Hotspots and hot moments of amino acid N in soil: Real-time insights using continuous microdialysis sampling

Hill, Elliot; Jones, Davey L.; Paterson, E.; Hill, Paul

Soil Biology and Biochemistry

DOI:
10.1016/j.soilbio.2018.12.026

Published: 01/04/2019

Peer reviewed version

Hotspots and hot moments of amino acid N in soil: real-time insights using continuous microdialysis sampling

Elliot J. Hill[a,*], Davey L. Jones[a,b], Eric Paterson[c], Paul W. Hill[a]

[a]*School of Natural Sciences, Bangor University, Gwynedd, LL57 2UW, UK*

[b]*UWA School of Agriculture and Environment, University of Western Australia, Crawley, WA 6009, Australia*

[c]*The James Hutton Institute, Craigiebuckler, Aberdeen, AB15 8QH, UK*

Corresponding author: Elliot J. Hill*

Corresponding author address: School of Natural Sciences, Bangor University, Bangor, Gwynedd, LL57 2UW, UK

Corresponding author Tel: +447980398914

Corresponding author E-mail: elliot.hill@bangor.ac.uk

Declarations of Conflict of Interest: None

Article type: Short Communication

ABSTRACT Protein hotspots in soil, such as those associated with decaying soil fauna or plant litter, may produce ephemeral patches of disproportionately high soil nutrients. These hotspots may occur at the macro and microscale in close proximity to plant roots, however, the likely concentration of soluble products produced in these hotspots remains poorly understood. To address this, we buried two contrasting biomass residues in soil, namely earthworm (*Lumbricus terrestris*) and clover (*Trifolium repens*). Their transformation to amino acids, NH_4^+ and NO_3^- was monitored continually over 6 days using microdialysis. All treatments showed greater soluble nitrogen (N) concentrations compared to the unamended controls. The highest concentrations of both amino acids (12.9 mM after 12 h) and NH_4^+ (45.3 mM after 6 h) were generated in the vicinity of decomposing earthworm. In comparison, dried clover residues yielded 2.7 mM of amino acids at 6 h. After 12 h, amino acid and NH_4^+ concentrations in both earthworm and dried clover treatments showed a steep decline, returning close to background levels (<20 µM). Through the use of microdialysis we are able to show that soil nutrient hotspots may provide nearby roots with concentrations of amino acids and NH_4^+ several orders of magnitude higher than found in the bulk soil solution.

Keywords: Dissolved organic nitrogen; DON; Mineralization; Plant-microbial competition; Proteases

Amino acids (AAs) and oligopeptides are the most abundant and first quantitatively-significant protein breakdown products to be directly available as nitrogen (N) sources for plants and soil microbes (Sauheitl et al., 2009; Farrell et al., 2011; Warren, 2013; Moran-Zuloaga et al., 2015). Consequently, transformation of proteins to AAs is a major factor limiting N availability in soil and competition between plant roots and soil microbes for AAs is fierce (Jones and Kielland, 2002; Bardgett et al., 2003; Jan et al., 2009; Hill et al., 2011; Hill and Jones, 2018). The concentration of AAs in soil appears to be a key determinant in

the outcome of competition, with higher concentrations probably favouring plant AA-N capture (Jones et al., 2005). Therefore establishing true concentrations of AAs in soil is crucial to our understanding of plant N acquisition, and N cycling in soil. Typically, total free AA concentrations in contrasting ecosystems have been reported to remain fairly constant at 23 ± 5 µM, with the concentration of individual AAs typically ranging from 0.1-5.0 µM (Jones et al., 2009). These measurements in the bulk soil reflect the balance between slow rates of AA production and, rapid microbial AA consumption. However, biogeochemical 'hotspots', ephemeral patches which yield disproportionately high nutrient levels relative to the surrounding soil matrix, may supply high quantities of AAs to nearby roots (McClain et al., 2003; Schimel and Bennett, 2004; Jones et al., 2005; Kuzyakov and Blagodatskaya, 2015).

