

# Microbial competition for nitrogen and carbon is as intense in the subsoil as in the topsoil

Jones, David; Magthab, Ebtsam; Gleeson, D.B.; Hill, Paul; Sanchez-Rodriguez, A.R.; Roberts, Paula; Ge, T.; Murphy, D.V.

## Soil Biology and Biochemistry

DOI: 10.1016/j.soilbio.2017.10.024

Published: 01/02/2018

Publisher's PDF, also known as Version of record

Cyswllt i'r cyhoeddiad / Link to publication

*Dyfyniad o'r fersiwn a gyhoeddwyd / Citation for published version (APA):* Jones, D., Magthab, E., Gleeson, D. B., Hill, P., Sanchez-Rodriguez, A. R., Roberts, P., Ge, T., & Murphy, D. V. (2018). Microbial competition for nitrogen and carbon is as intense in the subsoil as in the topsoil. Soil Biology and Biochemistry, 117, 72-82. https://doi.org/10.1016/j.soilbio.2017.10.024

Hawliau Cyffredinol / General rights Copyright and moral rights for the publications made accessible in the public portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

• Users may download and print one copy of any publication from the public portal for the purpose of private study or research.

- · You may not further distribute the material or use it for any profit-making activity or commercial gain
- You may freely distribute the URL identifying the publication in the public portal

Take down policy If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

Contents lists available at ScienceDirect





# Soil Biology and Biochemistry

journal homepage: www.elsevier.com/locate/soilbio

# Microbial competition for nitrogen and carbon is as intense in the subsoil as in the topsoil



D.L. Jones<sup>a,\*</sup>, E.A. Magthab<sup>a</sup>, D.B. Gleeson<sup>b</sup>, P.W. Hill<sup>a</sup>, A.R. Sánchez-Rodríguez<sup>a</sup>, P. Roberts<sup>a</sup>, T. Ge<sup>b</sup>, D.V. Murphy<sup>c</sup>

<sup>a</sup> School of Environment, Natural Resources & Geography, Bangor University, Gwynedd, LL57 2UW, UK

<sup>b</sup> Key Laboratory of Agro-ecological Processes in Subtropical Region & Changsha Research Station for Agricultural and Environmental Monitoring, Institute of Subtropical Agriculture, Chinese Academy of Sciences, Changsha, Hunan Province 410125, China

<sup>c</sup> Soil Biology and Molecular Ecology Group, School of Geography and Environmental Sciences, Institute of Agriculture, The University of Western Australia, 35 Stirling Highway, Crawley, WA 6009, Australia

## ARTICLE INFO

Keywords: Ammonium Dissolved organic nitrogen Nitrate Nitrification Rhizosphere

## ABSTRACT

Most studies on plant nutrition tend to focus on the topsoil (plough layer) and frequently neglect subsoil processes. However, cereal roots can potentially acquire nutrients including organic and inorganic nitrogen (N) from deep in the soil profile. Greater knowledge on the interaction of plants and microbes in subsoil environments is required to evaluate whether deep rooting traits in cereals will achieve greater nutrient use efficiency and greater soil carbon (C) storage in cropping systems. This study aimed to evaluate the relationship between root distribution, organic and inorganic N availability and potential N supply at the critical growth period during the wheat cropping cycle in a sand textured Eutric Cambisol. Our results provide evidence of significant microbial capacity in the subsoil. The rate of plant residue turnover and the mineralization of organic C and N substrates (glucose, amino acids, peptides, protein) declined slightly with increasing soil depth; however, these rates were not correlated with basal soil respiration, microbial biomass or community structure. This suggests that the microbial population in subsoil is more C limited but that its activity can be readily stimulated upon C substrate addition. A significant potential for organic and inorganic N turnover was also demonstrated at depth with a similar abundance of ammonifiers and ammonia oxidizing bacteria (AOB) and archaea (AOA) throughout the soil profile. Again, N mineralization in subsoils appears to be substrate limited. Root density declined rapidly down the soil profile with few roots present past 50 cm; suggesting that this is the major factor limiting C recharge of soil organic matter and microbial activity in subsoils. Greater root proliferation at depth could allow greater capture of water and the recapture of N lost by leaching; however, our results suggest that plant-microbial competition for C and N is as intense in the subsoil as in the topsoil. We conclude that while deeper rooting may improve nutrient and water use efficiency it may not lead to much greater C sequestration in subsoils, at least in the short term.

#### 1. Introduction

In high input agricultural systems, nitrogen (N) availability is largely controlled by fertilizer events and the subsequent transformation and redistribution of N within the soil (Van Egmond et al., 2002). Typically, however, only 50% of the N applied to the crop in temperate climates is taken up by the plant indicating low rates of N use efficiency (Lassaletta et al., 2014). In many countries, however, there is a move to reduce the reliance on mineral fertilizers and to use added and intrinsic soil N reserves more efficiently (Chen et al., 2016). Ultimately, this aims to reduce economic costs as well as simultaneously lowering losses via leaching  $(NO_3^-)$ , denitrification  $(N_2/N_2O)$  and volatilization  $(NH_3)$ . Increases in N efficiency can potentially be achieved using a range of plant-based strategies (e.g. changes in root architecture combined with deeper rooting, release of nitrification inhibitors, use of N<sub>2</sub>-fixers; Liu et al., 2013) as well as changes in agronomic practice (e.g. improvements in fertilizer timing, formulation, placement; Hoyle and Murphy, 2011; Sartain and Obrezai, 2010). Under some of these scenarios it is likely that plants will have to take up and utilise a wider range of organic and inorganic N forms (e.g. amino acids, peptides and polyamines). We hypothesize that this will increase the competition between plant roots and soil microbial community associated with both

https://doi.org/10.1016/j.soilbio.2017.10.024

Received 19 June 2016; Received in revised form 21 October 2017; Accepted 24 October 2017 Available online 20 November 2017

0038-0717/ © 2017 The Author(s). Published by Elsevier Ltd. This is an open access article under the CC BY license (http://creativecommons.org/licenses/BY/4.0/).

