ORGANIC NITROGEN UPTAKE: A NOVEL PATHWAY TO IMPROVE NITROGEN USE EFFICIENCY AND CROP PRODUCTIVITY

Kawsar Ali[1], Davey L Jones[2] and Muhammad Arif[1]

[1]Agriculture Department, Abdul Wali Khan University Mardan

[2]Environment Center Wales, Bangor University Wales, UK

Corresponding author Email: kawsar@aup.edu.pk

ABSTRACT

Formulation of amino acid N fertilizer and uptake of organic N by plants has the ability not only to ensure N availability to plants particularly in N-limiting environments but also can manipulate the environmental hazards associated with over inorganic N fertilization. To support this view, clear experimental evidence is still lacking. In addition, the current experiments aimed to evaluate the uptake of organic N (Amino acid based N fertilizer) by plants in comparison with inorganic fertilizer (ammonia and nitrate) and investigate the mineralization rate of amino acid fertilizers. Overall. commercial amino acid performed superior in terms of plant growth and mineralization over pure amino acid fertilizer and inorganic N fertilizer. All plants measured parameters (shoot and root biomass, leaf chlorophyll content and tissue N content) were highest in plant samples treated with commercial amino acid fertilizer as compared to pure amino acid and inorganic N fertilizer. It is therefore concluded that organic N may be of only limited consequence in high input agricultural systems.

INTRODUCTION

In cereal production systems, nitrogen (N) frequently represents the nutrient that most limits primary productivity(Li et al., 2008). If the rate of soil N supply is insufficient to meet crop demand, N deficiency occurs leading to reduced shoot growth, photosynthetic activity and low yields of poor quality (Jones, 1998). To circumvent this, farmers typically apply large amounts of N fertiliser (ca. 100 to 250 kg N ha^{-1}) to ensure it is always present in excess

of plant demand (Khan et al., 2008). As fertiliser is only applied once or twice a year there is no synchronisation between soil N availability and crop demand leading to low N use-efficiency (NUE) in cereals and large losses of N to the environment. This loss can occur in the form of NO_3^- to surface and ground water or via gaseous emissions from soil(e.g. NH_3,N_2O; Guillard et al., 1995; Gao et al., 2010). In response to this gross inefficiency in N use, there has been a shift towards the introduction of more sustainable agricultural practices which improve NUE in crops and which reduce the environmental impact of N losses (Stark and Richards, 2008; Arregui and Quemada, 2008).

Of the strategies proposed to improve NUE in cereal cropping systems, the most promising include the selection of plants with traits that (1) promote greater resource capture from soil (e.g. deeper or more branched root systems, alterations in N transporters expression, increased mycorrhizal colonization), (2) alter N use partitioning within the plant, and (3) the use of companion-planting with N-fixing plants (Clark and Myers, 1994; IITA, 1997; Aggarwale et al., 2002). However, it has been suggested that changes in soil fertiliser regime probably offer the greatest hope of reducing N losses and increasing NUE(reference). This may involve changes in fertiliser formulation, placement and timing as well as the co-application of urease or nitrification inhibitors to reduce the transformation of NH_4^+ to more mobile NO_3^-(Gioacchini et al.2002; Zaman et al.2008a; Soares et al. 2012). Consistent with both a soil- and plant-based approach to improving NUE is the potential for using alternative chemical forms of fertiliser N which are more plant available and less prone to loss in comparison to those conventionally employed (i.e. NO_3^-, NH_4^+, urea)

It is well established that plants can acquire a range of dissolved organic N (DON) forms directly from soil including protein, peptides, amino acids and nucleobases (Paungfoo-Lonhienne et al., 2008; Hill et al., 2011). Further, evidence suggests that plants may also be able to acquire particulate organic N(PON) via the direct ingestion of bacteria, although the ecological significance of this uptake pathway remains unclear (Raven et al., 2012; Hill et al., 2013). Work in some N-limiting ecosystems suggests that the uptake of DON could make a significant contribution to the plant's overall N balance (Xu et al., 2006; Henry and Jefferies, 2003), although good quantitative estimates of DON uptake are lacking due to difficulties in interpreting ^{15}N isotopic flux measurements (Jones et al., 2005; Warren, 2012). Despite this, it is clear that roots possess a range of constitutively expressed DON transporters that are expressed throughout the root system and which could be capitalised upon to supply plants with a range of DON-based fertilisers (Jones and Darrah, 1994). One potential problem with this N delivery strategy is that DON is frequently preferred over NO_3^- and NH_4^+ as a source

of N by the soil microbial community, implying that that significant competition for DON will occur between roots and microorganisms (Owen and Jones, 2001; Abaas et al., 2012). The microbial immobilization of applied DON compounds may temporarily reduce plant N availability and stimulate heterotrophic denitrification which may be viewed as negative attributes of the approach (Jones et al., 2013). Conversely, temporary immobilization may be seen as beneficial with DON immobilization-turnover acting as a slow release form of N which is less prone to leaching. Predicting the rate of return of NH_4^+ back to the soil, however, is hard to predict as its will be dependent upon a range of factors including the size, structure and activity of the soil microbial community, interactions of DON with the solid phase and the type, amount and C:N ratio of the applied DON substrate (Roberts et al., 2009).

Recent years has seen the release of a range of DON based fertilisers targeted towards the organic farming market, however, most of these are recommended for use as foliar N sprays and are typically applied at low dose rates (<5 kg N ha^{-1}). The aim of this study was therefore to investigate the behaviour of a commercial DON (amino acid) based fertiliser when applied directly to the soil and its potential to supply N to wheat. The experiments were undertaken in the context of cereals where NUE is typically low with direct comparison made to conventional inorganic based fertilisers (NH_4NO_3).