Using plant and soil fauna residues and microdialysis, we measured concentrations of protein breakdown products which may realistically occur close to roots. Microdialysis is a membrane-based sampling technique which offers non-invasive measurement of the soil solution phase, allowing probes to be positioned in close spatial proximity to samples and therefore yielding a high spatial resolution. We hypothesised that AAs generated from soil hotspots of protein breakdown transiently occur at concentrations greatly exceeding those found in measurements of bulk soil solution.

Microdialysis probes were single use, 100 kDa cut-off, 10 mm membrane length, 0.5 mm membrane diameter, 2.6 µL membrane internal volume and inlet internal volume of 1.4 µL (CMA Microdialysis, Torshamnsgatan, Sweden). Probes with a 100 kDa membrane pore size have been shown by other researchers to recover a great fraction of amino acids, compared to 20 kDa probes (Buckley et al., 2017). Probes were calibrated for relative recovery using amino acid (L-alanine), NH_4Cl and KNO_3 standard solutions of 0.1, 0.25, 0.5, 0.75, 1 and 25 mM (Inselsbacher et al., 2011; Lange, 2012). Probes (n = 8) were positioned

in standard solutions at room temperature, and perfused with ultra-pure deionised-water (5 µL min^{-1}). Aliquots of dialysate were collected at 30 min intervals during a 120 min perfusion period. Total amino acids in dialysate were measured fluorometrically according to Jones et al. (2002) while NH_4^+ and NO_3^- were determined colorimetrically according to Mulvaney (1996) and Miranda et al. (2001), respectively. Relative recovery was found to be 14.5 ± 5.5% of the supplied standard solution, irrespective of N-form, standard solution concentration or time. The relative recovery was later used to estimate actual concentrations of target analytes in the soil; although we cannot completely exclude some difference between standard solution calibration and measurements in soil solution. Collection vials were weighed before and after perfusion to ensure that there was no net loss or gain of water due to transmembrane flux or leakage.

Soil (0-15 cm) was collected from the Ah horizon of an agricultural grassland at Henfaes Agricultural Research Station, N. Wales, UK (53°23´N, 4°01´W, 19 m.a.s.l). The sandy clay loam textured soil is classified as a Eutric Cambisol (FAO), derived from post-glacial alluvial deposits. The main properties of the soil and added protein sources are shown in Table S1.

Soil was sieved to 2 mm, homogenised and added to 1.5 ml microcentrifuge tubes, each tube receiving *ca.*1.5 g of field-moist soil. Three protein sources were added to the soil in a 1:10 w/w protein source to soil ratio as follows: a) fresh foliage of *Trifolium repens* L. (clover), b) dried foliage of *T. repens*, and c) fresh necromass of the earthworm, *Lumbricus terrestris* L., with soil controls receiving no protein addition. All treatments were replicated three times. These protein sources, added by weight, represent different levels of N input, however our hypothesis is predicated on a greater understanding of realistically occurring hotspots in the soil, rather than a direct comparison between treatments. Protein sources were added to the centre of each tube. Each replicate was sealed using gas-permeable film

(Parafilm M®, Bemis Inc., USA) to restrict water loss by evaporation. Probes were positioned to a depth of 10 mm in the centre of the tube, using a syringe introducer and perfused with ultra-pure deionised water, at a continuous rate of 5 µL min^{-1}, using a multi-channel syringe pump (NE-1200 Multi-Phaser, New Era Pump Systems Inc., NY, USA) over a sampling period of 144 h. Samples were taken at hourly intervals for the first 6 h, then every 6 h thereafter (samples at 30 h were contaminated during storage and therefore omitted from analysis). Samples were analysed for amino acids and inorganic N as described above and for pH and electrical conductivity (EC) with standard microelectrodes. Outliers were identified using Grubbs' test (9 of 1008 measurements were removed). Data were analysed using a mixed two-way ANOVA with a Tukey HSD post-hoc test (SPSS v22; IBM, New York, USA), with a $p \leq 0.05$ cut-off for statistical significance.

