and NFA++ respectively. Alanine (Ala), GABA and Gluthamic acid (Glu) dominated the amino acid pool in *Lolium multiflorum* exposed to both NFA and NFA+ treatments in the first set of the fumigation experiment, by accounting for 2.71 nmol mg^{-1}, 2.18 nmol mg^{-1} and 2.35 nmol mg^{-1} respectively in the NFA treated plants and 5.66 nmol mg^{-1}, 4.33 nmol mg^{-1} and 1.88 nmol mg^{-1} in NFA+ treatment. However, in the same set of experiment, Glutamine (Gln), Asparagine (Asn) and Alanine (Ala), had the highest free amino acid concentrations with 175.50 nmol mg^{-1}, 90.95 nmol mg^{-1} and 16.44 nmol mg^{-1} respectively, and dominated the entire amino acid pool in the NFA++ treatment (Table 8A). In the second set of the fumigation experiment, Asn and Ala dominated the amino acid pools in *Lolium multiflorum* across all treatments with concentrations of 32.24 nmol mg^{-1} and 19.82 nmol mg^{-1} as well as 58.76 nmol mg^{-1} and 20.53 nmol mg^{-1} in both NFA and NFA+ treatments respectively. Whereas, in the NFA++ treatments Asn and Ala concentrations were 213.72 nmol $mg^{-1}$and 31.29 nmol mg^{-1} (Table 8B).

In *Echinochloa crus-galli*, Glu, Ala and GABA were the most prominent amino acid across the NFA and NFA+ treatments in the first set of the fumigation experiment, by constituting of 4.91 nmol mg^{-1}, 2.62 nmol mg^{-1} and 1.21 nmol mg^{-1} as well as 4.27 nmol mg^{-1}, 3.19 nmol mg^{-1} and 1.27 nmol mg^{-1} of the total free amino acids respectively. In

contrast to the NFA and NFA+ treatments, Ala, Gln and Glu were the most abundant amino acids in the NFA++ treatments with concentrations of 21.82 nmol mg^{-1}, 11.91 nmol mg^{-1} and 6.90 nmol mg^{-1} (Table 9A). Results obtained from *Echinochloa crus-galli*, in the second set of the fumigation experiment demonstrates Ala and Asn dominance in both NFA and NFA+ treatments, with concentrations of 44.50 nmol mg^{-1} and 33.48 nmol mg^{-1} as well as 31.07 nmol mg^{-1} and 13.11 nmol mg^{-1} respectively in both treatments. However, in the case of NFA++ *Echinochloa crus-galli* treated plants, Gln and Asn accounted for the highest amino acid concentrations of the total amino acid pool with 48.81 nmol mg^{-1} and 45.91 nmol mg^{-1} respectively (Table 9B).

Table 8A: Summary of free amino acid concentrations in *Lolium multiflorum* exposed to fumigation treatments at various concentrations of gaseous NH_3 in NFA, NFA+ and NFA++ treatments respectively during the first set of the fumigation experiment.

Amino acids (nmol mg^{-1})	Gaseous NH_3 treatment			
	NFA	NFA+	NFA++	*P* - level
Histidine	0.00 ± 0.00	0.00 ± 0.00	0.28 ± 0.09	*
Asparagine	0.26 ± 0.02	0.53 ± 0.08	90.95 ± 19.52	*
Serine	0.95 ± 0.11	1.38 ± 0.12	11.88 ± 2.14	*
Glutamine	0.73 ± 0.09	1.76 ± 0.58	175.50 ± 39.85	*
Arginine	0.15 ± 0.01	0.30 ± 0.04	1.34 ± 0.33	*
Glycine	0.22 ± 0.02	0.34 ± 0.06	1.69 ± 0.43	*
Aspartic acid	0.99 ± 0.12	0.60 ± 0.40	12.69 ± 1.47	*
Glutamic acid	2.35 ± 0.14	1.88 ± 0.56	6.74 ± 0.57	*
Threonine	0.63 ± 0.05	0.95 ± 0.04	5.39 ± 0.99	*
Alanine	2.71 ± 0.43	5.66 ± 0.83	16.44 ± 2.20	*
GABA	2.18 ± 0.34	4.33 ± 0.77	10.87 ± 1.65	*
Proline	0.15 ± 0.01	0.35 ± 0.04	1.99 ± 0.58	*
Lysine	0.39 ± 0.04	0.49 ± 0.04	0.83 ± 0.15	*
Tyrosine	0.21 ± 0.03	0.17 ± 0.20	0.00 ± 0.00	*ns*
Methionine	0.00 ± 0.00	0.09 ± 0.06	0.03 ± 0.00	*ns*
Valine	0.67 ± 0.07	1.02 ± 0.07	2.53 ± 0.40	*
Isoleucine	0.22 ± 0.05	0.44 ± 0.04	0.92 ± 0.13	*
Leucine	0.21 ± 0.03	0.52 ± 0.03	0.82 ± 0.09	*
Phenylalanine	0.43 ± 0.03	0.59 ± 0.06	1.16 ± 0.13	*
Tryptophan	0.66 ± 0.32	0.32 ± 0.04	0.87 ± 0.17	*

Above values represent means and standard deviations of the treatments (NFA; ambient NH_3 concentrations in chambers with non filtered air, NFA+; non filtered air fumigated with low NH_3 concentrations and NFA++; non filtered air fumigated with high NH_3 concentration respectively. Level of significance according to one-way analyses of variance (ANOVA); * $p \leq 0.05$, ns = no significance. The NH_3 concentrations achieved in the experiment are found in (Table 6).

Table 8B: Summary of free amino acid concentrations in *Lolium multiflorum* exposed to fumigation treatments at various concentrations of gaseous NH_3 in NFA, NFA+ and NFA++ treatments respectively during the second set of the fumigation experiment.

Amino acids (nmol mg^{-1})	Gaseous NH_3 treatment			
	NFA	NFA+	NFA++	*P* - level
Histidine	0.51 ± 0.25	0.68 ± 0.28	0.78 ± 0.17	ns
Asparagine	32.24 ± 26.32	58.76 ± 19.94	213.72 ± 39.23	*
Serine	6.06 ± 1.23	9.09 ± 1.16	20.17 ± 1.34	*
Glutamine	7.04 ± 2.87	11.76 ± 2.13	27.07 ± 5.10	*
Arginine	0.69 ± 0.19	0.82 ± 0.33	1.47 ± 0.25	*
Glycine	1.06 ± 0.21	1.38 ± 0.18	3.69 ± 0.55	*
Aspartic acid	5.15 ± 2.94	7.41 ± 2.49	8.32 ± 2.15	*ns*
Glutamic acid	4.78 ± 3.64	8.03 ± 4.40	6.76 ± 3.70	*ns*
Threonine	5.58 ± 0.80	7.46 ± 1.06	12.09 ± 0.58	*
Alanine	19.85 ± 4.66	20.53 ± 3.97	31.29 ± 6.21	*
GABA	10.42 ± 4.07	10.17 ± 3.31	14.78 ± 6.97	*ns*
Proline	0.82 ± 0.10	1.05 ± 0.46	1.64 ± 0.37	*
Lysine	0.68 ± 0.13	0.76 ± 0.32	1.20 ± 0.23	*
Tyrosine	1.39 ± 0.21	1.05 ± 0.22	0.42 ± 0.53	*
Methionine	0.15 ± 0.02	0.14 ± 0.01	0.14 ± 0.01	*ns*
Valine	2.84 ± 0.36	2.89 ± 0.31	4.27 ± 0.55	*
Isoleucine	1.36 ± 0.21	1.25 ± 0.19	1.56 ± 0.36	*ns*
Leucine	1.00 ± 0.08	1.05 ± 0.27	1.31 ± 0.33	*ns*
Phenylalanine	1.21 ± 0.15	1.16 ± 0.22	1.59 ± 0.33	*ns*
Tryptophan	1.38 ± 0.24	1.00 ± 0.24	1.33 ± 0.29	*ns*

Above values represent means and standard deviations of the treatments (NFA; ambient NH_3 concentrations in chambers with non filtered air, NFA+; non filtered air fumigated with low NH_3 concentrations and NFA++; non filtered air fumigated with high NH_3 concentration respectively. Level of significance according to one-way analyses of variance (ANOVA); * $p \leq 0.05$, ns = no significance. The NH_3 concentrations achieved in the experiment are found in (Table 7).

Table 9A: Summary of free amino acid concentrations in *Echinochloa crus-galli* exposed to fumigation treatments at various concentrations of gaseous NH_3 in NFA, NFA+ and NFA++ treatments respectively during the first set of the fumigation experiment.