MATERIAL AND METHODS

Soil characteristics

Soil was collected from the top layer (0-10cm depth) of a temperate oceanic agricultural site located at the Henfaes Experimental Station,Abergwyngregyn, Gwynedd, UK (53°14'N, 4°01'W). The soil has a sandy clay loam texture and is classified as a Eutric Cambisol (FAO) or Dystric Eutrudepts (US Soil Taxonomy). The mean annual soil temperature at 10cm is 11°Cand the mean annual rainfall is 1250mm(Glanville et al., 2012). Immediately after collection, the soil was transferred to the laboratory in gas permeable bags where it was sieved to pass 2mm to remove stones, plant roots and earthworms.The basic properties of the soil are presented in Table 1.

Plant response to the supply of different N forms

To evaluate the effect of different organic and inorganic forms of N on the growth of wheat (*Triticum aestivum* L. cv. Granary). The experiment consisted of six different fertilizer treatments namely: (1) Control, no N addition; (2) KNO_3;(3) NH_4Cl;(4) NH_4NO_3; (5)a commercial amino acid-based fertilizer; and (6) an artificial mixture of pure amino acids. The

commercial amino acid fertilizer (Compo; Avant Nature,Co, Spain) consisted of essential plant macro and micro nutrients (Table 2). The pure mixture of amino acid fertilizer was made with an amino acid composition identical to that of the commercial product but with no contaminants present (i.e. amino acids >99% purity). Plastic pots (1000 cm^3) were filled with soil (800 g fresh weight) and each pot sown with three wheat seeds. The pots were then placed in a climate-controlled room at 20°C, 70% relative humidity and 16 h photoperiod (ca. 500 µmol photons $m^{-2}s^{-1}$ PAR). The soil in the pots was maintained at its initial moisture content by weighing the pots on a daily basis and replacing and water lost with distilled water.With the exception of the control treatment, N was applied at the rate of 100 kg $ha^{-1}$for all treatments at day 13th after sowing. At the same time P and K were applied as KH_2PO_4 to all treatments at a rate equivalent to 30 and 75 kg ha^{-1} respectively. There were 5 replicate pots for each treatment.

Leaf chlorophyll content was measured weekly using aSPAD chlorophyll meter (SPAD-502Plus;Konica Minolta, Chiyoda, Tokyo, Japan). After 6 weeks the shoots were harvested, oven dried (80°C, 48 h) and their dry weight recorded. The soil in the pots was divided with one half being used to estimate root biomass and the other half used for soil analysis. Roots were separated from the soil by wet sieving and their dry weight determined after oven drying (80°C, 24 h). The roots and shoots were then ground to a fine powder for subsequent C and N determination using a TruSpec® CN Analyzer (Leco Corp., St Joseph MI, USA). At the end of the experiment the amount of NH_4^+ and NO_3^-remaining in the soil was determined by extracting the soil with 0.5 M K_2SO_4(1:5 w/v; 250 rev min^{-1}, 1 h), centrifuging the extracts (18,000 *g*, 10 min) and the amount of NH_4^+ and NO_3^- determined by colorimetrically using the methods of Mulvaney (1996) and Miranda et al. (2001) respectively.

C and N mineralization of the amino acid fertilizers in soil

To determine the rate of breakdown of the amino acids contained in the commercial and pure amino acid fertilizers, 5g of soil was placed in a 50 cm^3 polypropylene centrifuge tube. Subsequently, 500 µl of different concentrations of ^{14}C-labelled fertilizer were added to the soil surface. Different concentrations of each fertilizer (equivalent to 50 to 0.39 mg N l^{-1}) were prepared in distilled water and labelled with a ^{14}C-[U]-labelled amino acid mixture (PerkinElmer, Waltham , Massachusetts 02451, USA) at a specific activity of **2.3 ± 0.02 kBq** ml^{-1}. After addition of the ^{14}C-labelled fertilizer to the soil, a 1 M NaOH trap (1 ml) was placed inside the tube to catch any $^{14}CO_2$ evolved and the tubes sealed. The NaOH trap was

replaced after 1, 3, 6 and 9 h and then daily for 14 d. the amount of $^{14}CO_2$ in the NaOH traps was determined by liquid scintillation counting in a Wallac 1404 scintillation counter (Wallac EG&G, Milton Keynes,UK) with Optiphase-3 alkali compatible scintillation fluid (Wallac EG&G).All treatments were undertaken in quadruplicate.

To determine the rate of appearance of NH_4^+ and NO_3^- in soil from the commercial and artificial amino acid fertilizers a similar experiment to that described above was undertaken except that only one amino acid concentration was studied (**50** mg N l^{-1}). At known times after amino acid addition (0, 1, 3, 7, 10, 14, 21, 28, 42 and 56 d) the soil was extracted with 0.5 M K_2SO_4(1:5 w/v; 250 rev min^{-1}, 1 h) and the amount of NH_4^+ and NO_3^- determined as described above. There were 4 replicates of each treatment with the addition of distilled water used as a negative control.

Soil microbial response to the addition of amino acid based fertilizers

The response of the soil microbial community to the addition of the commercial and artificial amino acid-based fertilizers was determined by measuring changes in basal soil respiration following the addition of the fertilizer to soil. Briefly, 50 g of soil was placed in a 50cm^3 polypropylene tube and different concentration of the amino acid fertilizers **(5 ml; 50 to 0 mg N l^{-1})**added to the soil. The amount of CO_2 released from the soil was then continuously measured over a 14 d period using an automated multi channel SR1 infrared gas analyser (PP Systems Ltd., Hitchin, UK). All treatments were undertaken in duplicate.