Amino acid concentrations in controls did not exceed a mean concentration of 0.018 mM at any point during the experiment. Initially, levels of AA's were significantly higher in all treatments within the first 4 h following protein addition (Fig. 1). We attribute this to immediate loss of soluble components e.g. from damaged cells. All treatments had greater ($p \leq 0.05$) AA and NH_4^+ concentrations than controls during the majority of sampling intervals. Amino acids in dried clover and earthworm treatments showed a sharp rise in concentration after ~4 h, with those in dried clover rising to 2.7 mM after 6 h and to 12.9 mM in the earthworm treatment after 12 h, before sharply decreasing and levelling off after ~72 h. In contrast, fresh clover showed initially low AA concentrations (lowest mean concentration of 0.006 mM at 12 h), with only slightly higher concentrations than the control at some time points until ~54 h, thereafter reaching a peak of 0.099 mM at ~72 h, which was conversely the time at which the other treatments returned to levels comparable with controls. In comparison to the air-dried clover which was dead at the point of addition to the soil, it is likely that the fresh clover leaves underwent slow autolysis and death after excision. During

this autolysis, leaf protein and amino acids are catabolised internally for energy production (with plant cell walls probably remaining largely intact) and are less likely to be lost to the soil (Marella et al., 2017). The single highest concentration of AAs was observed in the earthworm treatment at 6 h, with one replicate of 29.3 mM of AAs, though this was identified as an outlier.

Concentrations of NH_4^+ in the control treatment did not exceed 0.21 mM during the entirety of the experiment. In contrast, in the earthworm treatment, concentrations peaked at 45.3 mM at 12 h within the same replicate as the greatest AA concentration. Generally, however, ammonium concentrations in other treatments were low in comparison to the earthworm, reaching peak mean concentrations of 2.67 mM and 1.71 mM for the dried and fresh clover respectively, with significantly ($p \leq 0.05$) greater concentrations than the control observed at every time point for dried clover, and from 24 h onwards for fresh clover (Fig. 2). Interestingly, mean nitrate concentrations of all treatments remained low throughout the sampling period (< 1 mM) with the highest concentration of 0.71 mM seen in the earthworm treatment at 6 h. We observed no clear relationship between pH and treatment and/or time (Fig. S1), however, EC was significantly higher in the earthworm and dried clover treatments than the control during the majority of sampling times (12-144 h) (Fig. S2).

The temporal pattern of our results suggests that the rise in levels of free AA's is matched by increased microbial consumption and growth that rapidly lowers the concentration. This suggests that a distinction should be made between concentration hotspots (where breakdown rate is high and exceeds microbial demand leading to accumulation in solution) and flux hotspots (where breakdown rate is high but is matched by microbial demand and where little change in solution concentration occurs). Likewise, microbial demand may have become saturated earlier on in the sampling period due in part to adding N at a higher concentrations (despite being the same weight) in earthworm and dried

clover treatments than that of fresh clover. However, hotspots occurring naturally within the soil would reflect this, yielding a range of concentrations from the macroscale, down to very fine microscales.

Results indicate that transformation of proteins to amino acids in ephemeral hotspots of protein addition may provide orders of magnitude greater levels of amino acids than previously measured in bulk soil solution (Jones et al., 2002; Jones et al., 2005; Hill et al., 2011). Further, although our experiment used relatively large fragments, it seems likely that hotspots occur at a much finer scale, for instance in the vicinity of dead microbial cells or damaged root cells in the detritusphere, as hypothesised by other researchers (Marschner et al., 2012; Kuzyakov and Blagodatskaya, 2015). As a consequence of this, it is therefore probable that the form and quantity of N acquired by plants varies considerably on a fine temporal and spatial scale.