Amino acids (nmol mg^{-1})	Gaseous NH_3 treatment			
	NFA	NFA+	NFA++	*P* - level
Histidine	0.03 ± 0.05	0.00 ± 0.00	0.50 ± 0.43	*
Asparagine	0.57 ± 0.35	0.35 ± 0.16	3.27 ± 1.33	*
Serine	0.93 ± 0.18	0.69 ± 0.08	3.76 ± 0.49	*
Glutamine	0.84 ± 0.13	0.82 ± 0.14	11.91 ± 2.79	*
Arginine	0.19 ± 0.04	0.16 ± 0.03	0.33 ± 0.05	*
Glycine	0.27 ± 0.01	0.24 ± 0.03	1.45 ± 0.28	*
Aspartic acid	0.84 ± 0.05	0.48 ± 0.08	1.75 ± 0.64	*
Glutamic acid	4.91 ± 0.23	4.27 ± 0.43	6.90 ± 1.26	*
Threonine	0.60 ± 0.04	0.51 ± 0.04	2.17 ± 0.21	*
Alanine	2.62 ± 0.34	3.19 ± 0.59	21.82 ± 3.18	*
GABA	1.21 ± 0.25	1.27 ± 0.25	3.84 ± 1.19	*
Proline	0.24 ± 0.04	0.25 ± 0.04	0.48 ± 0.06	*
Lysine	0.32 ± 0.12	0.19 ± 0.05	0.56 ± 0.08	*
Tyrosine	0.27 ± 0.01	0.21 ± 0.02	0.70 ± 0.06	*
Methionine	0.18 ± 0.02	0.17 ± 0.02	0.13 ± 0.08	*ns*
Valine	0.82 ± 0.06	0.65 ± 0.05	2.43 ± 0.32	*
Isoleucine	0.28 ± 0.02	0.22 ± 0.03	0.68 ± 0.08	*
Leucine	0.31 ± 0.07	0.27 ± 0.03	0.78 ± 0.12	*
Phenylalanine	0.50 ± 0.03	0.38 ± 0.02	1.24 ± 0.08	*
Tryptophan	0.33 ± 0.05	0.19 ± 0.04	0.57 ± 0.07	*

Above values represent means and standard deviations of the treatments (NFA; ambient NH_3 concentrations in chambers with non filtered air, NFA+; non filtered air fumigated with low NH_3 concentrations and NFA++; non filtered air fumigated with high NH_3 concentration respectively. Level of significance according to one-way analyses of variance (ANOVA); * $p \leq 0.05$, ns = no significance. The NH_3 concentrations achieved in the experiment are found in (Table 6).

Table 9B: Summary of free amino acid concentrations in *Echinochloa crus-galli* exposed to fumigation treatments at various concentrations of gaseous NH_3 in NFA, NFA+ and NFA++ treatments respectively during the second set of the fumigation experiment.

Amino acids (nmol mg^{-1})	Gaseous NH_3 treatment			
	NFA	NFA+	NFA++	*P* - level
Histidine	1.72 ± 0.98	0.66 ± 0.24	0.52 ± 0.63	*ns*
Asparagine	33.48 ± 23.46	13.11 ± 8.79	45.91 ± 30.16	*ns*
Serine	10.77 ± 6.07	7.70 ± 3.27	12.14 ± 3.77	*ns*
Glutamine	15.42 ± 5.94	7.71 ± 2.89	48.81 ± 23.08	*
Arginine	1.54 ± 1.23	0.70 ± 0.21	0.66 ± 0.86	*ns*
Glycine	4.18 ± 2.51	3.16 ± 1.11	5.67 ± 1.63	*ns*
Aspartic acid	3.74 ± 3.38	2.64 ± 2.56	5.05 ± 2.26	*ns*
Glutamic acid	9.82 ± 3.31	6.88 ± 3.92	10.75 ± 1.49	*ns*
Threonine	5.89 ± 2.89	3.79 ± 1.23	5.51 ± 1.96	*ns*
Alanine	44.50 ± 24.57	31.07 ± 8.87	40.45 ± 10.09	*ns*
GABA	5.43 ± 0.60	4.82 ± 2.75	5.43 ± 0.59	*ns*
Proline	0.75 ± 0.21	0.55 ± 0.07	0.60 ± 0.12	*ns*
Lysine	1.36 ± 0.88	0.76 ± 0.13	0.69 ± 0.48	*ns*
Tyrosine	2.46 ± 1.31	1.05 ± 0.36	1.02 ± 1.10	*ns*
Methionine	0.15 ± 0.01	0.13 ± 0.01	0.15 ± 0.02	*ns*
Valine	11.65 ± 3.98	8.44 ± 1.57	13.43 ± 0.94	*ns*
Isoleucine	2.84 ± 1.72	1.53 ± 0.45	1.50 ± 1.14	*ns*
Leucine	1.87 ± 0.81	1.13 ± 0.14	1.12 ± 0.50	*ns*
Phenylalanine	4.48 ± 2.32	1.65 ± 0.42	2.27 ± 2.10	*ns*
Tryptophan	2.24 ± 0.95	0.93 ± 0.31	1.13 ± 1.23	*ns*

Above values represent means and standard deviations of the treatments (NFA; ambient NH_3 concentrations in chambers with Non filtered air, NFA+; Non filtered air fumigated with low NH_3 concentrations and NFA++; Non filtered air fumigated with high NH_3 concentration respectively. Level of significance according to one-way analyses of variance (ANOVA); * $p \leq 0.05$, ns = no significance. The NH_3 concentrations achieved in the experiment are found in (Table 7).

Of all the twenty free amino acids analysed, the increase in gaseous NH_3 across the NFA, NFA+ and NFA++ treatments induced significant effects on all the amino acids in *Lolium multiflorum* except in Tyr and Met in the first set of the fumigation experiment and in His, Asp, Glu, GABA, Met, Ile, Leu, Phe and Trp in the second set of the fumigation experiment (Tables 8A and 8B). However, in *Echinochloa crus-galli*, significant effects were observed in all amino acids except in Met in the first set of the fumigation experiment, whereas in the second set no significant effect was observed except in Gln (Tables 9A and 9B).

The percentage composition of each amino acid in *Lolium multiflorum* in both the first and second set of the fumigation experiment is shown in (Figures 17A and 17B). Ala, Glu and GABA were the most prominent amino acids in *Lolium multiflorum* at the NFA treatment, in the first set of the fumigation experiment. They accounted for 19.2 %, 16.7 % and 15.4 % respectively, of the total free amino acids in the plant. Furthermore, in the NFA+ treatment, Ala and GABA had the highest amino acid compositions with 26.1 % and 20 % followed by Glu comprising of 8.7 % composition of the total amino acids. However, at the NFA++ treatment, only Gln and Asn accounted for over 70% of the total amino acids with compositions of 51.2 % and 26.5 % respectively. In the second set of the fumigation experiment, Asn and Ala dominated the amino acid compositions in *Lolium multiflorum* across the NFA, NFA+ and NFA++ treatments, with compositions of 30.9 % and 19 % at the NFA treatments and 40.1 % and 14 % respectively at the NFA+ treated plants. Whereas, at the NFA++ treatment, Asn composition was over half of the total amino acids in *Lolium multiflorum*, by accounting for 60.4 % of the entire amino acids, followed by Ala with 8.8 %.

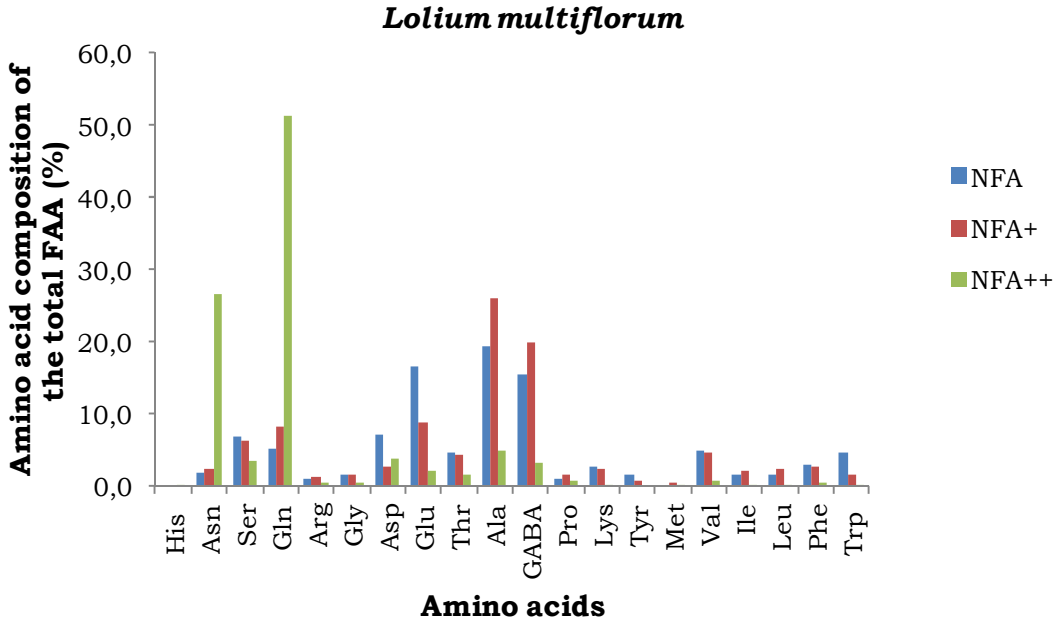

Fig 17A: Percentage composition of individual amino acids of the total free amino acids (FAA) in *Lolium multiflorum* exposed to three different treatments of NH_3 concentrations in the first set of the fumigation experiment. Values are means (n = 4). NFA, NFA+ and NFA++ represent different NH_3 treatments.