Effect of amino acid fertilizer on the priming of native soil organic matter

The effect of amino acid fertilization on the mineralization of native soil organic matter content was determined by adding the commercial and artificial fertilisers to soil in which the native soil organic matter was isotopically labelled. The same soil was labelled 4 years previously by the addition of ^{14}C-labelled glucose (1 MBq m^{-2}) as described in Rousk et al. (2014). Briefly, the ^{14}C-labelled soilwas collected from the field and 10 mm sieved to minimise disruption to the microbial community (Jones and Willett, 2006). Replicate portions of the soil (120 g) were then placed in glass jars. Solutions (5 ml, 50 mg N l^{-1}) containing either the commercial or artificial amino acid fertiliser, NH_4Cl, KNO_3 or NH_4NO_3 were added to the soil.The amount of$^{14}CO_2$evolved from the soil was captured in a 1M NaOH trap (4 ml) placed inside the jar and the trap replaced twice per week. The amount of 14C in the NaOH traps was determined as described above.

Statistical analysis

After the harvesting of wheat crop and amino acid mineralisation the data collected were statistically analysed using the procedure appropriate for CR design suing Excel software. Standard error mean were calculated and Sigma Plot (12.5) were used for creating graphs for comparing mean (Jan et al., 2009). For measuring soil respiration repeated measure 2 way ANOVA was used. The SPSS v 20.0 (SPSS Inc., Chicago, IL) was used for calculating repeated measure ANOVA.

Table 1. Physio-chemical properties of soil used in these experiments are given below. Values represent means ± Standard error mean (n = 3)

Soil character	Measured quantity
Water content (%)	16.48 ± 0.29
pH (1:2 H_2O)	5.81 ± 0.01
EC (1:2 H_2O μScm^{-1})	48.93 ± 0.53
Available NO_3^- (mg N l^{-1} soil solution)	4.51 ± 0.07
Available NH_4^+ (mg N l^{-1} soil solution)	0.1 ± 0.01
Free amino acids (mg C l^{-1} soil solution)	1.14 ± 0.43

Table 2. Chemical characteristics of commercial and pure amino acid fertilizer used in experiments.

Parameters	Pure amino acid fertilizer	Commercial amino acid fertilizer
Total N (%)	22.16	27.73
Total C (%)	1.183	1.234
Ca (mg l^{-1})	28.2	626.7
K (mg l^{-1})	15.0	187.4
P (mg l^{-1})	1.5	80.7
Na (mg l^{-1})	21.8	10174
Mg (mg l^{-1})	1.1	353.5

Zn (mg l^{-1})	0.3	1.7
Cu (mg l^{-1})	0.4	0.6
Fe (mg l^{-1})	2.0	145.4
Al (mg l^{-1})	1.0	1.3
NH_4(µ g^{-1})	4.79	0.58
NO_3(µ g^{-1})	26	40
EC (mS)	4.98	28.2
pH	4.62	5.67

RESULTS

Plant growth response to the addition of amino acid and inorganic N based fertilizers

Wheat above-ground biomass was significantly affected by the application of organic and inorganic N sources in comparison to the control treatment which received no extra N (P<0.001; Fig. 1). Among the different N treatments, the greatest shoot biomass was produced in the plants treated with the commercial amino acid fertilizers followed by those amended with $NO_3^-$and NH_4NO_3. Application of the pure amino acid fertilizers resulted in a reduced shoot biomass in comparison to its commercial counterpart (P<0.001). The worst overall performance in the N fertiliser treatments was in the plants supplied with NH_4^+. A similar response to that observed in the shoots was also found for below-ground (root)biomass (Fig. 1). However, the commercial amino acid fertilizer appeared to promote root proliferation in comparison to the other N treatments, although no such response was observed for the pure amino acid fertilizer.

Foliar chlorophyll and N response to the addition of amino acid and inorganic N based fertilizers

As expected, the addition of N fertiliser significantly increased leaf chlorophyll content (P< 0.001). Leaf chlorophyll content was also significantly affected by the form of N fertiliser supplied to the wheat plants over the 6 week monitoring period (P< 0.001; Fig. 2). At all measurement times, leaf chlorophyll content was significantly greater in the commercial amino acid fertilizer treatment in comparison to the NO_3^-, NH_4NO_3 and the pure amino acid fertiliser which were similar. The lowest chlorophyll content of the N addition treatments was seen in the plants supplied with NH_4^+.

Influence of inorganic N fertilisers and two amino acid-based fertilisers on above-ground N content and C-to-N ratio in *Triticumaestivum*.

The effect of inorganic N fertiliser and two amino acid-based fertiliser on above ground N content and C-to-N ratio in wheat (*Triticum Aestivum*) are shown in Table 3. As expected all treatments (NH_4, NO_3, commercial and pure amino acid based fertilizer caused significant variation in wheat above ground N content and C-to-N ratio as compared to control ($P<0.001$). Higher above ground N content was observed in wheat sample treated with NH_4^+ as source of N fertiliser followed by commercial N fertiliser. There was no significant difference between NH_4NO_3 and pure amino acid fertiliser, however both of these treatments resulted in lower wheat tissue N content than commercial amino acid fertiliser. Although NO_3 as source of N fertiliser resulted in higher N content than control (no N addition), however; the performance of NO_3 as source of N fertiliser was worsted as compared to other treatments. Likewise, all treatments significantly affected wheat C-to-N ratio ($P<0.001$). Overall the C-to N ratio was highest in control treatment than fertilised plant. Among the treatments, the lower C-to-N ratio was recorded in wheat samples treated with NH_4+ as N fertiliser source followed by commercial fertilizer. The performance of pure amino acid fertilizer was at par with NH_4NO_3 while among the treatments, NO_3 resulted in higher C-to-N ratio however, it was lower than control.