<sup>\*</sup> Corresponding author.

E-mail address: d.jones@bangor.ac.uk (D.L. Jones).

the mineralization of N contained in soil organic matter (SOM) (via the direct release of root proteases or stimulation of SOM priming) and the capture of any N released in both the topsoil and subsoil (Bardgett et al., 2003; Farrell et al., 2013; Kaiser et al., 2015).

As soils frequently become progressively drier during the growing season, there is a decreased root capture of water and nutrients from the topsoil, leading to the growth of a few roots to depths often in excess of 1 m (DuPont et al., 2014). This suggests that the subsoil may play a more significant role in N supply later in the season, especially under reduced fertilizer input regimes. This may also promote carbon (C) sequestration in subsoils although the evidence to support this remains controversial (Agostini et al., 2015; Menichetti et al., 2015). Plant and microbial N cycling in deeper soil horizons, however, have received much less attention than in surface soils. If we are to capitalize on the deep rooting phenomenon of most cereals and the potential to manipulate root architecture (breeding, genetic modification; Fang et al., 2017), it is important that we understand water and nutrient availability in deeper soil layers as well as the microbial processes that control them (e.g. SOM dynamics; Zhang et al., 2014).

Agronomic estimates of N supply to plants are typically predicted from the amount of inorganic N released during the laboratory incubation of soils collected within the plough layer (0-30 cm). These mineralization rates are unlikely to be representative of deeper soil layers and ways of integrating potential N supply from subsoil is therefore needed. The amount and turnover of N in subsoil will largely depend on its exchange capacity, structure, organic material availability and microbial activity. It is well established that significant microbial activity may occur at depth (Doran, 1987; Soudi et al., 1990), albeit at much lower levels and with a different community structure than occurs in topsoil (Federle et al., 1986). When considering microbial processes at depth a key component with respect to N cycling is the abundance of ammonia-oxidizing archaea (AOA) and bacteria (AOB) that are responsible for the rate limiting step in nitrification and thus potential N loss. Dominance of AOA relative to AOB in the amoA (ammonia monooxygenase) soil gene pool has been reported in many ecosystems globally. Substrate availability and pH have been identified as the major drivers of niche specialization between AOA and AOB, with AOA being reported to be more competitive in acidic, organic matter depleted soil conditions at depth than AOB (He et al., 2012; Zhang et al., 2012; Banning et al., 2015). However, variation in soil factors such as water and oxygen availability are also important factors which differ in subsoil and which may play a role in regulating population abundances to depth. The quantity and quality of organic inputs to subsoil may also be different to the soil surface due to lower rates of root and microbial turnover and the lack of leaf litter and crop residue inputs. Subsoil soil organic matter has also been suggested to be older and more recalcitrant than in the topsoil (Schrumpf et al., 2013; Torres-Sallan et al., 2017). While this may favour C sequestration, it may conversely limit N supply to the plant.

Root length density (RLD) has been used as a proxy to predict water and nutrient uptake by plants (Taylor and Klepper, 1975; Herkelrath et al., 1977). This relationship can work well when there is adequate soil moisture available; however, it lacks precision when surface soils become prone to drying. The root systems of mature wheat plants typically extend deeper than 120 cm by the end of the growing season. However, the time at which maximal crop N demand and subsoil exploitation coincide is earlier in the season (i.e. Growth Stages GS31-71, stem elongation to the start of flowering; AHDB, 2015). Further, even though roots may extend deeper into the subsoil, their density may be extremely low (Li et al., 2017). This study therefore aimed to evaluate the relationship between root distribution, organic and inorganic N availability and potential N supply at this critical period during a wheat growing cycle. We hypothesized that the subsoil microbial population would be very low due to the lack of supply of available C and N from plant roots and associated mycorrhizas. Further, this nutrient limitation would lead to a more fungal and Gram + dominated community and

that this would be slow to respond to C substrate addition leading to a greater potential to retain C in subsoils. We also hypothesized that slow rates of organic N addition would lead to low populations of AOA and AOB and little potential to generate  $NO_3^-$ , thus also favouring N retention in subsoils.

#### 2. Materials and methods

#### 2.1. Site characteristics

Soil was collected from a replicated wheat field trial site located in Abergwyngregyn, North Wales (53°14′29″N, 4°01′15″W) and is classified as a sand textured Eutric Cambisol. The soil pH is 6.3 and does not vary significantly with depth (0–60 cm; P > 0.05). The bulk density in the topsoil (0–30 cm) is 1.48 ± 0.12 g cm<sup>-3</sup> and in the subsoil (30–60 cm) 1.63 ± 0.10 g cm<sup>-3</sup>. The climate at the site is classed as temperate-oceanic with a mean annual soil temperature of 11 °C at 10 cm depth and a mean annual rainfall of 1250 mm yr<sup>-1</sup>. The field trial consisted of six replicated plots (12.5 × 3 m) which were ploughed (0–30 cm) and planted with spring wheat (*Triticum aestivum* L. cv. Granary) in May 2013. Fertilizer was added after crop emergence (60 kg N ha<sup>-1</sup> as ammonium nitrate, 80 kg K ha<sup>-1</sup>, 28 kg P ha<sup>-1</sup>) and dicot herbicides applied following standard agronomic practice.

Soil water content, crop height and biomass were determined weekly by destructive sampling throughout the growing season. Briefly, in six replicate plots, all the crop biomass was removed within a subplot ( $0.5 \text{ m} \times 0.5 \text{ m}$ ), the samples placed in paper bags and the harvested biomass dried at 80 °C for 7 d to determine dry weight. At the same time, crop height was recorded at 5 points (1 m apart) within each of the six plots. Soil water content was determined weekly by destructive sampling throughout the growing season. Briefly, topsoil (0–30 cm) and subsoil (30–60 cm) samples were taken from six replicate plots, sieved to pass 2 mm and a subsample used to determine moisture content by drying at 105 °C overnight.