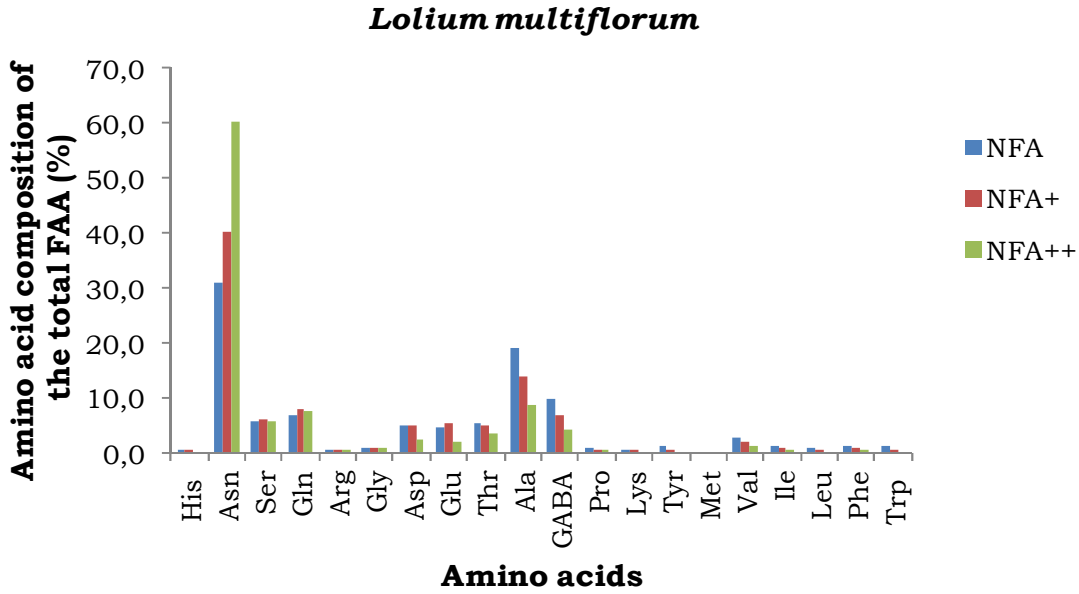

Fig 17B: Percentage composition of individual amino acids of the total free amino acids (FAA) in *Lolium multiflorum* exposed to three different treatments of NH_3 concentrations in the second set of the fumigation experiment. Values are means (n = 4). NFA, NFA+ and NFA++ represent different NH_3 treatments.

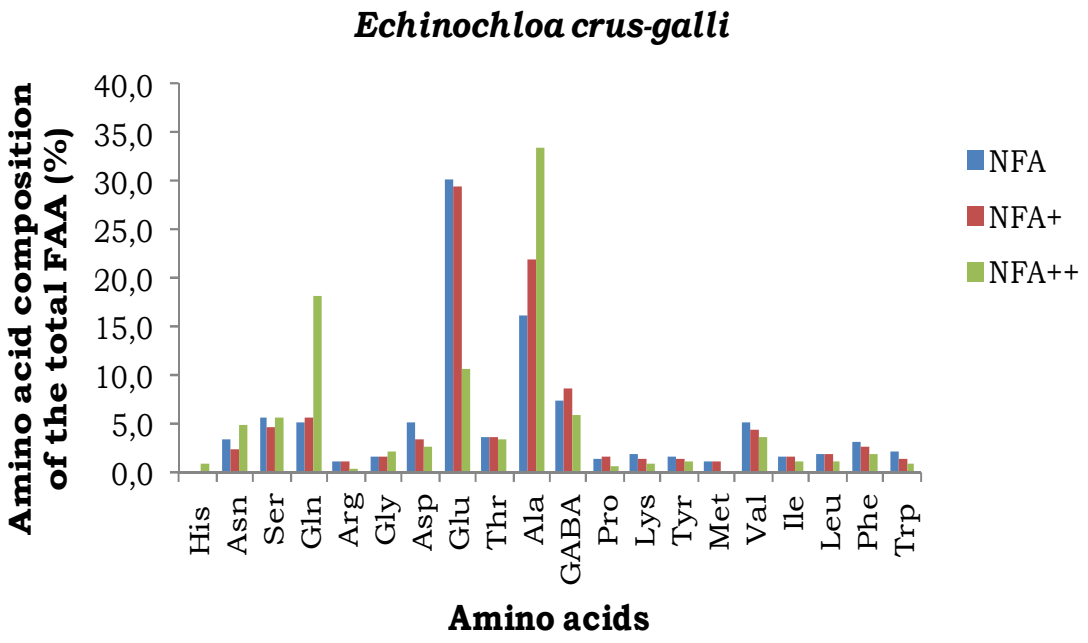

Fig 18A: Percentage composition of individual amino acids of the total free amino acids (FAA) in *Echinochloa crus-galli* exposed to three different treatments of NH_3 concentrations in the first set of the fumigation experiment. Values are means (n = 4). NFA, NFA+ and NFA++ represent different NH_3 treatments.

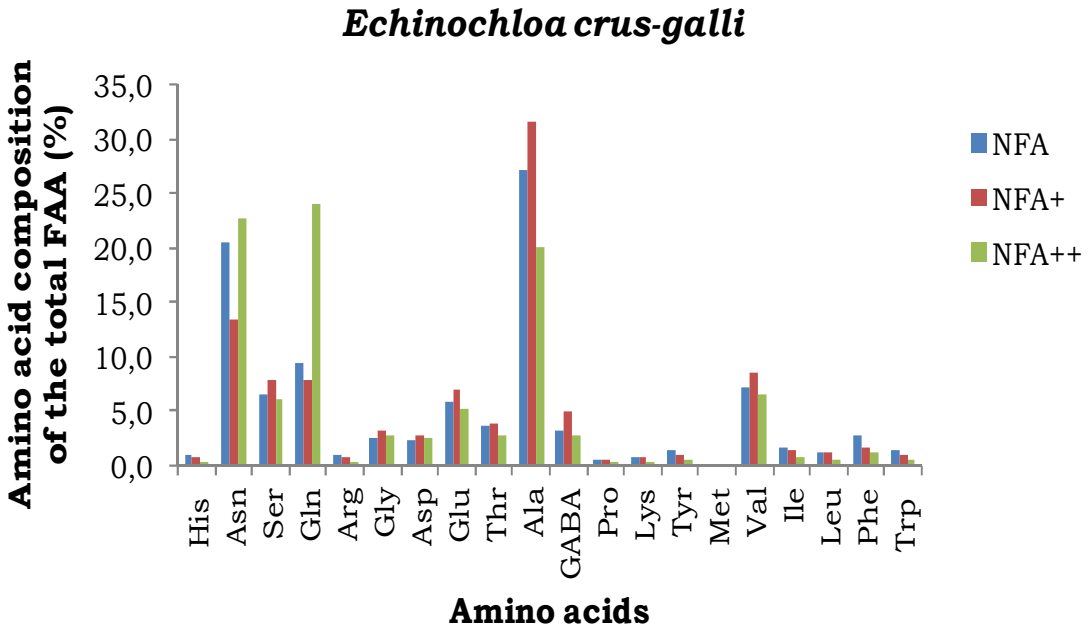

Fig 18B: Percentage composition of individual amino acids of the total free amino acids (FAA) in *Echinochloa crus-galli* exposed to three different treatments of NH_3 concentrations in the second set of the fumigation experiment. Values are means (n = 4). NFA, NFA+ and NFA++ represent different NH_3 treatments.

Results obtained from the first set of the fumigation experiment in *Echinochloa crus-galli* showed Glu and Ala dominance at the NFA treatment, with percentage compositions of 30.3 % and 16.1 % of the total free amino acid compositions in the plant. However, at the NFA+ treated plants Glu had the highest percentage composition of 29.5 %; followed by Ala recording 22 %. In the NFA++ *Echinochloa crus-galli* treated plants, Ala and Gln dominated the percentage compositions of the total amino acids, by accounting for 33.4 % and 18.3 respectively (Figure 18A). Ala, Asn and Gln were also prominent amino acids observed in *Echinochloa crus-galli* across the NFA, NFA+ and NFA++ treatments in the second set of the fumigation experiment. In *Echinochloa crus-galli* Ala, Asn and Gln accounted for over 50 % percentage compositions of the total amino acids in the NFA treatment by accounting for 27.1 %, 20.4 % and 9.4 % respectively. Whereas, Ala composition at the NFA+ treatment was 31.6 %, while Asn and Gln compositions were 13.3 % and 7.8 %. Furthermore, at the NFA++ treatment, Gln dominated the amino acid percentage compositions in *Echinochloa crus-galli* with 24.1 %, followed by Asn and Ala accounting for 22.6 % and 19.9 % respectively of the total amino acid compositions (Figure 18B).

Figure 19 demonstrates the variation in trend of the total free amino acids in *Echinochloa crus-galli* and *Lolium multiflorum* plants with increase in gaseous NH_3 concentrations in the first set of the fumigation experiment at the end of a four weeks fumigation period. The total free amino acids in *Lolium multiflorum* plants increased across NFA, NFA+ and NFA++ NH_3 concentrations by accounting for 14.11 nmol mg^{-1}, 21.69 nmol mg^{-1} and 342.89 nmol mg^{-1} respectively. However, in *Echinochloa crus-galli* the total free amino acids contents at NFA, NFA+ and NFA++ treatments were 16.22 nmol mg^{-1}, 14.5 nmol mg^{-1} and 65.24 nmol mg^{-1}. In the second set of the fumigation experiment, the total free amino acids in *Lolium multiflorum* increased across the gaseous NH_3 treatments of NFA, NFA+ and NFA++ by accounting for concentrations of 104.1 nmol mg^{-1}, 146.4 nmol mg^{-1} and 353.5 nmol mg^{-1}. In *Echinochloa crus-galli* the total free amino acids was highest at the NFA++ treatments by recording 202.7 nmol mg^{-1}, while the NFA and NFA+ treatments recorded 164.2 nmol mg^{-1} and 98.3 nmol mg^{-1} respectively (Figure 20).