Mineralization of the fertiliser-based amino acids in soil

The mineralization of the ^{14}C-labelled amino acids contained within the pure and commercial amino acid fertilisers is shown in Figure 3. Overall, the mineralization of the amino acids was faster in the pure fertilizer in comparison to the commercial fertilizer ($P<$ 0.001). When the initial mineralization rate measured at 1 h was expressed as a rate (mg kg^{-1} h^{-1}), mineralization was positively correlated with concentration for both fertilizer types and aMichaelis-Menten equation conformed well to the experimental data ($r^2>$ 0.99 for both; on-line Supplementary Fig. S1). At the low amino acid concentrations (<25%), mineralization was characterised by an initial rapid phase of $^{14}CO_2$ loss which lasted ca. 24 h after which a slowed secondary phase was observed. At the higher amino acid concentrations (>25%) mineralization showed a temporary lag phase after ca. 24 h, however, subsequently mineralization rate increased again. By the end of the experiment, proportionally more of the amino acids had been respired at the higher addition rates (>12.5%) in comparison to the lower addition rates.

N mineralization after the addition of amino acid fertilizers to soil

The production of NH_4^+ and NO_3^-in soil after the addition of either a commercial or pure amino acid fertiliser is shown in Figure 5. At all measurement times in the unamended (control) soil the concentration of NH_4^+ remained very low and relatively constant (0.4 ± 0.1 mg N kg^{-1}). In comparison, the concentration of NO_3^-in the control treatment progressively increased over the 56 d incubation period. Upon the addition of the amino acid fertilisers to soil an initial increase in NH_4^+ was seen, particularly in the commercial amino acid treatment. After incubation for 3 d, however, NH_4^+had dropped to background levels in both amino acid treatments while by d 28, no significant treatment differences were apparent (P> 0.05). In contrast to NH_4^-, the concentration of NO_3^- in soil progressively increased in all treatments over the 56 d incubation. In both amino acid fertiliser treatments this increase was non-linear and was characterised by an initial rapid rise (0-3 d), a stabilisation phase (3-12 d) and then a progressive increase until day 56. Although the pattern was the same for both amino acids significantly more NO_3^- was produced in the commercial amino acid treatment.

Response of the soil microbial community to the addition of amino acid fertilizers

Treatment of the soil with the both amino acid fertilizers caused a rapid increase in soil respiration relative to the unamended control treatment. Overall, the respiration response was more rapid and initially greater in the pure fertiliser in comparison to the commercial formulation (P< 0.001). In the commercial fertiliser treatments, a significant lag phase in respiration occurred, especially at the high addition rates, however, the duration of the respiration response was longer than in the pure formulation. With the exception of the highest addition rate treatment which appeared incomplete after 14 d, the amount of the CO_2 produced over the 14 d period was extremely similar in both fertiliser types (P> 0.05). In addition, the total amount of respiration was linearly correlated with amino acid dose in both the pure formulation (r^2 = 0.98, P< 0.001) and the commercial formulation (r^2 = 0.90, P< 0.01) across all concentrations.

DISCUSSION

The need to augment fertilizer use efficiency and to discover the most stable form of fertilizers is clearly evident. The use of inorganic fertilizer not only bring surge in the production cost but may also prove to be instrumental in creating a large number of environmental problems alongside. It is likely that climate change and ecosystem degradation inflict new restrictions, accordingly sustainable agriculture and organic sources of nutrients has an essential function to perform in conserving natural resources. To eliminate such like

problems, the use of organic sources of inputs is considered to be the only viable solution for not only increasing crop production and yield but also play an important role in ameliorating soil fertility and productivity. Consequently, this study was designed and executed to find out the capacity of easily available input (Mineral N fertilizer) and amino acid based commercial and pure fertilizers. Young and Aldag (1982) examined and judged that 30- 40% N is retrieved in amino acid form on account of complete hydrolysis of soil organic matter, and accordingly, they established that soil contains more amino acid- N reserves than NH_4 and NO_3 –N. Though, the absorption of amino acid and microbial integration is much smaller than its existence. However, only a small quantity of amino-N low molecular weight (MW < 200) is available form which is suitable for plant and microbe assimilation. Characteristically amino acids content in the soil solution can vary from as low as 1 mM to a number of mM habituated to soil types and proximity to the rhizosphere. Plant litter is the major form of organic-N inputs in many soils (Stevenson, 1982) of which root turnover may characterize one of the greatest inputs in the form of free amino acids and polymeric amino acids (i.e. proteins and polypeptides). Fundamentally, complete root cells have a free amino acid content of 110 mM displays that upon epidermal cell lysis a huge quantity of amino acids will be taken into the rhizosphere (11±5 mM).