Acknowledgments

This work was supported by the Biotechnology and Biological Sciences Research Council; and the Natural Environment Research Council [Grant number NE/M009106/1], by a Soils Training and Research Studentships (STARS) grant to Elliot J. Hill. STARS is a consortium consisting of Bangor University, British Geological Survey, Centre for Ecology and Hydrology, Cranfield University, James Hutton Institute, Lancaster University, Rothamsted Research and the University of Nottingham.

References

Bardgett, R., Streeter, T., Bol, R., 2003. Soil microbes compete effectively with plants for organic-nitrogen inputs to temperate grasslands. Ecology 84, 1277-1287.

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

de Lange, E.C.M., 2012. Recovery and calibration techniques: Toward quantitative microdialysis. In: Microdialysis in Drug Development, (M. Müller, ed.), AAPS Advances, Pharmaceutical Sciences Series, pp. 13-33. Springer Nature, Switzerland.

Farrell, M., Hill, P., Wanniarachchi, S., Farrar, J., Bardgett, R., Jones, D., 2011. Rapid peptide metabolism: A major component of soil nitrogen cycling? Global Biogeochemical Cycles 25, GB3014.

Hill, P.W., Jones, D.L., 2018. Plant-microbe competition: does injection of isotopes of C and N into the rhizosphere effectively characterise plant use of soil N? New Phytologist, In Press.

Hill, P., Quilliam, R., DeLuca, T., Farrar, J., Farrell, M., Roberts, P., Newsham, K., Hopkins, D., Bardgett, R., Jones, D., 2011. Acquisition and assimilation of nitrogen as peptide-bound and D-enantiomers of amino acids by Wheat. PLoS ONE 6,e19220.

Inselsbacher, E., Öhlund, J., Jämtgård, S., Huss-Danell, K. and Näsholm, T., 2011. The potential of microdialysis to monitor organic and inorganic nitrogen compounds in soil. Soil Biology and Biochemistry 43, 1321-1332.

Jan, M., Roberts, P., Tonheim, S., Jones, D.L., 2009. Protein breakdown represents a major bottleneck in nitrogen cycling in grassland soils. Soil Biology and Biochemistry 41, 2272-2282.

Jones, D.L., Owen, A., Farrar, J., 2002. Simple method to enable the high resolution determination of total free amino acids in soil solutions and soil extracts. Soil Biology and Biochemistry 34, 1893-1902.

Jones, D.L., Kielland, K., 2002. Soil amino acid turnover dominates the nitrogen flux in permafrost-dominated taiga forest soils. Soil Biology and Biochemistry 34, 209 - 219.

Jones, D.L., Kielland, K., Sinclair, F.L., Dahlgren, R.A., Newsham, K.K., Farrar, J.F., Murphy, D.V., 2009. Soil organic nitrogen mineralization across a global latitudinal gradient. Global Biogeochemical Cycles 23, G81016.

Jones, D.L., Owen, A., Farrar, J., 2002. Simple method to enable the high resolution determination of total free amino acids in soil solutions and soil extracts. Soil Biology and Biochemistry 34, 1893-1902.

Jones, D.L, Shannon, D., Junvee-Fortune, T., Farrar, J., 2005. Plant capture of free amino acids is maximized under high soil amino acid concentrations. Soil Biology and Biochemistry 37, 179-181.

Kuzyakov, Y., Blagodatskaya, E., 2015. Microbial hotspots and hot moments in soil: Concept and Review. Soil Biology and Biochemistry 83, 184-199.

Marella, V.S., Roberts, P., Hill, P., Jones, D.L., 2017. Different ways in which CO_2 can be released during the turnover of roots in soil. Biology and Fertility of Soils 53, 369-374.

Marschner, P., Marhan, S., Kandeler, E., 2012. Microscale distribution and function of soil microorganisms in the interface between rhizosphere and detritusphere. Soil Biology and Biochemistry 49, 174-183.