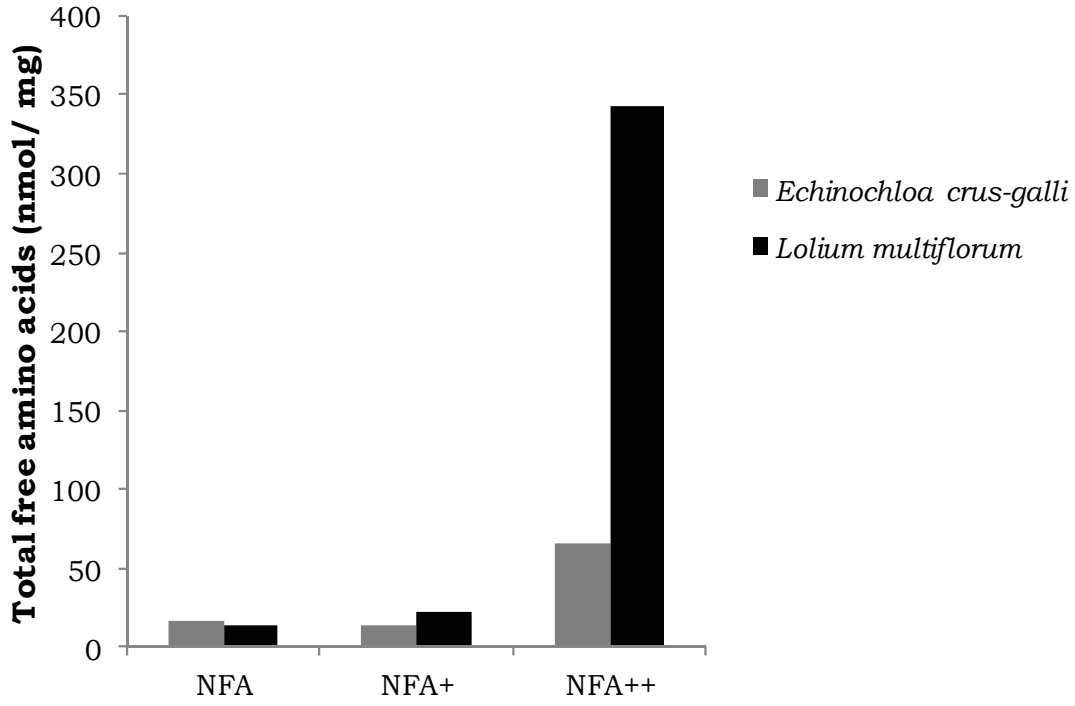

Fig 19: Total free amino acid concentrations in *Echinochloa crus-galli* and *Lolium multiflorum* exposed to three different treatments of NH_3 concentrations in the first set of the fumigation experiment. Values are means (n = 4). NFA, NFA+ and NFA++ represent different NH_3 treatments.

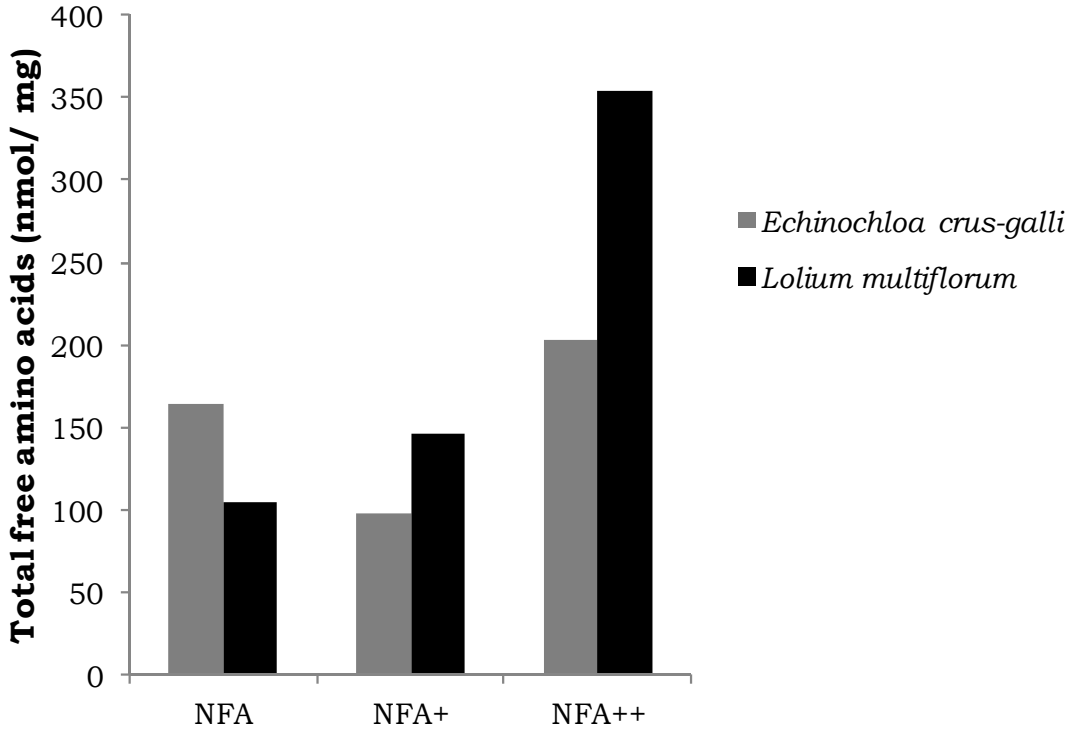

Fig 20: Total free amino acid concentrations in *Echinochloa crus-galli* and *Lolium multiflorum* exposed to three different treatments of NH_3 concentrations in the second set of the fumigation experiment. Values are means (n = 4). NFA, NFA+ and NFA++ represent different NH_3 treatments

5. DISCUSSION

5.1 The effect of ambient NH_3 concentrations in the accumulation of free amino acids in *Lolium multiflorum*, *Chenopodium album*, *Echinochloa crus-galli* and *Urtica dioica*

The increasing ambient NH_3 concentration with close proximity to the stable, induced changes in free amino acid concentrations in the four plant species studied. The changes in amino acid concentrations with the prevailing ambient NH_3 concentration during field exposure, indicates that the plants were able to incorporate and take up NH_3 from the air into the metabolism of the plants located at different sites along the selected transect. This also possibly indicates that, the mesophyllic NH_3 concentration obtained through the plant stomata, a means of NH_3 entry and uptake (van Hove *et al*., 1987), was quite lower when compared with the ambient NH_3 concentrations which facilitated the uptake of atmospheric NH_3, from the environment into the plants (Fangmeier *et al*., 1994; Schjoerring *et al*., 2000). Results obtained in this study, demonstrated that additional nitrogen from nitrogen deposition was assimilated in these plants into free amino acids compounds possessing mostly surplus nitrogen atoms and with a low carbon to nitrogen ratio. The assimilation of NH_4^+ is primarily by the glutamine synthetase (GS) and glutamate synthase pathway (GOGAT), which leads to the production of glutamine as the first product and a subsequent generation of two molecules of glutamate of which one molecule serves as a substrate for (GS) while the other glutamate molecule is used for the further production of amino acids (Calanni *et al*., 1999).

The free amino acid composition in *Lolium multiflorum* showed a remarkable response to increasing NH_3 concentration. A sequential rise in all the free amino acids from biochemically analysed samples of *Lolium multiflorum* exposed to the increasing NH_3 concentration was observed except in tryptophan. Gln dominated the amino acid pool at 67 m from the stable with the highest NH_3 concentration and accounted for 19.1% of the entire total amino acid. A shift in dominance to Ala was observed at further distances from the stable with lower NH_3 concentrations of 11.35 and 1.5µg m^{-3} at distances of 149 m and 804 m respectively. The ability of some plants to respond to an increase in N supply, by revealing changes in amino acid dominance has also been reported (Nordin *et al*., 1998; Ohlson *et al*., 1995). Previous studies by (Näsholm *et al*., 1994) demonstrated a relationship between amino acid accumulation and increased nitrogen availability in some Swedish forest species. They observed that only some of the amino

acids Asn, Gln and Arg accounted for most of the total amino acid in all the species studied.

It was an entire different scenario with *Echinochola crus- galli* and *Urtica dioica*. Ala dominated the entire amino acid pool in *Echinochola crus- galli* at all distances studied by accounting for 25-28% of total amino acid concentration at NH_3 concentrations of 15.25, 8.25 and 1.15µg m^{-3}. The dominance of Ala in *Echinochola crus- galli* a C4 plant might be as a result of its function in C4 photosynthesis (Kennedy and Laetsch., 1974; Lea & Azevedo., 2007). Lys constituted a major proportion of the total amino acid, in *Urtica dioica* with 17-29% followed by Ala accounting for 15-18% across all distances with increase in NH_3 concentration. Other amino acids in *Urtica dioica* such as Asn, Gln, Glu and GABA were also observed in high quantities at all NH_3 concentrations across the selected transect, compared to the remaining amino acids.

Results obtained in this study, showed variability in amino acid accumulation across different plants, exposed to NH_3 from the least to the highest concentrations. At the highest NH_3 concentration, Gln was the most prominent amino acid in *Lolium multiflorum and Chenopodium album*, while in *Echinochola crus-galli* and *Urtica dioica*, Ala and Lys accounted for the highest proportion of free amino acids. However, Asn, Gln, Ala and Lys with a low C/N ratio constituted a major proportion of the total amino acids in all the plant species studied (Nordin *et al*., 1998).