Putrefaction of amino acid is chiefly determined by soil conditions (moisture, temperature and microbial population) though; the decomposition process was very swift in comparison to other more complex plant constituents (Rutigliano et al., 1996). It highlights the point that no extra cellular enzymes are needed previous to transfer into the microbial cells and the fact that majority of amino acids are feebly absorbed to the soil's solid phase (Anraku, 1980; Jones et al., 1994). It is also observed that soil microbial biomass quickened mineralization and enhanced absorption of free amino acid (Jones, 1999; Jones and Hodge, 1999). Both soil microorganisms and plant roots have even potential to absorb free amino acids from the soil solution, though plant roots are minor challengers for amino acid in comparison to soil micro-organisms. As micro-organisms due to its full treatment in soil populated places in the ectorhizosphere which resourcefully take out amino acids, and therefore, are able to move towards flashpoints of substrate. The maximum comparative diffusion of N solutes in the rhizosphere may be another vital characteristic for greater plant microbial competition. From a theoretical perspective, dissemination rate of all N solutes in water (NO^-_3, NH^+_4 and amino acids) is virtually the same. Nevertheless, N form such as NH_4

and amino acids (e.g. lysine, arginine) are absorbed to the solid phase of soil, thus appreciably decreased the diffusion rate (Jones and Hodge, 1999).

The advantage of amino acids to the N pool for crop production can be brought about in comparison to other forms of N, and yet its content in the soil solution is characteristically in the 1±100mM range (Stevenson, 1982; Monreal and McGill, 1985). In a survey of Reisenauer (1964), about 879 soil solution samples were examined and deduced that 95% have a NO_3 concentration more than 1 mM with distinctive concentrations varied from 2 to 5 mM, while Barber (1995) testified that soil NH_4 solution may range from 20 to 200mM. In addition, the lowermost sugar and free amino acids substance stand for less than 5% of the total DOC. The reason of the smallest substances of sugar and amino acid was the result of their quick elimination from the soil by the microbes. Thus, this study is in full agreement with previous studies proposing that the blockage in soil C and N cycling is the result of extracellular collapse of macromolecules rather than the application of their LMW breakdown products (Jones et al., 2004). Plant roots have the capacity to soak up LMW DOC from soil through different means stated as active proton co-transport systems (Jones et al., 2005a). These systems are not only applied to retrieve C and N to soil through root secretions, but are also important for direct immersion of organic N from the soil (Jones et al., 2005a). This has been postulated to make available plants with a procedure to short circuit the N cycle as it invalidates the requirement for organic N to be especially microbially pulverized to NH_4+and NO_3 before root absorption.

Table 3. Influence of inorganic N fertilisers and two amino acid-based fertilisers on above-ground N content and C-to-N ratio in *Triticumaestivum*. Values represent means ± SEM (n = 5). Different superscript letters represent significant differences at the $P< 0.05$ level.

	Shoot N content (%)	Shoot C-to-N ratio
Control (no N addition)	0.80 ±0.03^{a}	53.3 ±1.7^{a}
NH_4^+	1.59 ±0.07^{c}	26.5 ±1.3^{c}
NH_4NO_3	1.38 ±0.08^{ab}	31.2 ±1.7^{bc}
NO_3^-	1.26 ±0.05^{b}	34.1 ±1.4^{b}
Commercial amino acid fertiliser	1.42 ±0.12^{ab}	30.8 ±2.6^{bc}
Pure amino acid fertiliser	1.32 ±0.05^{ab}	32.4 ±1.4^{bc}
ANOVA result	$P<0.001$	$P< 0.001$

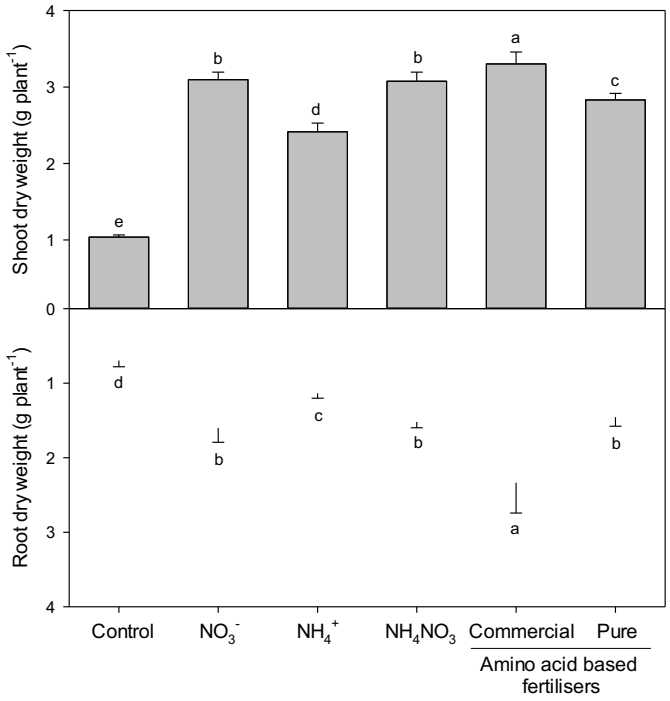

Figure 1. Wheat root and shoot biomass in response to the application of three conventional inorganic N fertilisers and two amino acid based N fertilisers. No N was added in the control treatment. Values represent means ± SEM (n = 4). Different letters represent significant differences between treatments at the $P < 0.05$ level.