Duplicate soil samples were collected from 4 of the 6 plots in July 2013, when the plants had reached late stem extension (Feekes growth stage 9, Zadoks growth stage 39; Large, 1954; Zadoks et al., 1974) corresponding to the period of maximum plant N demand (AHDB, 2015). To estimate root density, intact soil cores were taken to a depth of 80 cm using a Cobra-TT percussion hammer corer (Eijkelkamp Agrisearch Equipment, 6987 EM Giesbeek, The Netherlands). After removal from the soil, the intact cores were split into 10 cm sections, the samples transferred to CO<sub>2</sub> permeable polythene bags and placed at 4 °C to await root recovery and soil analysis. As there were very few roots in the 60-80 cm layer, soils were only analyzed to 60 cm for the microbial N cycling and N pool size estimates. For root analysis, one of the duplicate cores was maintained intact, however, for the remaining soil analyses, the second soil core was sieved to pass 2 mm, removing any vegetation, stones and earthworms and experiments started within 48 h of field collection.

#### 2.2. Quantification of root length density and soil respiration

Roots were washed from the soil cores by a combination of mechanical shaking and flotation using a 1 mm mesh to capture roots. The roots were then placed on  $20 \times 20$  cm clear plastic plates and root length determined with WinRhizo<sup>\*</sup> (Regent Instruments Inc., Ville de Québec, Canada).

Basal respiration was determined on field-moist soil (50 cm<sup>3</sup>) in the laboratory at 20 °C over 24 h using an SR1 automated multichannel soil respirometer (PP Systems Ltd, Hitchin, UK). Visible roots were removed prior to analysis. The mean respiration rate was determined for the last 6 h of the measurement period when the  $CO_2$  efflux rates had quasistabilized.

#### 2.3. Soil solution extraction and soil chemical analysis

Soil N availability was estimated according to Jones and Willett (2006). Briefly, 5 g of field-moist soil was extracted with 25 ml of 0.5 M K<sub>2</sub>SO<sub>4</sub> on a reciprocating shaker (Edmund Bühler GmbH, SM-30, Germany; 200 rev min<sup>-1</sup>) for 60 min. After shaking, samples were centrifuged (10 min; 1699 g) and the supernatant recovered and stored at -20 °C to await analysis.

Soil solution samples were analyzed for dissolved organic C and total dissolved N (TDN) using a Multi N/C 21005 (Analytik-Jena AG, Jena, Germany). Total amino acid-N was determined fluorometrically using the *o*-phthaldialdehyde- $\beta$ -mercaptoethanol procedure of Jones et al. (2002). Nitrate and NH<sub>4</sub><sup>+</sup> were analyzed colorimetrically using the methods of Miranda et al. (2001) and Mulvaney (1996) respectively. Dissolved organic N (DON) was calculated by subtraction of inorganic N (NO<sub>3</sub><sup>-</sup> and NH<sub>4</sub><sup>+</sup>) from TDN.

Total C and N of soils were determined on ground soil using a Truspec CN analyzer (Leco Corp., St Joseph, MI, USA). Soil pH and electrical conductivity (EC) were determined in soil:distilled water extracts (1:5 v/v) with standard electrodes, while moisture content was determined by oven drying (105 °C, 24 h). The gravimetric moisture contents were corrected for stone-corrected bulk density to allow expression of water content on a volumetric basis.

#### 2.4. Net N mineralization and nitrification

Net N mineralization was determined by anaerobic incubation according to Waring and Bremner (1964) and Kresović et al. (2005). Briefly, 10 g of field-moist soil was placed in 50 cm<sup>3</sup> polypropylene tubes and anaerobic conditions imposed by filling the tubes with distilled water and then sealing the tubes. Soil samples were then incubated for 7 d in the dark at 40 °C. Subsequently, solid KCl was added to achieve a final concentration of 1 M KCl and the samples extracted by shaking for 60 min (200 rev min<sup>-1</sup>). The extracts were then centrifuged (1699 g, 10 min) and NH<sub>4</sub><sup>+</sup> determined as described previously. Net ammonification was calculated as the amount of NH<sub>4</sub><sup>+</sup> present after 7 d minus that present at the start of the incubation.

Net nitrification was determined according to Hart et al. (1994). Briefly, 5 g of field-moist soil from each soil layer was placed in a 50 cm<sup>3</sup> polypropylene tube. The tubes were then loosely sealed and the samples incubated in the dark at 20 °C. After 30 d, the soil was subsequently extracted with 0.5 M K<sub>2</sub>SO<sub>4</sub> and NO<sub>3</sub><sup>-</sup> and NH<sub>4</sub><sup>+</sup> determined as described above. Net ammonification and nitrification was calculated as the amount of NO<sub>3</sub><sup>-</sup> and NH<sub>4</sub><sup>+</sup> present after 30 d minus that present at the start of the incubation.