The classification of amino acids Asn, Lys, Thr, and Meth belonging to the aspartate family (Azevedo *et al*., 2006), in all plant species studied, sheds more light on their response pattern in each plant. In *Lolium multiflorum*, a slight increase in Asn was observed with increase in NH_3. However, at the highest NH_3 concentration, Asn increased remarkably suggesting that, the increase in NH_3 concentration stimulated the production and induced Asn proportion in the total amino acids in *Lolium multiflorum*. Asn formed from its precursor aspartate is considered as a vital storage compound of nitrogen, which is capable of accumulating in various plant organs (Lea *et al*., 2007). Although the accumulation of Asn is known to occur under different conditions, studies have also shown its ability to accumulate with the availability of large amounts of reduced nitrogen (Lea *et al*., 2007). The ability of ammonium to induce Asn production has also been indicated by several studies (Masclaux-Daubresse *et al*., 2006; Stewart., 1979). Certain enzymes involved in Asn production have also been reported to be

induced in the presence of high NH_3 concentrations (Givan., 1979; Lam *et al*., 1998). As an enzyme involved in this process, asparagine synthetase plays a vital role in the formation of glutamate and asparagine by catalysing the transfer from glutamate, an amide amino group to its precursor aspartate (Lea & Azevedo., 2007). A similar result was obtained in *Echinochola crus-galli* and *Urtica dioica*. Although, Asn concentrations increased with increase in NH_3 concentrations but, was lower compared to Lys in both *Echinochola crus-galli* and *Urtica dioica* respectively and Thr in the former plant. Contrary to results obtained in other plants, all the amino acids belonging to the aspartate family in *Chenopodium album* decreased at the highest NH_3 concentration except aspartate.

Gln concentration increased with increased NH_3 concentration and accounted for the highest proportion of amino acids in the glutamate family in all plant species studied. The predominant proportion of Gln in all plant species studied probably indicates the preference of certain amino acids for nitrogen storage and transport (Näsholm *et al*.,1994). Increases in amino acids especially the accumulation of Gln could occur as a result of short term N effects either as nitrates or NH_3 supply on leaves, thereby demonstrating the effects of N up-take on Gln content in leaves (Foyer *et al*., 1994). Therefore the increases in Gln concentration observed under increasing NH_3 concentration, possibly suggest the supply of excess available N for Gln synthesis (Ruan *et al*., 2010). The availability of 2-oxoglutarate and the rate of NH_3 supply are major constituents influencing Gln in leaves (Novitskaya *et al*., 2002) while 2-oxoglutarate also plays a vital role in NH_3 incorporation and the supply of C skeletons during amino acid synthesis (Foyer *et al*., 1994). The accumulation of Asn and Gln in both aspartate and glutamate families, especially at high NH_3 concentrations possibly suggests NH_3 detoxification into Asn and Gln compounds (Givan, 1979). Such a detoxification process is considered as a means getting rid of reduced nitrogen by plants thereby storing nitrogen in low C/N ratio amino acid compounds (Fangmeier *et al*., 1994).

The percentage increase in free amino acids, in all plant species exposed to various NH_3 concentrations, ranging from the least to the highest concentration, was dominated predominantly by low C/N ratio compounds. Gln responded sharply with increase in concentration from the least to the highest NH_3 concentration in *Lolium multiflorum* and

Chenopodium album, whereas it was Arg in *Urtica dioica* and Asn in both *Echinochola crus-galli* and also *Lolium multiflorum*. The large scale accumulation of Asn in both grasses *Lolium multiflorum and Echinochola crus- galli* by 413 and 287% respectively, support previous studies on Asn accumulation in another grass *Deschampsia flexuosa* under increasing Nitrogen supply (Näsholm *et al*., 1994; Nordin *et al*., 1998; Ohlson *et al*., 1995).

Urtica dioica a nitrophilic species has been reported to accumulate Asn and Arg respectively in roots under elevated ammonium conditions (Ohlson *et al*., 1995; Rosnitschek – Schimmel, 1985). Atanasova, (2008), investigated the effects of different nitrogen sources and levels on amino acid composition in head cabbage. Arg composition increased up to eight folds at the highest nitrogen application rate compared to the control plant, regardless of the nitrogen source. Edfast *et al*., (1996) reported high Arg levels in needles of Scots pine trees exposed to high nitrogen input. They suggested the suitability of Arg as a tool in the determination of nitrogen status in pine trees. In another study involving pine needles, mineral imbalances in nutrients and nitrogen were found to trigger Arg concentrations (van Dijk and Roelofs 1988; Edfast *et al*., 1996). Of all the plant species studied Arg concentration in *Urtica dioica* had the highest percentage increase by 1400% under increasing NH_3 conditions. This backs up previous studies connoting the use of Arg as an indicator of atmospheric nitrogen deposition.

Furthermore, the increase in NH_3 concentration with close distance to the stable, not only showed an increase in total free amino acids but also a strong relationship with individual amino acids in all the plants studied with few exceptions. This indicates the plants were able to respond positively with subsequent incorporation of atmospheric nitrogen into amino acids (Calanni *et al*., 1999). However, in *Lolium multiflorum,* Arg, Glu, Gly and His showed statistically significant effects with increase in NH_3 concentration, while it was Ala, Pro and Thr in *Urtica dioica.* Imbalances in nitrogen nutrition have been attributed to such possible increases in Ala and Pro (Atanasova, 2008). However, in contrast to *Lolium multiflorum* and *Urtica dioica*, there were no significant effects in *Echinochola crus- galli* and *Chenopodium album.*

The responses of all the plant species in this study with regards to its free amino acid composition, sheds more light on its possible suitability as biomonitors of nitrogen deposition. The alterations observed in the free amino acid concentrations in the plant species studied, with increasing distance from the stable and increasing NH_3 concentration at closer distances to the stable, showed the plants were suitable and able to respond to a source of nitrogen deposition (Näsholm *et al*., 1994). This is evidenced from the substantial linear relationships observed between the total free amino acid compositions and the ambient NH_3 concentrations in the various plant species studied. The observed changes in total free amino acid composition as a result of alterations in plant metabolism shows the potential impact of nitrogen deposition (Pitcairn *et al*., 2003) and the ability of the plants studied to detect and respond as biomonitors of nitrogen deposition.

The ability of atmospheric NH_3 to induce toxic effects such as visible injuries on vegetation is well documented (Fangmeier *et al*., 1994; Pearson and Stewart, 1993; van der Eerden, 1982; van Dijk and Roelofs 1988). This has been reported to occur due to its capacity to function in the electron transport system of plants by accepting electron and being able to uncouple the photosynthetic electron transport system (Fangmeier *et al*., 1994; Pearson and Stewart, 1993). Therefore, in order to avert toxicity, under conditions of surplus availability of atmospheric NH_3, plants tend to assimilate NH_3 into other nitrogen compounds such as the free amino acids as observed in this study (Fangmeier *et al*., 1994. This could only be a temporary measure of averting toxicity, as a result of the alteration in cell pH that would arise due the H^+ produced sequel to NH_3 assimilation, which might induce devastating effects on enzymatic processes if H^+ is not properly translocated from the plant via the roots (Pearson and Stewart, 1993). However, in all the selected indicator plants investigated in this study, there was no case visible injury even at the highest ambient NH_3 concentration of 32.6 µg m^{-3} in the field or 119.5 µg m^{-3} in the greenhouse chambers. In the case of *Lolium multiflorum* this finding is consistent with the results obtained by (van der Eerden, 1982) after the fumigation of the cultivar Optima with NH_3 for 30 days at a concentration of 0.6 mg m^{-3}. Similarly, Whitehead and Lockyer (1987) conducted a fumigation study on the cultivar Lam and observed no visible injuries on *Lolium multiflorum* even at the highest NH_3 concentration of 709 µg m^{-3} after exposing the plants for a period of 33days.

5.2. The influence of ambient NH_3 concentrations on the above ground biomass accumulation in *Lolium multiflorum*, *Chenopodium album*, *Echinochloa crus-galli* and *Urtica dioica*

In this study the influence of ambient NH_3 concentrations on the aboveground biomass of the various plant species studied, were observed regardless of the source of NH_3 exposure either in the field or through chamber studies with gaseous NH_3 fumigation. Plants involved in the field experiment were exposed to almost virtually different ambient NH_3 concentrations as well as climatic and environmental conditions compared to the fumigation experiments conducted under controlled greenhouse conditions. The average atmospheric temperature between July and September when the field exposures for each plant was carried out were 13.5 to 16^oC. An increase in temperature up to 21^oC would possibly have induced an increase in number of tillers and yield in *Lolium multiflorum* (Hill *et al*., 1985) while in *Echinochloa crus-galli*, temperature has been reported to induce a significant effect on increase in plant height (Maun and Barrett, 1986).

5.2.1. Dry matter accumulation in field exposed plants

It is assumed the low temperatures in the field, had a positive impact on NH_3 uptake by the plants rather than being a source of emission which could possibly arise under higher temperature conditions (Schjoerring *et al*., 2000). In this study the biomass production of *Lolium multiflorum* and *Echinochloa crus-galli* across the selected sites from highest distance increased by 6.7 %, 33.64% and 13.04 %, 23.36 % respectively in both plants with decreasing distance closer to the source of NH_3 emission. The exposure of the selected plants to a source of NH_3 emissions, on different dates resulted in the interaction of varying ambient NH_3 concentrations with these plants after an exposure of four weeks. Since all the plant species were not exposed same day at each site, the ambient NH_3 concentrations at the various sites were higher during the exposure periods of *Urtica dioica* followed by *Lolium multiflorum*, *Chenopodium album* and then *Echinochloa crus-galli*. However, amongst all the plant species studied, *Urtica dioica* demonstrated the highest increase in biomass production with increasing NH_3 availability by accounting for 17.97 % and 56.18 % increase in biomass production respectively. At the end of the exposure period, the ambient NH_3 concentrations induced a significant effect on the above ground biomass in *Lolium multiflorum*, *Echinochloa crus-galli* and *Urtica dioica*. Therefore as the NH_3 concentrations

increased with a closer distance to the source of emissions the above ground biomass also increased significantly, while in *Chenopodium album* a different observation was made, indicating an increase in above ground biomass of the plant as NH_3 concentration decreased. *Urtica dioica* is a generally acknowledged nitrophilic species (Taylor, 2009), known to thrive in the presence of high nitrogen availability and has been observed among dominant species located in close proximity to sources of NH_3 emission and nitrogen deposition (Pitcairn *et al*., 1998). This present study confirms through active biomonitoring, the sensitivity of *Urtica dioica* to nitrogen availability from sources of NH_3 pollution and its ability to incorporate this nitrogen source for growth and biomass production. In the case of *Chenopodium album* it is difficult to conclude this result as an outcome of the plants response to interactions with increasing ambient NH_3 concentrations due to an obvious loss in biomass observed during field study as a result of the wind speed intensity at the studied sites.