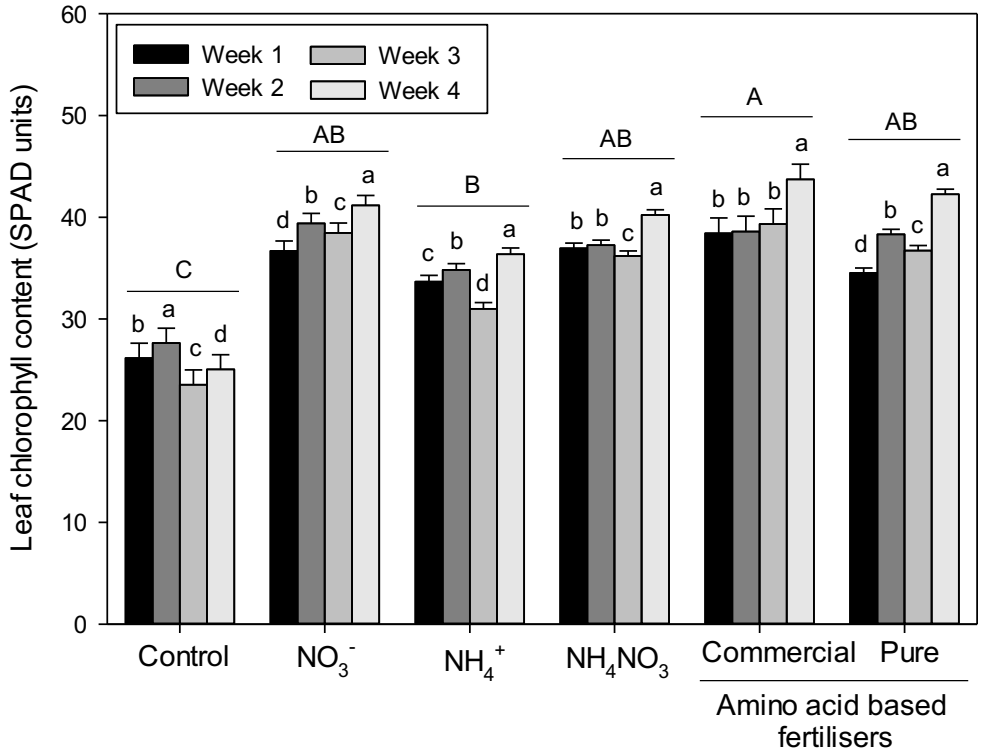

Figure 2. Changes over time in wheat leaf chlorophyll content in response to the application of three conventional inorganic N fertilisers and two amino acid based N fertilisers. No N was added in the control treatment. Values represent means ± SEM (n = 4). Different capital letters above the represent significant differences between treatments at the $P < 0.05$ level, while lowercase letters represent differences within an individual treatment at the $P < 0.05$ level.

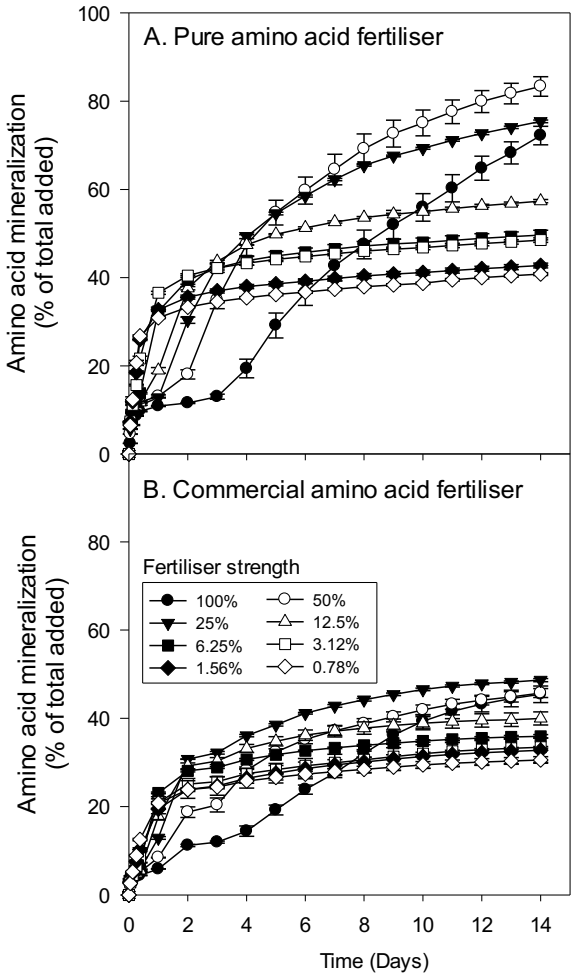

Figure 3. Time-dependent mineralization of ^{14}C-labelled amino acids contained within different strengths of either a pure (Panel A) or commercial (Panel B) amino acid based fertiliser when added to an agricultural soil. Values represent means ± SEM (n = 3).

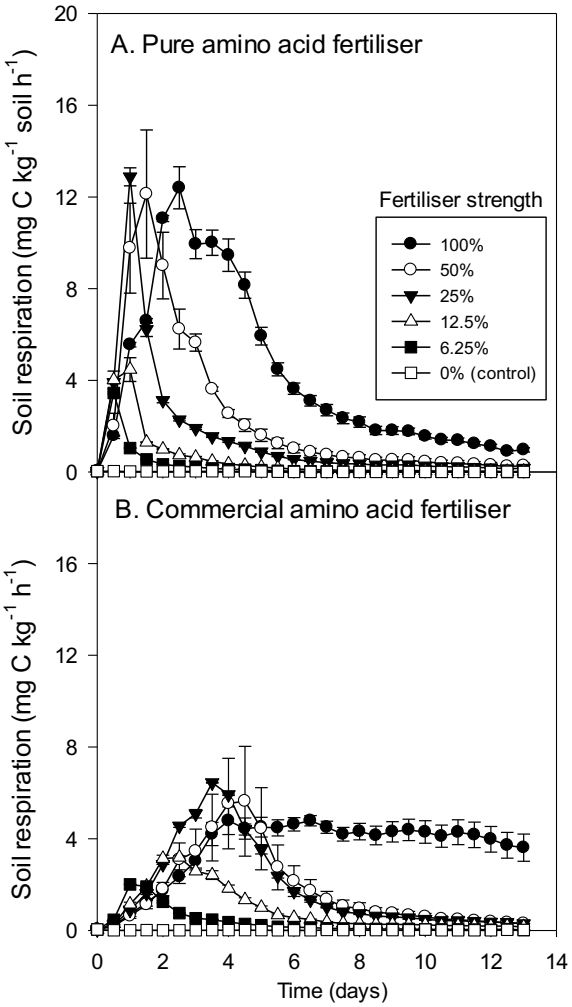

Figure 4. Temporal dynamics of CO_2 production after the addition of different strengths of either a pure (Panel A) or commercial (Panel B) amino acid based fertiliser to an agricultural soil. Values represent means ± SEM (n = 3).