#### 2.5. Amino acid, peptide, protein and glucose turnover

To estimate rates of DON turnover, the mineralization of amino acids, oligopeptides and protein were determined. For comparison, the turnover of glucose was also used as a general reporter of soil microbial activity (Coody et al., 1986). Briefly, field-moist soil (5 g) was placed in 50 cm<sup>3</sup> polypropylene containers and 0.5 ml of either <sup>14</sup>C-labelled glucose (25 mM, 1.85 kBq ml<sup>-1</sup>), amino acids (10 mM, 1.55 kBq ml<sup>-1</sup>), peptides (25 mM, 1 kBq ml<sup>-1</sup>) or protein (13.2 mg l<sup>-1</sup>, 51 kBq ml<sup>-1</sup>) added to the soil surface (Farrell et al., 2011). After the addition of each <sup>14</sup>C-substrate to the soil, a <sup>14</sup>CO<sub>2</sub> trap containing 1 ml of 1 M NaOH was placed above the soil and the tubes sealed. With the exception of protein, the tubes were then incubated at 20 °C for 30 min after which the NaOH traps were removed to determine the amount of substrate mineralized. In the case of protein, the procedure was identical except that the incubation period was 24 h. The <sup>14</sup>C content of the NaOH traps was determined with Wallac 1404 liquid scintillation counter (Wallac EG&G, Milton Keynes, UK) after mixing with Scintisafe3 scintillation cocktail (Fisher Scientific, Loughborough, UK). The amino acids consisted of an equimolar mix of 20 different L-amino acids (L-glycine, L-

isoleucine, L-arginine, L-glutamine, L-phenylalanine, L-histidine, L-asparagine, L-valine, L-threonine, L-leucine, L-alanine, L-methionine, L-cysteine, L-lysine, L-tryptophan, L-serine, L-proline, L-glutamate, L-aspartate acid, L-ornithine) while the L-peptides consisted of a mixture of equimolar L-dialanine and L-trialanine. The mixed soluble plant protein was purified from <sup>14</sup>C-labelled tobacco leaves (American Radiolabeled Chemicals Inc., St Louis, MO, USA).

To determine the rate of arginine mineralization, 0.5 ml of a  $^{14}\text{C}$ -labelled L-arginine solution (25 mM; 2.17 kBq ml $^{-1}$ ; Amersham Biosciences UK Ltd, Chalfont St Giles, Bucks, UK) was added to 5 g of field-moist soil and the rate of  $^{14}\text{CO}_2$  evolution measured over a 48 h as described in Kemmitt et al. (2006). After 48 h, the net amount of NH<sub>4</sub>  $^+$  and NO<sub>3</sub> $^-$  produced from the added arginine was determined by extracting the soil with 25 ml 0.5 M K<sub>2</sub>SO<sub>4</sub> and subsequent analysis as described previously.

#### 2.6. Mineralization of plant-derived C

The microbial turnover of complex, plant-derived C across the different soil depths was evaluated according to Glanville et al. (2012). Briefly, high molecular weight (MW) plant material was prepared by heating 2.5 g of <sup>14</sup>C-labelled *Lolium perenne* L. shoots (Hill et al., 2007) in distilled water (25 ml, 80 °C) for 2 h. The extract was then centrifuged (1118 g, 5 min) and the soluble fraction removed. The pellet was then resuspended in distilled water and the heating and washing procedure repeated twice more until > 95% of the water soluble fraction had been removed. The pellet remaining was dried overnight at 80 °C and ground to a fine powder.

The mineralization dynamics of the high MW plant material was determined by mixing 100 mg of <sup>14</sup>C-labelled plant material with 5 g of field-moist soil. The production of <sup>14</sup>CO<sub>2</sub> was monitored as described above for the low MW substrates but over 40 d. To ensure that water was not limiting, the experiment was also repeated but after the simultaneous addition of distilled water (to reach field capacity) and the <sup>14</sup>C-labelled plant material.

#### 2.7. Nucleic acid extraction and quantitative PCR (qPCR)

For each soil sample, DNA was extracted from duplicate 800 mg sub-samples using UltraClean<sup>\*</sup> DNA Isolation Kit (MoBio Laboratories Inc., Carlsbad, CA, USA). Cell lysis was performed using a Mini Bead beater (BioSpec Products Inc., Bartlesville, OK) at 2500 rev min<sup>-1</sup> for 2 min. Duplicate DNA extractions were combined to give a total extract volume of 100  $\mu$ l.

Functional genes, archaeal and bacterial amoA, were quantified using a ViiA7 qPCR machine (Thermo Fisher Scientific, Scoresby, Australia). Each 20 µl qPCR reaction contained 10 µl of Power SYBR® Green PCR Master Mix (Thermo Fisher Scientific). 0.2 µl of the specific forward and reverse primer at a concentration of 10 µM, 2 µl BSA (Ambion UltraPure BSA; 5 mg ml $^{-1}$ ; Thermo Fisher Scientific), 8 ng template DNA and sterile water to 20 µl. Primers and thermal cycling conditions for both bacterial (primers amoA-1F and amoA-2R) and archaeal (primers Arch-amoAF and Arch-amoAR) amoA genes were as described previously (Banning et al., 2015). Melting curves were generated for each qPCR run and fluorescence data was collected at 78 °C to verify product specificity. Each qPCR reaction was run in triplicate. Standard curves were generated using dilutions of linearized cloned plasmids. Template amplified with each primer pair described above, was cloned with the P-GEM T-easy system (Promega Inc., Madison, WI), plasmid DNA extracted and inserts sequenced using Big Dye Terminator chemistry (Australian Genome Research Facility, Western Australia) to confirm correct length and identity. The standard curve gene sequences were as described previously (Barton et al., 2013). Standard curves generated in each reaction were linear over four orders of magnitude  $(10^4 \text{ to } 10^7 \text{ gene copies})$  with  $r^2$  values greater than 0.99. Efficiencies for all quantification reactions were 80-100%.