5.2.2. Dry matter accumulation of chamber fumigated plants

In the chamber studies, gaseous NH_3 had positive effects on the above ground biomass of *Lolium multiflorum* and *Echinochloa crus-galli* in both sets of the fumigation studies, by inducing increases in the above ground biomass of the plants in the following treatment order of NFA, NFA+ and NFA++ with the exception of *Lolium multiflorum* in the second set of fumigation study conducted. However, such effects were not statistically significant except in leaves of *Lolium multiflorum* in the first set of fumigation experiment. The decrease in the biomass of *Lolium multiflorum* in the second set of the fumigation study especially at NFA+ and NFA++ treatments, was probably due to the complete shading of some sides of the plant by the rapidly growing *Echinochloa crus-galli* plants exposed under NFA+ and NFA++ treatment condition. Results from these studies however, demonstrates a possible uptake and assimilation of NH_3 by these plants as have been observed in some other plants (Pearson and Stewart, 1993; van Hove *et al*., 1987). Furthermore, the nitrogen content of a plant arising from the uptake of atmospheric NH_3 could also be increased with increasing NH_3 concentrations as shown in a previous study on *Lolium multiflorum* (Whitehead and Lockyer, 1987). This resulted not only in a linear relationship between the nitrogen contents available in the plant shoots with NH_3 concentrations in their study, but also induced an influence on shoot dry weight of the plants (Whitehead and Lockyer, 1987). Ashraf *et al*. (2003) reported the foliar uptake of $^{15}NH_3$ in three rice cultivars when

exposed to $15NH_3$ at various growth stages. They were able to observe $^{15}NH_3$ recoveries from the plant parameters indicating a possible uptake and transport of ^{15}N across various plant parts. According to previous studies by Perez-Soba and van der Eerden (1993) such foliar uptake of labelled^{15}N induced an increase in the biomass composition of needles in *Pinus sylvestris* after a one year exposure to atmospheric NH_3. Based on results obtained in this study, the ability to convert increased nitrogen availability across the various treatments either with increasing distance to the source of NH_3 emission or with fumigation with increasing NH_3 concentrations into additional biomass production is an evidence of the plants ability to take up and utilize these nitrogen sources as nitrogen nutrient for growth (Stevens and Tilman, 2010; Castro *et al*., 2006; Perez-Soba and van der Eerden, 1993). However, climatic factors such as temperature and relative humidity could also play a role in determining how a plant responds to a source of atmospheric NH_3 either as a source of emission or as a channel in the uptake of atmospheric NH_3. Husted *et al*. (1996) reported the emission of NH_3 from *Brassica napus* plants when exposed to a low ambient NH_3 concentrations under increasing leaf temperature conditions, whereas in the case of relative humidity, NH_3 absorption was facilitated by increasing light intensities at a high relative humidity. The above ground biomass in *Lolium multiflorum* and *Echinochloa crus-galli* in the first set of the fumigation experiment, increased from 4 to 10 % and 10 to 14 % respectively in both plants when exposed to the same low and high gaseous NH_3 fumigation compared to those treated with non - filtered air. Although, *Lolium multiflorum* decreased by 10.7 % and 8.5 % in the second set of the fumigation experiment due to shading effects, the *Echinochloa crus-galli* plants showed similar increases of 9.7 % and 10 % from NFA to NFA+ and NFA++ treatments respectively. The higher increases observed in the biomass of *Echinochloa crus-galli* an ideal C4 plant is an indication of its ability to allocate extra nitrogen into leaf production and growth development, under increasing nitrogen availability compared to *Lolium multiflorum* a C3 plant (Sage and Pearcy, 1987) signifying an uptake in NH_3 based on the available ambient NH_3 concentration in the air.

5.2.3. Effects of NH_3 on above ground biomass partitioning in both field and chamber studies.

The aboveground biomass fractions of the plant materials studied were evaluated and amongst the plants exposed in the field and chamber studies, only in leaves of *Urtica dioica* and *Lolium multiflorum* was a significant effect observed. In the fumigation study, it was also observed that, in *Lolium multiflorum* the leaf biomass to the total above ground biomass increased with the increasing treatment level of NFA, NFA+ and NFA++. This increase was accompanied also by a subsequent decrease in the proportion of stem biomass to the total above ground biomass. Similarly, in the same study *Echinochloa crus-galli* showed a negligible increase in the proportion of leaf biomass and a decrease in stem biomass to the total above ground biomass across the NFA, NFA+ and NFA++ treatments. Remarkably, within the end of the 4 weeks fumigation of *Echinochloa crus-galli*, growth development had progressed to the next phase with the production of seed heads which increased with the increasing concentration of NH_3 available to the plants. This is an indication of the positive effects additional nitrogen, resulting in the development of seed heads (Burkle and Irwin, 2010) due to the uptake of gaseous NH_3 by the plants. *Echinochloa crus-galli* was responsive to its interaction with NH_3 treatments by the development of seed head production and on the other hand signifying a positive influence of NH_3 on its biomass accumulation. This observation in seed head development is typical of any perfect weed especially when the conditions are favorable enough to support an accelerated development across the various growth phases (Baker, 1974). This finding at both first and second sets of the fumigation study, especially at the highest NFA++ treatments could be attributed to the increase in the leaf nitrogen contents of the plants which therefore enhances its photosynthetic ability enabling the plant to produce sufficient carbohydrates required in meeting certain requirements for growth and development of the plants (Trlica, 2006). In other words, increased nitrogen accumulation in plants is influential in its response to growth and biomass accumulation when exposed to an abundant source of available nitrogen (Gastal and Lemaire, 2002; Perez-Soba and van der Eerden, 1993) as its observed for instance in grasses, where the growth and elongation rate in leaves is determined by the availability of nitrogen (Gastal and Nelson, 1994). Besides, atmospheric NH_3 has the capacity of enhancing photosynthesis in plants and this could subsequently correspond positively with increase in leaf biomass production and ultimately plant growth (van der Eerden and Perez-Soba, 1992; Whitehead and Lockyer, 1987). Additionally, it is

important to note that regardless of the concentration of atmospheric NH_3 exposed to plants in both sets of the fumigation study, the plants in the second set had higher biomass compared to the first set due to the differences in nitrogen contents in the soil types used.

An increase in seed to biomass ratio in *Echinochloa crus-galli* with NH_3 concentration was observed, suggesting an increase in biomass of *Echinochloa crus-galli* was accompanied with increase in seed production (Perron and Legere, 2000). In addition, the proportion of leaf to stem ratio in the plants studied so far, in both experiments were increased with increasing NH_3 availability. Different parameters such as the specie of a plant, coupled with its growth and environmental conditions, could influence the biomass allocation of such a given plant, but above all external factors, the nutrient availability, plays a dominant role in determining how its biomass is eventually distributed either above or below ground (Poorter and Nagel, 2000). Having established that plants have the ability to utilize atmospheric NH_3 as nutrients (Asman *et al*., 1998; Castro *et al*., 2006), increases in biomass accumulation of leaves across the various NH_3 concentrations could be as a result of increases in leaf area (Trapani and Hall, 1996), specific leaf area (SLA) and biomass per unit time and leaf area also known as unit leaf rate (ULR) (Poorter and Nagel, 2000). This is based on the assertion that, the differences between plants of the same species placed at several distances from the point of NH_3 emission and exposed for the same duration was basically, the interaction with the ambient NH_3 concentrations, along those distances. This also applies to the chamber experiments where fumigation with different concentrations of gaseous NH_3 was conducted.