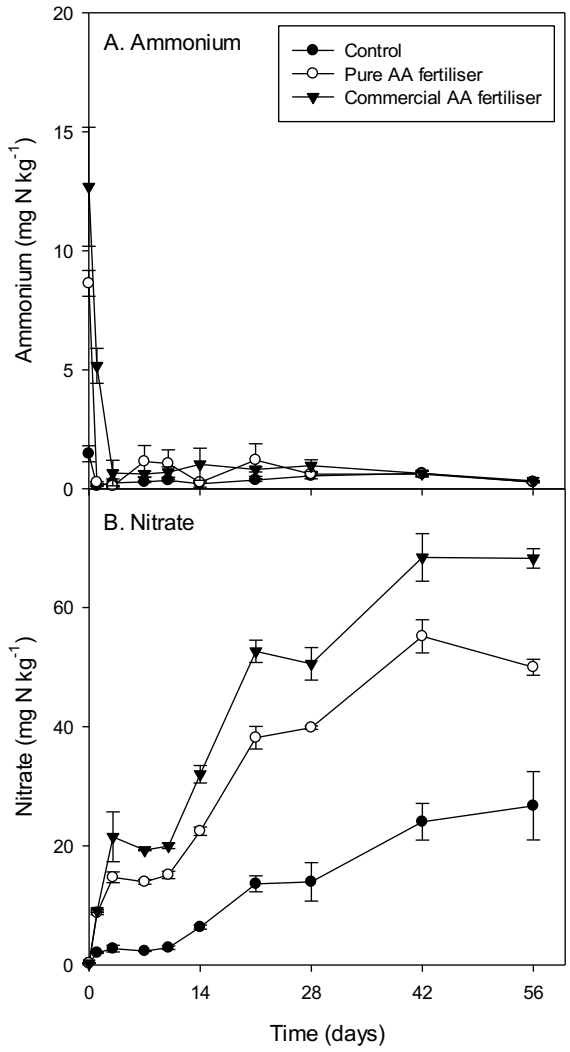

Figure 5. Temporal dynamics of NH_4^+ and NO_3^- production after the addition of either a pure or commercial amino acid based fertiliser to an agricultural soil. The control treatment only received distilled water in place of the amino acid fertiliser. Values represent means ± SEM (n = 3).

LITERATURE CITED

Abaas, E., P. W. Hill, P. Roberts, D. V. Murphy, D. L. Jones. 2012. Microbial activity differentially regulates the vertical mobility of nitrogen compounds in soil. Soil Biol. & Biochem. 53:120–123.

Aggarwal, P. K., O. P. Qarrity, S. P. Liboon and R. A. Morris. 2002. Resource use and plant interaction in a rice-mugbean intercrop. Agron. J. 84: 71-78.

Anraku, Y. 1980. Transport and utilization of amino acids by bacteria. In: Payne, J.W. (Ed.), Microorganisms and Nitrogen Sources. John Wiley, London, pp. 9-33.

Arregui, L. M. and M. Quemada. 2008. Strategies to Improve Nitrogen Use Efficiency in Winter Cereal Crops under Rainfed Conditions. Agron. J. 100: 277–284.

Barber, S.A. 1995. Soil Nutrient Bioavailability: A Mechanistic Approach. John Wiley, New York.

Clark, K.M., and R. I. Myers. 1994. Intercrop performance of Pearl, Millet, Amaranth, Cowpea, Soybean and quay response to planting pattern and Nitrogen fertilizer. Agron. J. 86: 1097-1102.

Gao, H. F. Schreiber, G. Collins, M. M. Jensen, J. E. Kostka, G. Lavik, D. de Beer, H. Zhou and M. M. M. Kuypers.2010.Aerobic denitrification in permeable Wadden Sea sediments. The ISME Journal. 4: 417–426.

Gioacchini, P., A. Nastri and C. Marzodori. 2002: Influence of urease and nitrification inhibitors on N losses from soil fertilized with urea. Biol. Fertil. Soils. 36: 125–131.

Guillard, K., G. F. Griffin, D. W. Allinson, M. M. Rafey, W. R. Yamartino and S. W. Pietrzyk. 1995. Nitrogen utilization of selected cropping systems in the U.S. Northeast. I. Dry matter yield, N uptake, apparent N recovery, and N use efficiency. Agron. J. 87: 193–199.

Henry, H. A. L., R. L. Jefferies. 2003. Interactions in the uptake of amino acids, ammonium and nitrate ions in the Arctic salt-marsh grass, *Puccinellia phryganodes*. Plant, Cell & Environ. 26(3): 419-428.

Hill, P.W., J. Farrar, P. Roberts, M. Farrell,H. Grant, K. K. Newsham, D. W. Hopkins, R. D. Bardgett and D. L. Jones. 2011. Vascular plant success in a warming Antarcticmay be due to efficient nitrogen acquisition. Nature Climate Change. 1: 50-53.

Hill, P.W., K.A. Marsden and D.L. Jones. 2013. How significant to plant N nutrition is the direct consumption of soil microbes by roots? New Phytol.199: 948-955.