#### 2.8. Microbial community structure

Microbial community structure was measured by phospholipid fatty acid (PLFA) analysis following the method of Buyer and Sasser (2012). Briefly, samples (2 g) were freeze-dried and Bligh-Dyer extractant (4.0 ml) containing an internal standard added. Tubes were sonicated in an ultrasonic bath for 10 min at room temperature before rotating endover-end for 2 h. After centrifuging (10 min) the liquid phase was transferred to clean 13 mm  $\times$  100 mm screw-cap test tubes and 1.0 ml each of chloroform and water added. The upper phase was removed by aspiration and discarded while the lower phase, containing the extracted lipids, was evaporated at 30 °C. Lipid classes were separated by solid phase extraction (SPE) using a 96-well SPE plate containing 50 mg of silica per well (Phenomenex, Torrance, CA). Phospholipids were eluted with 0.5 ml of 5:5:1 methanol:chloroform:H<sub>2</sub>O (Findlay, 2004) into glass vials, the solution evaporated (70 °C, 30 min). Transesterification reagent (0.2 ml) was added to each vial, the vials sealed and incubated (37 °C, 15 min). Acetic acid (0.075 M) and chloroform (0.4 ml each) were added. The chloroform was evaporated just to dryness and the samples dissolved in hexane. The samples were analyzed with a 6890 gas chromatograph (Agilent Technologies, Wilmington, DE) equipped with autosampler, split-splitless inlet, and flame ionization detector. Fatty acid methyl esters were separated on an Agilent Ultra 2 column, 25 m long  $\times$  0.2 mm internal diameter  $\,\times\,$  0.33  $\mu m$  film thickness. Standard nomenclature was followed for fatty acids (Frostegård et al., 1993).

#### 2.9. Statistical and data analysis

Statistical analysis of the results was carried out by ANOVA followed by Tukey HSD post hoc test and linear regression using SPSS v14 (IBM UK Ltd, Hampshire, UK) with P < 0.05 used as the level to define significance. Analysis of differences in qPCR abundances of bacterial and archaeal *amoA* across soil depth was performed by analysis of variance (one-way ANOVA) using GenStat (15th edition; Lawes Trust, Harpenden, UK). Principal component analysis was performed in R'.

#### 3. Results

#### 3.1. Crop and soil characteristics

As expected, crop height showed a sigmoidal extension pattern over the growing season with full stem extension evident after 8 weeks (Fig. 1a). Crop biomass also showed a sigmoidal growth pattern, however, above-ground biomass continued to increase up until week 13 due to progressive grain filling (Fig. 1a).

Corresponding with the period of maximum crop development and low rainfall, soil water content declined dramatically between weeks 5–8 in both the topsoil and subsoil; with soil water in the subsoil being consistently lower than in the topsoil (P < 0.05; Fig. 1b). At week 9, significant amounts of rainfall caused recharge of the soil profile with the topsoil retaining significantly more water than the subsoil (P < 0.01; Fig. 1b).

Root length density decreased down the soil profile, with the vast majority located in the topsoil (Fig. 2a). Less than 4% of total root length density was in the subsoil below 30 cm. Soil total and bio-available C pools also decreased with increasing depth (Table 1). Soil basal respiration was significantly greater (P < 0.05) in the 0–20 cm layer (Fig. 2b) with the pattern matching that of root density distribution.

#### 3.2. Mineral N cycling

Ammonium and nitrate concentrations in the field-collected samples were significantly greater in the surface (0–10 cm) layer (P < 0.05) than in the deeper soil horizons (Table 1). Overall, the



**Fig. 1.** Crop biomass and height (Panel A) and soil water content in the topsoil (0–30 cm) and subsoil (30–60 cm) layers (Panel B) during the wheat cropping cycle from planting to harvest. Values represent mean  $\pm$  SEM (n = 6). \* and \*\* in Panel B indicate significant differences between depths at the P < 0.05 level and P < 0.01 level respectively. Note: Soil profiles for biochemical and molecular analysis were collected 8 weeks after planting.

patterns of N mineralization in the aerobic and anaerobic incubations were similar, decreasing in an exponential pattern down the soil profile (Fig. 3). The concentration of NH<sub>4</sub><sup>+</sup> after 30 d of aerobic incubation only increased significantly in the 10–20 cm soil layer (Fig. 3). Aerobic net N mineralization within the 0–20 cm layer of the soil profile was significantly greater (P < 0.05) compared to the 40–60 cm layer (Fig. 3). In contrast to the aerobic incubation, the anaerobically incubated soils showed large increases in NH<sub>4</sub><sup>+</sup> concentration at all depths, with the largest increase occurring in the surface soil layer (Fig. 3).

#### 3.3. Low molecular weight carbon substrate mineralization

Mineralization rates of low molecular weight C molecules tended to decrease slightly with depth (Fig. 4). While substrate mineralization in the topsoil (0–30 cm) was significantly greater compared to the subsoil there was still considerable mineralization occurring at 50–60 cm (Fig. 4). There was a 10,000 fold difference between protein and amino acid mineralization rates, with rates in the order amino acid > peptide > glucose > protein.



**Fig. 2.** Density of primary (first order) and lateral (second and third order) roots (Panel A) and basal soil respiration at different depths in an agricultural wheat cropping soil (Panel B). Values are means  $\pm$  SEM (n = 5). All measurements were made 8 weeks after planting. Different letters indicate significant differences between depths at the P < 0.05 level (Tukey's HSD).

#### 3.4. Arginine and plant residue turnover

The initial (0–6 h) arginine C mineralization rate decreased with soil depth (P < 0.05) (Fig. 5a). However, by 48 h the amount of arginine mineralization was statistically similar at all soil depths. While the rate of mineralization was linear in the topsoil, however, a lag phase in mineralization was observed in the subsoil horizons (data not shown). The net amount of NH<sub>4</sub><sup>+</sup> produced from the added arginine significantly increased with soil depth (Table 2). In contrast, however, the net amount of NO<sub>3</sub><sup>-</sup> decreased significantly with increasing soil depth. Overall, the ratio of C mineralization to N immobilization was greater in the topsoil than in the subsoil.

The rate of <sup>14</sup>C-labelled plant residue mineralization was much slower than those of the simple C substrates. Notably there was no significant difference in turnover rates between soil depths (P > 0.05; Fig. 5b).