McClain, M., Boyer, E., Dent, C., Gergel, S., Grimm, N., Groffman, P., Hart, S., Harvey, J., Johnston, C., Mayorga, E., McDowell, W., Pinay, G., 2003. Biogeochemical hot spots and hot moments at the interface of terrestrial and aquatic ecosystems. Ecosystems 6, 301-312.

Miranda, K.M., Espey, M.G., Wink, D.A., 2001. A rapid simple spectrophotometric method for simultaneous detection of nitrate and nitrite. Biology and Chemistry 5, 62-71.

Moran-Zuloaga, D., Dippold, M., Glaser, B., Kuzyakov, Y., 2015. Organic nitrogen uptake by plants: reevaluation by position-specific labeling of amino acids. Biogeochemistry 125, 359-374.

Mulvaney, R., 1996. Nitrogen—inorganic forms. In: Sparks, D.L. (Ed.). Methods of Soil Analysis. Part 3. Chemical Methods. Soil Science Society of America, Madison, WI, USA, pp. 1123–1184.

Rousk, J., Jones, D., 2010. Loss of low molecular weight dissolved organic carbon (DOC) and nitrogen (DON) in H_2O and 0.5 M K_2SO_4 soil extracts. Soil Biology and Biochemistry 42, 2331-2335.

Sauheitl, L., Glaser, B., Weigelt, A., 2009. Uptake of intact amino acids by plants depends on soil amino acid concentrations. Environmental and Experimental Botany 66, 145-152.

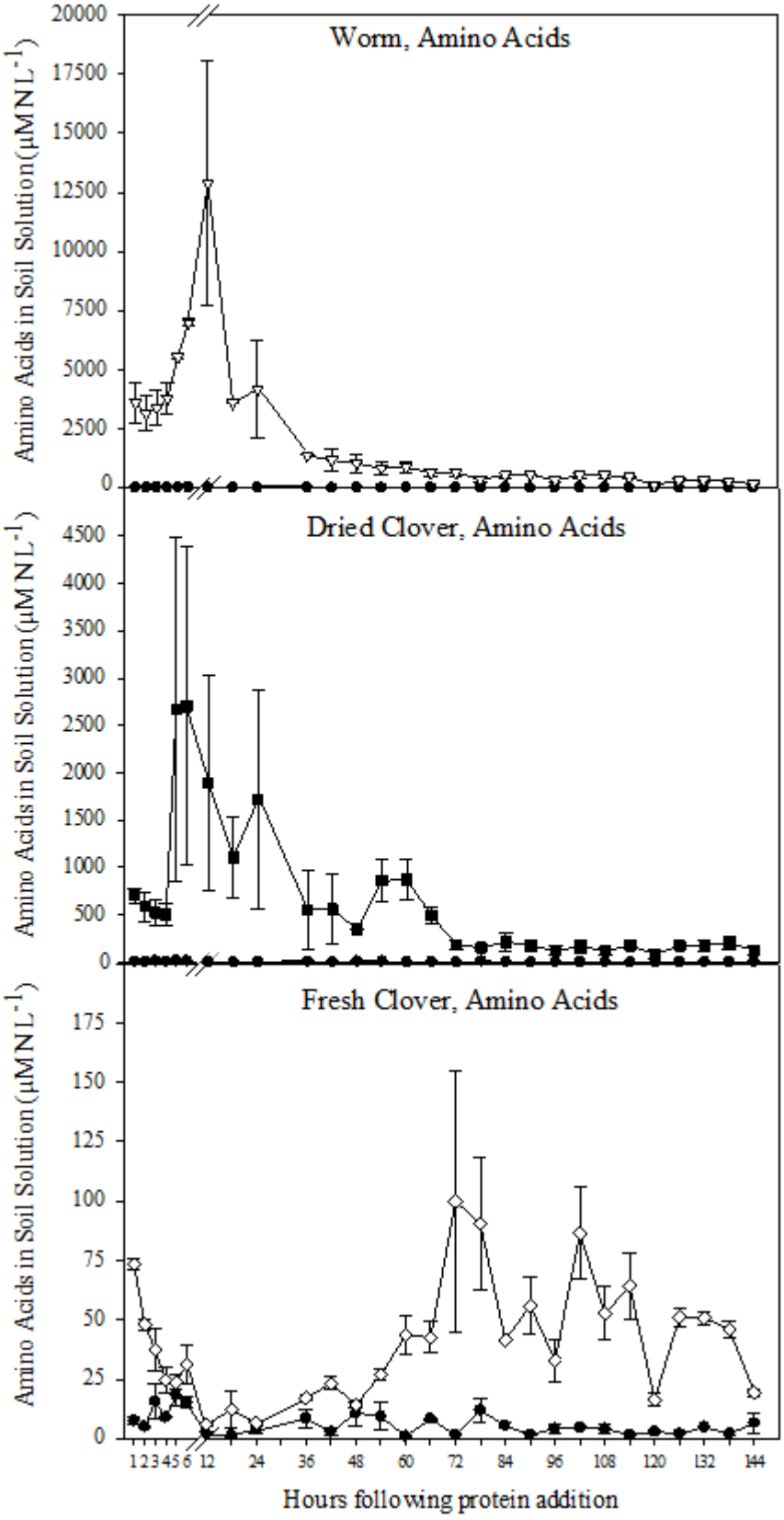
Schimel, J., Bennett, J., 2004. Nitrogen mineralization: Challenges of a changing paradigm. Ecology 85, 591-602.