International Institute of Tropical Agriculture (IITA) Annual Report (1997). Restoring nutrients to sub-Saharan soils.

Jones, D. L. and P. R. Darrah. 1994. Amino-acid influx at the soil-root interface of *Zea mays* L. and its implications in the rhizosphere. Plant Soil. 163:1–12.

Jones, D.L., A. C. Edwards, K. Donachie and P. R. Darrah. 1994. Role of proteinaceous amino acids released in root exudates in nutrient acquisition from the rhizosphere. Plant and Soil. 158: 183-192.

Jones, D. L. 1998. Organic acids in the rhizosphere - a critical review. Plant Soil 205:25-44

Jones, D.L. 1999. Amino acid biodegradation and its potential effects on organic nitrogen capture by plants. Soil Biol. & Biochem. 31: 613-622.

Jones, D. L. and A. Hodge. 1999. Biodegradation kinetics and sorption reactions of three differently charged amino acids in soil and their effects on plant organic nitrogen availability. Soil Biol. & Biochem. 31, 1331-1342.

Jones, D. L., A. Hodge. and Y. Kuzakov. 2004. Plant and mycorrhizal regulation of rhizodeposition. New Phytol. 163: 459-480.

Jones, D. L., J. R. Healey, V. B. Willet, J. F. Farrar and A. Hodge. 2005a. Dissolved organic nitrogen uptake by plants− an important N uptake pathway. Soil Biol. & Biochem. 37:413-423.

Jones, D. L., J. R. Healey, V. B. Willett, J. F. Farrar, and A. Hodge. 2005. Dissolved organic nitrogen uptake by plants - an important N uptake pathway. Soil Biol. Biochem. 37. 413–423.

Jones, D. L., P. L. Clode, M. R. Kilburn, E. A. Stockdale, and D. V. Murphy. 2013. Competition between plant and bacterial cells at the microscale regulates the dynamics of nitrogen acquisition in wheat (*Triticum aestivum*). New Phytol. 200(3): 796–807.

Khan, F.S., Z.I. Ahmed, M. Ansar H. Shah. 2008. Response of mungbean genotypes to Rhizobium inoculum and varying levels of nitrogen fertilizer. Pak. J. Agric. Res., 21(1-4): 33-44.

Li, Y., M. Horsman, B. Wang, N. Wu and C. Q. Lan. 2008. Effects of nitrogen sources on cell growth and lipid accumulation of green alga *Neochloris oleoabundans*. App. Microbiol. & biotech. 81(4): 629-636.

Monreal, C.M. and W. B. McGill. 1985. Centrifugal extraction and determination of free amino acids in soil solutions by TLC using tritiated 1-fluoro-2,4-dinitrobenzene. Soil Biol. & Biochem. 17: 533-539.

Owen, A. G., Jones, D. L., 2001. Competition for amino acids between wheat roots and rhizosphere microorganisms and the role of amino acids in plant N acquisition. Soil Biol. & Biochem. 33: 651–657.

Paungfoo-Lonhienne, C. T. G. A. Lonhienne, D. Rentsch, N. Robinson, M. Christie, R. I. Webb, H. K. Gamage, B. J. Carroll, P. M. Schenk, and S. Schmidt. 2008.Plants can use protein as a nitrogen source without assistance from other organisms. PNAS. 105(11): 4524–4529.

Reisenauer, H. M. 1964. Mineral nutrients in soil solution. In: Altman, P.L., Dittmer, D.S. (Eds.). Environmental Biology. Federation of the American Society for Experimental Biology, Bethesda, MD, pp. 507-508.

Roberts, P., R. Stockdale, M. Khalid, Z. Iqbal and D. L. Jones. 2009. Carbon-to-nitrogen ratio is a poor predictor of low molecular weight organic nitrogen mineralization in soil. Soil Biol. & Biochem. 41:1750–1752.

Rutigliano, F.A., A. V. Desanto, B. Berg, A. Alfani and A. Fioretto. 1996. Lignin decomposition in decaying leaves of *Fagus sylvatica* L. and needles of Abies alba Mill. Soil Biology & Biochem. 28: 101-106.

Soares, J. R., H. Cantarella and M. L. C. Menegale. 2012: Ammonia volatilization losses of surface–applied urea with urease and nitrification inhibitors. Soil Biol. Biochem. 52: 82-89.

Stark, C. H. and K. G. Richards. 2008. The continuing challenge of agricultural Nitrogen loss to environment in the context of global change and advancing research. Dynamic soil, dynamic plant. 2(1): 1-12.

Stevenson, F. J. 1982. Organic forms of soil nitrogen. In: Nitrogen in Agricultural Soils. American Society of Agronomy, Madison.

Warren, C. R. 2012. Post-uptake metabolism affects quantification of amino acid uptake. New Phytol. 193:522–531.

Xu, X., H. Ouyang, Y. Kuzyakov, A. Richter, and W. Wanek. 2006. Significance of organic nitrogen acquisition for dominant plant species in an alpine meadow on the Tibet plateau, China. Plant Soil. 285: 221-231.

Young, J. L. and R. W. Aldag. 1982. Inorganic forms of nitrogen in soil. In: Nitrogen in Agricultural Soils. American Society of Agronomy, Madison.

Zaman, M., M. L. Nguyen, J. D. Blennerhassett and B. F. Quin. 2008a: Reducing NH_3,N_2O and NO_3 N losses from a pasture soil with urease or nitrification inhibitors and elemental S-amended nitrogenous fertilizers. Biol. Fertil. Soils. 44: 693-705.