#### 3.5. AOA and AOB gene abundances

Nitrification capacity, as assessed by amoA gene abundance, was present throughout the soil profile. At every soil depth AOA gene abundance was significantly lower (P < 0.01) than AOB (Fig. 6). For AOB *amoA* gene copies ranged from  $1 \times 10^7$  to  $2 \times 10^8$  g<sup>-1</sup> dry soil

while AOA *amoA* gene copies ranged from 2 to  $5 \times 10^5 \text{ g}^{-1}$  dry soil. There was no significant effect of depth on AOA population abundance (P > 0.05) but there was a significant effect of depth on AOB population abundance (P < 0.05) whereby AOB gene abundance was significantly lower (P < 0.05) in the subsoil below 30 cm than in topsoil (Fig. 6).

#### 3.6. Microbial community structure

Total PLFA significantly decreased below 30 cm depth; with the amount of total PLFA relatively constant within topsoil and subsoil layers (Fig. 7a). Overall, the relative proportion of major microbial groups was quite similar at the different soil depths. The proportion of fungi and actinomycetes significantly increased with soil depth (P < 0.01 and 0.001 respectively) while the relative abundance of Gram-positive and Gram-negative bacteria both reduced (P < 0.01). The relative abundance of putative arbuscular mycorrhizal PLFAs (16:1 w5c) was similar at all depths (data not presented). Principal component analysis of the PLFA data revealed a separation of the topsoil and subsoil microbial communities (Fig. 8).

#### Table 1

Soil properties at different soil depths in an agricultural wheat cropping soil. Different superscript letters indicate significant differences between depths (P < 0.05). Values represent mean  $\pm$  SEM (n = 4).

Soil depth	Moisture content	pH <sub>1:5</sub>	EC <sub>1:5</sub>	Total C	DOC	Total N	DON	Amino acids	NH4 <sup>+</sup>	NO3 <sup>-</sup>
(cm)	(g kg <sup>-1</sup> )		(μS cm <sup>-1</sup> )	(g N kg <sup>-1</sup> )	(mg C kg <sup>-1</sup> )	(g N kg <sup>-1</sup> )	(mg N kg <sup>-1</sup> )	(mg N kg <sup>-1</sup> )	(mg N kg <sup>-1</sup> )	(mg N kg <sup>-1</sup> )
0-10 10-20 20-30 30-40 40-50 50-60	$\begin{array}{rrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrr$	$\begin{array}{rrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrr$	$\begin{array}{rrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrr$	$\begin{array}{rrrr} 19.4 \ \pm \ 1.5^{a} \\ 16.3 \ \pm \ 2.4^{ab} \\ 11.6 \ \pm \ 2.2^{bc} \\ 3.9 \ \pm \ 0.6^{d} \\ 5.3 \ \pm \ 1.7^{d} \\ 3.0 \ \pm \ 0.8^{d} \end{array}$	$\begin{array}{rrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrr$	$\begin{array}{rrrrr} 2.4 \ \pm \ 0.2^{a} \\ 2.0 \ \pm \ 0.3^{ab} \\ 1.6 \ \pm \ 0.2^{bc} \\ 0.8 \ \pm \ 0.1^{d} \\ 1.1 \ \pm \ 0.2^{d} \\ 0.8 \ \pm \ 0.1^{d} \end{array}$	$\begin{array}{rrrr} 14.3 \ \pm \ 3.7^{a} \\ 10.6 \ \pm \ 1.3^{ab} \\ 6.9 \ \pm \ 0.8^{abc} \\ 5.8 \ \pm \ 1.6^{bc} \\ 1.0 \ \pm \ 0.4^{cd} \\ 1.6 \ \pm \ 0.4^{d} \end{array}$	$\begin{array}{rrrrr} 0.14 \ \pm \ 0.02^{a} \\ 0.10 \ \pm \ 0.02^{ab} \\ 0.09 \ \pm \ 0.02^{ab} \\ 0.04 \ \pm \ 0.01^{bc} \\ 0.02 \ \pm \ 0.01^{c} \\ 0.02 \ \pm \ 0.01^{c} \end{array}$	$\begin{array}{rrrrr} 2.6 & \pm & 1.0^{a} \\ 1.0 & \pm & 0.3^{b} \\ 0.6 & \pm & 0.2^{b} \\ 0.6 & \pm & 0.2^{b} \\ 0.6 & \pm & 0.3^{b} \\ 0.6 & \pm & 0.1^{b} \end{array}$	$\begin{array}{rrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrr$



**Fig. 3.** Net ammonification after incubation under anaerobic conditions for 7 days or net mineralization (ammonification and nitrification) after incubation under aerobic conditions for 30 days at different soil depths in an agricultural wheat cropping soil. Values represent means  $\pm$  SEM (n = 4). Different letters indicate significant differences between depths at the P < 0.05 level (Tukey's HSD; lowercase for anaerobic incubation and uppercase for aerobic incubation).



**Fig. 4.** Mineralization of <sup>14</sup>C-labelled glucose, amino acids, oligopeptides and protein at different soil depths in an agricultural wheat cropping soil. Values represent means  $\pm$  SEM (n = 4). Different letters indicate significant differences between depths at the P < 0.05 level ((Tukey's HSD).



**Fig. 5.** Cumulative percentage of <sup>14</sup>C- arginine mineralization (Panel A) and cumulative percentage of <sup>14</sup>C-*Lolium perenne* shoots mineralization (Panel B) at different soil depths in an agricultural wheat cropping soil. Values represent means  $\pm$  SEM (n = 4).

#### Table 2

Net amount of ammonium  $(NH_4^+)$  and nitrate  $(NO_3^-)$  released as a function of soil depth after the addition of arginine and incubation for 48 h. Values represent means  $\pm$  SEM (n = 4). Different superscript letters indicate significant differences between depths at the P < 0.05 level.