5.2.4 Effects of different parameters in field exposed and chamber fumigated plants

The results observed in this study, showed a quite difference in the responses of *Echinochloa crus-galli* and *Lolium multiflorum* plants with response to field exposures and fumigation studies. This is owing to the fact that both plants were exposed and grown under completely distinct conditions, prior and during field exposures or gaseous NH_3 fumigation. The differences observed in the field and fumigation chamber studies of *Echinochloa crus-galli* and *Lolium multiflorum* besides the effects of NH_3

concentrations on the above ground biomass and on various plant parameters could be attributed to several factors. An important factor is the level of light intensity and this has a profound effect on plant growth (Guenni *et al*., 2008) as well as in the uptake of atmospheric NH_3 especially during conditions of high relative humidity (Husted *et al*., 1996; van Hove *et al*., 1987). The average relative humidity in all the growth chambers was above 90 % and as a result of the condensation inside the chambers the light transmission into the chambers was impaired, thereby reducing the incident, photon flux density of the light reaching the plant surface to an average of 283 µmol m^{-2} s^{-1} during the fumigation period. A lower incident PPFD could also have a profound effect on tiller development in grasses (Gautier *et al*., 1999). Although the penetration of light radiation into the greenhouse could have been an added advantage to the plants, however this was not feasible due to shading. In contrast to the fumigation chamber experiment, the field grown plants were not obscured from light radiation in the environment thereby exposing the plant surface to a higher photosynthetic flux density (PPFD) ushering a possible rise in the net photosynthetic rate of field exposed plants compared to their counterparts grown in fumigation chambers (Mebrahtu *et al*., 1993). In addition, under conditions of ample light availability NH_3 could trigger photosynthesis in the plant thereby possibily increasing its net photosynthetic rate and also facilitating an increase in NH_3 uptake and also in the biomass production of leaves (van der Eerden and Perez-Soba, 1992). Studies have demonstrated the ability of different plants to preferentially respond to certain levels of light conditions for growth (Mahoney and Swanton, 2008; Monaco *et al*., 2005). For instance, a low light intensity would result in a reduction in growth rate in *Lolium multiflorum* notwithstanding its ability to possibly exhibit a higher photosynthetic rate under higher conditions (Blackman and Jack, 1959). In addition, the defoliation of the field exposed plants prior to exposure could possibly have influenced the differences observed in the responses of both plants species to the availability of different sources of atmospheric NH_3. Aoyagi and Akimoto (2009) observed an increase in the resource allocation of above ground structures in *Lolium multiflorum* plants in response to clipping. In this study, defoliated plants exposed in the field were able to demonstrate a positive effect significantly and a higher increase in the percentage biomass of above ground structures between treatments compared to the chamber grown non defoliated plants. This indicates the positive influence of the ambient NH_3 concentration in the growth rate of defoliated

plants despite being lower in concentration to that exposed to the non defoliated plants in the fumigation chamber experiment.

5.3 The influence of gaseous NH_3 fumigations on the accumulation of free amino acids in *Lolium multiflorum* and *Echinochloa crus-galli.*

The gaseous NH_3 fumigation of *Lolium multiflorum* and *Echinochloa crus-galli* plants in this study with the following NFA, NFA+ and NFA++ treatments, induced alterations in the concentrations and compositions of free amino acid metabolites in the plants. In *Lolium multiflorum* the total amino acids increased with increasing gaseous NH_3 concentrations, in both the first and second set of the fumigation experiment, implying an uptake in gaseous NH_3 (Perez-Soba *et al*., 1994; Whitehead and Lockyer, 1987). Consequently, such increases in total amino acids could be associated with the increase in assimilation and subsequent detoxification of readily available NH_3 in the ambient air taken up by the plants (Fangmeier *et al*., 1994).

Results obtained from this study, clearly demonstrates a shift in nitrogen accumulation in the amino acids under the different fumigation treatments applied, especially in the first set of the fumigation experiment. For instance in *Lolium multiflorum* plants, which was dominated by Ala, followed by GABA and Glu in both NFA and NFA+ treatments, demonstrated a shift in nitrogen accumulation at the highest gaseous NH_3 concentration in the NFA++ treatment, to Gln, Asn and Ala. This shift in nitrogen accumulation to Gln, Asn and Ala dominance at the NFA++ treatment, not only produced an increase in total free amino acids in the plant but also demonstrates a shift in nitrogen accumulation as a result of the uptake of high NH_3 concentrations by the plants and subsequent storage into low C/N ratio and nitrogen rich compounds (Fangmeier *et al*., 1994). A similar shift was observed in *Echinochloa crus-galli* in both first and second set of the fumigation experiment. In the first set of the fumigation experiment, the initial dominance of Glu, Ala and GABA in both NFA and NFA+ treatments was observed, whereas in the NFA++ treatment Ala, Gln and Glu dominated the amino acid pool. Similarly, Gln, Asn and Ala were the most dominant amino acid in the second set of the fumigation experiment at the NFA++ treatment of *Echinochloa crus-galli*, whereas the Ala, Asn and Glu dominated the NFA treatment, while Ala, Asn and Val were most prominent at the NFA+ treatment. This shift in nitrogen storage and amino acid

dominance is also an indication of the preference for nitrogen storage into amino acid compounds with abundant nitrogen availability and low C/N ratio (Coruzzi and Last, 2000; Fangmeier *et al*., 1994). For instance, Asn could be a preferred choice for nitrogen storage and transport under conditions of limited carbon availability, as a result of its possession of a large number of nitrogen atoms per unit carbon (Coruzzi and Last, 2000). Additionally, Ala synthesis have been reported to be accompanied with the accumulation of GABA (Wallace *et al*., 1984) and this was evident in *Lolium multiflorum* and *Echinochloa crus-galli* plants and might possibly be responsible for its higher concentration compared to some other amino acids. Futhermore, the presence of Gln as one of the dominant amino acids in the plants besides its low C/N ratio is as a result of its position as the first reaction product upon the assimilation of NH_4^+, in the two way enzyme system comprising of GS and GOGAT pathway (Calanni *et al*., 1999) and was found to increase under conditions of higher NH_3 concentrations.

In this study significant effects on most of the amino acids across treatments were observed in *Lolium multiflorum* and *Echinochloa crus-galli* plants in the first set of the fumigation experiment after a 4 weeks exposure to gaseous NH_3, compared to the second set. At the end of 4 weeks exposure to NH_3 *Lolium multiflorum* showed more statistically significant effect in the second set of the fumigation experiment compared to *Echinochloa crus-galli* exposed for 2 weeks in the second set. It is also important to note, that the differences in total amino acids produced by both plants in the first and second sets of the fumigation experiment were probably partly as a result of the different soil types used in the two fumigation experiments which contained different nitrogen levels. Generally, results obtained from this fumigation experiment showed differences in the response pattern of *Lolium multiflorum* and *Echinochloa crus-galli* plants to gaseous NH_3 and this is clearly evident in the amino acid profiles of both plants. This might be attributed to the different patterns in the CO_2 fixation of *Lolium multiflorum* a C_3 plant and *Echinochloa crus-galli* a C_4 into carbohydrate which is important in providing the carbon skeleton for nitrogen storage coupled with the large amount of energy required for the synthesis of a molecule of glucose by C_4 compared to C_3 plants (Coruzzi and Last, 2000). In addition, increased nitrogen availability could increase contents of ribulose-1,5-bisphosphate carboxylase (RuBisCo), (Warren *et al*., 2003) which is responsible for catalyzing the carboxylation of ribulose-1,5-bisphosphate (RuBP). However, the dominance of certain nitrogen compounds such as

Ala, Asn and Gln in both plants especially, under high ambient NH_3 concentrations are comparable as these were evident in both the fumigation study and field trials conducted in this study.

5.4. Modification of responses to NH_3 by different soil nitrogen supply.

The results from the first and second set of the fumigation study showed a modification of the plant responses to NH_3 by the different nitrogen contents of the soil types used in the fumigation experiments. Thus, these differences in the responses of *Lolium multiflorum* and *Echinochloa crus-galli* to gaseous NH_3 was observed in their biomass production and accumulation of free amino acids. This finding may be explained by the fact that since it was possible for plants to take up nitrogen from the soil and as well as from gaseous NH_3, (Whitehead and Lockyer, 1987) the plants in the second set of the fumigation experiment were therefore able to produce more biomass and accumulated more amino acids compared to those in the first set (Nordin *et al*., 1998). This is as a result of the higher nitrogen content in the soil used in the second set compared to the nitrogen content of those in the first set of the fumigation experiment. Therefore the plants were able to respond as well to the perturbations in soil nitrogen content in the two sets of the fumigation study. In addition, the effects of the different nitrogen contents of the two soil types used is consistent with results obtained in simulated nitrogen deposition studies and nitrogen addition experiments whereby additional nitrogen contents have influenced increases in biomass production and free amino acid accumulation (Näsholm et al., 1994; Nordin *et al*., 1998; Whitehead and Lockyer, 1987). Obviously, results obtained in each set of the fumigation experiment, clearly shows the influence of NH_3 interaction on the plants across the NFA, NFA+, NFA++ treatments either in the first and second set of the fumigation study. However, when considering the free amino acid compositions and biomass accumulation in both sets of the fumigation experiments, the effects of soil nitrogen contents are seen implying that the plants were able to take up nitrogen from the soil as well as from the various concentrations of gaseous NH_3 they were exposed to in the fumigation chambers (Whitehead and Lockyer, 1987).

5.5. Potential for biomonitoring of NH_3 pollution in ambient air.

At the initial stage of this study, the need to look out for certain changes in the selected indicator plants either in plant growth or biochemical metabolites such as in above ground biomass and free amino acid accumulation, were considered as important parameters in determining the potentials of the selected plants as biomonitors of NH_3 pollution. Previous studies have reported the ability of certain plants to take up nitrogen under conditions of abundant nitrogen supply and incorporate this into biomass accumulation or the synthesis of metabolites such as free amino acids when protein is not being produced (Pitcairn *et al*., 2003). In order words, the acumulation of above ground biomass and free amino acids in the plants were considered as indicators of nitrogen accumulation arising sources of NH_3 pollution.