Warren, C., 2013. Organic N molecules in the soil solution: what is known, what is unknown and the path forwards. Plant and Soil 375, 1-19.

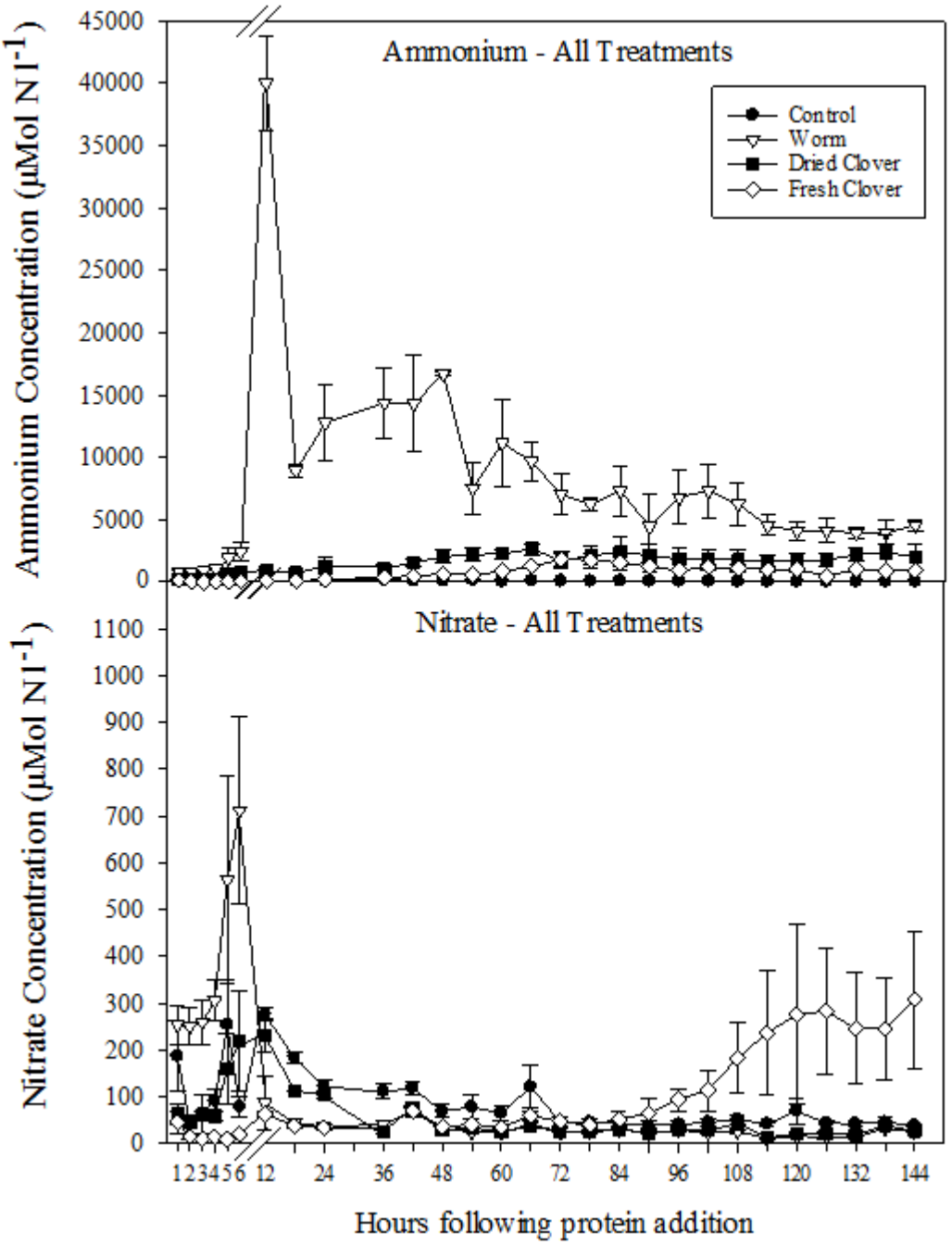
Figure legends

Figure 1. Temporal dynamics of soil solution amino acid concentrations in response to the addition of protein-rich hotspots (dead earthworm (A), dried (B) or fresh clover leaves (C)) to soil and measurement by microdialysis. Values represent means + SEM (n=4).

Figure 2. Temporal dynamics of soil solution ammonium (A) and nitrate concentrations (B) in response to the addition of protein-rich hotspots (dead earthworm, dried or fresh clover leaves) to soil and measurement by microdialysis. Values represent means + SEM (n=4).


Worm, Amino Acids
Dried Clover, Amino Acids
Fresh Clover, Amino Acids
Amino Acids in Soil Solution (µM N L-1)
Hours following protein addition
0
2500
5000
7500
10000
12500
15000
17500
20000
500
1000
1500
2000
2500
3000
3500
4000
4500
25
50
75
100