Soil depth (cm)	$\mathrm{NH_4}^+$ after 48 h (mg N kg <sup>-1</sup> )	$NO_3^-$ after 48 h (mg N kg <sup>-1</sup> )
0-10 10-20 20-30 30-40 40-50 50-60	$72.2 \pm 1.9^{a}$ $72.1 \pm 2.7^{a}$ $76.4 \pm 6.7^{bc}$ $93.0 \pm 3.8^{cd}$ $95.7 \pm 3.2^{d}$ $124.2 \pm 2.8^{e}$	$\begin{array}{rrrrr} 18.3 \ \pm \ 2.1^{a} \\ 12.4 \ \pm \ 1.4^{b} \\ 7.8 \ \pm \ 0.5^{bc} \\ 4.2 \ \pm \ 0.5^{c} \\ 4.5 \ \pm \ 1.2^{c} \\ 5.2 \ \pm \ 0.9^{c} \end{array}$

*amoA* gene abundance (copy number g<sup>-1</sup> soil)



**Fig. 6.** Bacterial (AOB) and archaeal (AOA) *amoA* gene copy numbers at different soil depths in an agricultural wheat cropping soil. Values are means  $\pm$  SEM (n = 4). Different letters indicate significant differences between depths at the P < 0.05 level (Tukey's HSD).

#### 4. Discussion

4.1. Changes in microbial biomass, activity and community structure with depth

As expected, root abundance, microbial biomass and basal respiration all declined with soil depth (Kramer et al., 2013; Li et al., 2014;



Fig. 8. Principal component analysis for PLFAs (taxonomic groups based on PLFAs) as a function of soil depth. Two scales are used, the  $\pm$  3.0 scale refers to the loadings of the samples at different depths and the  $\pm$  1.0 scale refers to the loadings of the different taxonomic groups (variables). The percent of variation is included on each Principal Component (PC).

Loeppmann et al., 2016). In many cases, these changes can be attributable to excess acidity and toxic levels of  $Al^{3+}$  in the subsoil (Tang et al., 2011). In our study, however, soil pH did not vary down the profile and therefore this does not represent a confounding factor. Microbial community structure also shifted down the soil profile with the fungal-to-bacterial ratio increasing with depth, presumably due to increased C limitation and the lower N requirement of fungi rather than due to a shift in soil pH. This is in agreement with the results of Sanaullah et al. (2016) in grasslands but contrasts with the results of Kramer et al. (2013) and Stone et al. (2014) who showed either no effect or a strong decrease in fungal-to-bacterial ratio with depth. Based



**Fig. 7.** Total microbial PLFA (Panel A) and the relative abundance of specific microbial PLFA markers (Panel B) at different soil depths in an agricultural wheat cropping soil. Values are means  $\pm$  SEM (n = 4). In Panel B the fungal PLFA marker data have been multiplied  $\times 10$  for scaling purposes. Different letters indicate significant differences between depths at the P < 0.05 level (Tukey's HSD).

on the slow growth of Gram + bacteria and there greater ability to survive C starvation (De Vries and Shade, 2013), we expected to see an increased Gram + -to-Gram- ratio with depth. Although our results do support this to some extent, the overall effect was quite small. Despite the low microbial biomass, however, we demonstrate that high rates of both soluble and insoluble C and N turnover can occur at depth. Generally, however, microbial processes have a tendency to be greatest in surface layers, especially when soil disturbance is minimised (Murphy et al., 1998). Numerous factors could contribute to lower microbial activity in deep soils. The results obtained here show a much lower abundance of roots at depth so it is likely that there is less soluble organic C or fresh particulate C being delivered to the subsoil via root exudation and root/mycorrhizal turnover (Fontaine et al., 2007). The lack of earthworm presence in our soil also prevents the bioturbationdriven delivery of C to the subsoil and limits subsoil biological hotspots in the form of deep vertical earthworm burrows (Uksa et al., 2015; Hoang et al., 2016). Microbes at depth therefore experience strong C limitation which is supported by the decrease in C-to-N ratio with depth in some soils (Rumpel and Kogel-Knabner, 2011). In addition, the increasing DOC-to-DON ratio with depth suggests that the DOC may be becoming more chemically recalcitrant (i.e. humic-like) down the soil profile. Other mechanisms which may also restrict microbial activity in subsoils include: (1) an increased bulk density which may suppress root growth; (2) greater structural aggregation which may both restrict root access and promote the physical protection of C; (3) a greater abundance of clay and oxyhydroxides which may stimulate the chemical protection of C; and (4) greater moisture contents and resulting anoxia which may supress root and microbial activity (Kinyangi et al., 2006; Rumpel and Kogel-Knabner, 2011). In the context of our well drained, sandy-textured soil we expect the influence of these factors to be relatively low.

Soluble organic N concentrations decreased with soil depth suggesting that the microbial community could also be N limited at depth. Based on the evidence presented, we ascribe these low concentrations to the low rate of DON supply from rhizodeposition and SOM turnover combined with the rapid microbial removal of labile DON from solution. As added soluble-N was readily mineralized to NH4<sup>+</sup> in our subsoils we conclude that subsoil microbial activity is driven more by C limitation rather than by N limitation. This view is also supported by Jones et al. (2005) who demonstrated that the microbial use of DON compounds was largely insensitive to N fertilizer regime and more related to C availability than N availability in a range of agricultural soils. In addition, we observed a relatively high concentration of NO<sub>3</sub><sup>-</sup> at depth. NO3<sup>-</sup> tends only to be utilized in large amounts by microorganisms under severe N deficiency due to the energetic costs associated with its assimilation (in comparison to DON and NH4<sup>+</sup>), again suggesting that the subsoil microbial community is not N limited (Abaas et al., 2012).