Results obtained from this study confirmed previous observations made in field and chamber studies on the possible uptake of NH_3 by plants (Pitcairn *et al*., 2003; van Hove *et al*., 1991; Whitehead and Lockyer, 1987). In the field studies, plant materials especially *Lolium multiflorum*, *Echinochloa crus-galli* and *Urtica dioica* exposed along various distances around the source of NH_3 emission showed a strong relationship in its above ground biomass with ambient NH_3 concentration at distances from the point source of NH_3 emission. This relationship demonstrates a positive effect of the ambient NH_3 concentration at each site on NH_3 uptake by the plant leaves (van Hove *et al*., 1991). On the other hand, this observation demonstrates the potential of these plants as biomonitors of NH_3 pollution, bearing in mind the responses of the above ground parameter of the plants to the ambient NH_3 concentration at each site. Nothwithstanding the few number of sites used for this study, the 804 m long transect investigated, was large enough to reflect the exponential decrease in ambient NH_3 concentration from a closer distance to the stable to the sites further away and its subsequent influence on the above ground biomass of plant materials mounted across the transect. In the case of *Lolium multiflorum* and *Echinochloa crus-galli* plants exposed to gaseous NH_3 in the fumigation experiment, slight increases in above ground biomass were observed with increasing NH_3 treatment in the first set of the experiment. However, in the second set *Lolium multiflorum* showed a decline in above ground biomass at the NFA+ and NFA ++ treatments compared to the NFA treatment, probably because the plants were shaded

by the *Echinochloa crus-galli* plants in the NFA+ and NFA++ treatments, which had a bigger biomass compared to those treated with NFA.

It has previously been reported that in addition to biomass accumulation, some of the NH_3 assimilated into the plants could also be metabolized into free amino acid compounds (Pearson and Stewart, 1993). Observations made in the field exposed plants as well as the chamber fumigated plants showed a positive influence of NH_3 availability on the free amino contents of the plants. This could be attributed to the trends observed in free amino acid compositions and accumulation of certain nitrogen compounds in plant materials exposed to increasing ambient NH_3 concentrations in the field and those fumigated with increasing gaseous NH_3 concentration in the chamber experiments (Huhn and Schulz, 1996; Näsholm *et al*., 1994).

Before now, previous studies have been conducted whereby responses of plants to nitrogen deposition based on parameters such biomass production and free amino acid compositions were investigated in NH_3 fumigation experiments, NH_4^+ misting and nitrogen addition experiments as well as in transect studies close to a source of NH_3 pollution. Early studies by van Dijk and Roelofs (1988) investigated the effects of NH_4^+ deposition on the needles of Scots pine (*Pinus sylvestris* L.) from 17 stands in the Netherlands. They observed significantly high amounts of Arg which dominated the amino acid pool in both young and old needles. In a nitrogen addition experiment, Näsholm *et al*. (1994) investigated the effects of the application of 500 kg N ha^{-1} on the accumulation of amino acids on four boreal forest plants namely, *Vaccinium myrtillus* L., *Vaccinium vitis-idaea*, *Deschampsia flexuosa* and *Epilobium angustifolium.* Significant increases in amino acids were observed in the plants as a result of increased nitrogen availability. They discovered variability with respect to amino acid dominance in the plants. For instance, in *Vaccinium myrtillus* L. and *Vaccinium vitis-idaea* Gln and Arg were most prominent, while in *Deschampsia flexuosa* it was Asn and Gln, and then in *Epilobium angustifolium* Gln and Arg were most dominant after fertilization. Similarly, in a passive monitoring study Pitcairn *et al*. (2003) investigated the influence of NH_3 pollution around a livestock farm on the free amino acid accumulation in *Rhytidiadelphus triquetrus*, *Pseudoscleropodium purum* and *Brachythecium rutabulum* along a transect of 276 m. They found a good relationship in the free amino acids in the mosses studied with NH_3 concentration and observed increases in amino acids

especially Arg with closer proximity to the stable. These observations are in agreement with the results obtained with higher plants, through active monitoring in this study in which increases in free amino acids were observed in plants located at distances very close to the stable with higher NH_3 concentrations.

It is quite interesting to note that despite the short duration period in which the plants were exposed in the field or fumigated in the chamber experiments they were able to respond to changes in NH_3 availability in their biomass production. However, this could be attributed to a higher uptake in nitrogen from NH_3 under conditions of a higher NH_3 concentration in the ambient air or during gaseous fumigation (Whitehead and Lockyer, 1987). This assumption is based on results obtained by (Whitehead and Lockyer, 1987) in which they observed a linear relationship between uptake of gaseous NH_3 nitrogen and concentration in their study on *Lolium multiflorum* exposed to nine different NH_3 concentrations. In the field studies across the selected distances of 67 m, 149 m and 804 m, NH_3 concentrations ranged between 1.1-1.5 µg m^{-3}, 8.3-12.4 µg m^{-3} and 15.3-32.6 µg m^{-3} respectively during the 4 weeks exposure period. Additionally, in the fumigation studies some of the gaseous NH_3 concentrations and the exposure period applied across the NFA, NFA+ and NFA+ treatments were quite lower compared to those reported in other studies (Perez-Soba *et al*., 1993; van der Eerden, 1982; Whitehead and Lockyer, 1987). Irrespective of these differences, results obtained from this study were consistent with results obtained in previous studies on the influence of gaseous NH_3 on increased biomass production and free amino acid accumulation in plants (Lockyer and Whitehead, 1986; Perez-Soba *et al*., 1993; Perez-Soba *et al*., 1994).

The potentials exhibited by the selected indicator plants in this study, is quite promising as possible biomonitors of nitrogen deposition. However, having subjected these plants to field studies and the two grasses to fumigation chamber studies here in Germany, they could also be used in the measuring nitrogen deposition in the North China Plain (NCP) which is one of the major objectives of the sub-project 1.3 within the framework of the International Research Training Group (IRTG). Previous studies using standardized physical methods in the measuring of Nitrogen pollution in the NCP have been carried out within the sub-project 1.3 of the IRTG (Liu *et al*., 2006; Shen *et al*., 2009; Shen *et al*., 2011). Shen *et al*., (2011) conducted a monitoring study of atmospheric NH_3 and particulate NH_4^+ using passive diffusion tube samplers and

particulate samplers, at six different locations within the NCP namely, Dongbeiwang, Shouguang, Shangzhuang, Quzhou, Huimin and Wuqiao. They reported an average annual NH_3 concentration of 15.6 µg m^{-3} and particulate NH_4^+ concentration of 12.4 µg m^{-3} respectively across the six sites with Shouguang accounting for the highest mean NH_3 concentration of 24.2 µg m^{-3}. The high concentrations in these sites were as a result of the intensive agricultural activity across the sites within the NCP coupled with the often large applications of nitrogen fertilizer (Shen *et al*., 2011). Hence the selected indicator plants could also serve as a biomonitoring tool in complementing physical methods used in the monitoring of nitrogen deposition in the NCP, having been exposed to mean ambient NH_3 concentrations below and above 15.6 µg m^{-3} in the field trial and also in the chamber experiments.

6. CONCLUSION

In order to complement well known and established techniques in the monitoring of atmospheric pollution through a biomonitoring method, it is therefore important to investigate different possible indicators in a bid to discover if any positive response or responses to such pollutant by the selected indicators are observed. In so doing the sensitivity of a selected bioindicator species is identified. This study demonstrates the potentials of the selected indicator plants as possible biomonitors of NH_3 pollution and nitrogen deposition not only on growth and biomass production but also on the synthesis of biochemical metabolites such as free amino acids. The ability of these plants to utilize a substantial part of the nitrogen obtained when exposed to conditions of high atmospheric NH_3 concentration for growth and also storage into organic nitrogen compounds such as free amino acids were demonstrated in this study (Lockyer and Whitehead, 1986; Pearson and Stewart, 1993; Perez-Soba *et al*., 1994; van der Eerden and Perez-Soba, 1992; Whitehead and Lockyer, 1987). NH_3 induced increases in biomass accumulation and alterations in free amino acid concentrations and compositions under conditions of increasing ambient NH_3 concentrations both in the field and fumigation study, and this clearly suggests an uptake of atmospheric NH_3. This observation have previously been reported in other studies where nitrogen deposition from various sources such as in field and fumigation experiments have also led to the alterations in free amino acid compositions and stimulated biomass production in the plants (Castro *et al*., 2006; Huhn and Schulz, 1996; Näsholm *et al*., 1994; Perez-Soba and van der Eerden, 1993; van Dijk and Roelofs, 1988; Whitehead and Lockyer, 1987). The overall significance of the responses demonstrated by the selected indicator plants in this study, is shown by the relationships exhibited between the the above ground biomass and free amino acid parameters of the various plants studied and NH_3 concentrations in the field and fumigation chambers. Besides exhibiting increases in free amino acid concentrations and compositions, it is of significance to note that NH_3 induced the accumulation of free amino acids mostly in low carbon to nitrogen compounds such as Arg, Gln, Asn, Ala and Glu in the selected plants studied. The selected indicator plants studied were able to withstand field exposures regardless of the differences in environmental conditions when compared to the controlled green house conditions where they were initially propagated and grown prior to being exposed in the field. In addition the plants were able to demonstrate

different sensitivities to NH_3 availability in both the field exposed and fumigated plants as evidenced from the alteration in free amino acid pool composition and concentrations of plants exposed at different sites along the 804 m transect and across the NFA, NFA+ and NFA++ treatments in a short period of 4 weeks. Likewise, it is interesting to note the sensitivity of these plants especially *Lolium multiflorum*, *Echinochloa crus-galli* and *Urtica dioica* in response to a small difference in ambient NH_3 availability especially between the distances of 67 m and 149 m in the field and in NFA and NFA+ treatments in the greenhouse chambers with respect to the two grasses. In view of the results obtained in this study, the plants showed prospects as potential biomonitors of nitrogen deposition but however, further studies need to be done in which the potentials demonstrated by these plants are further tested by subjecting them to a large scale monitoring study with higher NH_3 concentrations and nitrogen deposition rates.

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