125
150
175
1 2 3 4 5 6 12 24 36 48 60 72 84 96 108 120 132 144

244


Ammonium - All Treatments
Control
Worm
Dried Clover
Fresh Clover
Ammonium Concentration (µMol N l-1)
45000
40000
35000
30000
25000
20000
15000
10000
5000
0
Nitrate - All Treatments
Nitrate Concentration (µMol N l-1)
1100
1000
900
800
700
600
500
400
300
200
100
0
1 2 3 4 5 6 12 24 36 48 60 72 84 96 108 120 132 144
Hours following protein addition

245

246

247

248 **Table 1.** Physiochemical properties of the A-horizon of the Eutric Cambisol (Means + SEM).

Soil parameters	Values
Soil moisture (%)	31.47 ± 0.22
pH	5.33 ± 0.04
EC (µS cm^{-1})	45.62 ± 3.25
Total carbon (%)	2.55 ± 0.18
Total nitrogen (%)	0.28 ± 0.02
Nitrate (N mg kg^{-1} oven dry soil)	0.872 ± 0.10
Ammonium (N mg kg^{-1} oven dry soil)	3.94 ± 1.02

249

250

251 **Table 2.** Total C & N ratios of protein additions (Means + SEM).

	Carbon (%)	**Nitrogen (%)**
Clover	48.6 ± 0.2	4.81 ± 0.06
Earthworm	43.73 ± 3.78	9.72 ± 1.03

252