#### 4.2. Implications for subsoil C storage

Reduced microbial activity at depth has led to the suggestion that subsoils may have the potential to lock up additional C and that this could help offset anthropogenically derived greenhouse gas emissions (Lynch and Wojciechowski, 2015; Pierret et al., 2016; Gocke et al., 2017; Torres-Sallan et al., 2017). One widely proposed mechanism to stimulate this transfer of C into subsoils is the use of crops with deep rooting traits (Lavania and Lavania, 2009; Kell, 2011) or shifts towards less intensive land management systems (Ward et al., 2016). It should be noted, however, that much controversy surrounds the stability of C in subsoils with many reports suggesting it persists for long time periods and is more stable than C in topsoils (Kramer and Gleixner, 2008; Muller et al., 2016). The evidence presented here clearly showed that while the rates of plant residue turnover were initially slower in subsoils in comparison to topsoils (0–24 h), these differences disappeared over longer incubation times (e.g. 30 d). This suggests that the subsoil microbial community quickly adapted to an increased C supply. This directly challenges the assumption that increasing the rate of C supply to subsoils will lead to greater long term C storage. It is also consistent with measurements showing that most subsoil C is of recent origin and not very stable (Hobley et al., 2017; Zhang et al., 2014). Our results also support the results from Brauer et al. (2013) and Matus et al. (2014) who suggest that C storage in subsoils is mainly driven by microbially-processed C being translocated down the soil profile as DOC and then becoming chemically protected, rather that C generated in situ within subsoils.

It should be emphasized that the discussion above mainly relates to the potential for accumulating subsoil C over a limited number of cropping cycles (i.e. 1–10 v). Over longer time scales it is conceivable that small amounts of C may become progressively stabilized in subsoils leading to substantial C increases over decadal time scales. Current evidence suggests that long-term shifts in agronomic management (> 40 years) targeted at surface residue management and tillage regime can substantially increase topsoil C levels, but that they have limited capacity to alter subsoil C storage (Jarvis et al., 2017; Kinoshita et al., 2017). This provides strong evidence that C migration from top-to subsoils is not an effective mechanism for promoting C storage in deeper soil layers. An alternative to relying on roots to deliver C to subsoils is the deep incorporation (> 50 cm depth) of crop residues into soil (Alcantara et al., 2017; Cui et al., 2017). Unlike cereal roots whose C-to-N ratio ranges from 15 to 30, the low N content of crop residues (C-to-N ratio = 50-80) is more likely to favour C retention (and may additionally suppress N losses via leaching). It is clear, however, that more long-term field trials are required to critically address whether deeper rooting crops lead to enhanced C sequestration.

#### 4.3. Variation in soil N cycling with depth

Protein represents the major input of organic N into cropping soil systems. Therefore, the mineralization of protein, oligopeptides and amino acids is an important part of the N cycle and supplies the substrate for inorganic N production and therefore root N uptake (Jones et al., 2013). In topsoils it has been proposed that the breakdown of proteins to peptides is the main rate limiting step in the soil N cycle (Jan et al., 2009) and the evidence presented here clearly suggests that this is also the case for subsoils. However, when expressed per unit of microbial biomass, protein breakdown rate was much greater in subsoils than topsoils. Contrary to Loeppmann et al. (2016), this could either imply that the proteases have a greater substrate affinity at depth or it may relate to greater substrate availability (i.e. less substrate sorption to the solid phase). More work is therefore required to understand the factors regulating the production and behaviour of proteases in subsoils. Pinggera et al. (2015) recently demonstrated that subsoil protease activity was upregulated when abundant substrate was available but was repressed if sufficient inorganic N was present. In line with our results, this suggests that the microbial community will readily respond to substrate addition. Further, it also suggests that addition of high C:N residues (e.g. values > 20) might stimulate positive priming and the mining of subsoil SOM to release N.

Arginine addition caused the rapid mineralization of amino acid-N to  $NH_4^+$  at all soil depths, again demonstrating that ammonification was not a rate limiting step at any point in our soil profile. In addition, our soil incubation results showed that  $NH_4^+$  only increased under anaerobic conditions, when C degradation and nitrification are oxygen limited. As rapid ammonium oxidation occurred readily under aerobic conditions it also supports the premise that N cycling in both topsoils and subsoils is limited by upstream elements in the N cycle (i.e. substrate availability for protease action).

Our results also reveal much greater nitrification potential in surface soils than at depth which may be indicative of a larger active community of nitrifiers. Therefore, more  $NH_4^+$  would be transformed to  $NO_3^-$  in the surface soil and potentially more could be lost as  $N_2O$  or

N<sub>2</sub>. We show that the vertical distribution of both AOA and AOB were strongly correlated with each other ( $r^2 = 0.92, P < 0.01$ ) and also with total microbial PLFA ( $r^2 > 0.82$ , P < 0.05). Further, AOB abundance closely correlated with the wider Gram-bacterial community of which it forms part ( $r^2 = 0.87, P < 0.05$ ). This implies that nitrification has no specialist niche in the soil profile relative to the more general aspects of soil organic C and N cycling. The results also do not support the proposal that AOB and AOA communities behave differently in different soil layers (Wang et al., 2014). In surface soils we found an increased abundance of AOB when compared to lower depths, and additionally report that AOA abundance was much lower than for AOB (ca. 200-fold) and did not vary as greatly with depth. Our results contrast with Fischer et al. (2013), Uksa et al. (2014), Wang et al. (2014) and Liu et al. (2016) who all showed that AOA abundance was much greater than AOB, particularly in subsoils. From our results, we infer that AOB are likely driving nitrification in this system due to the increase in measured nitrification in conjunction with an increase in AOB but not AOA abundance in surface soils. It is also likely that AOB are more active and can respond more quickly to additions of organic N and  $NH_4^+$  derived from this (Di et al., 2010). This is consistent with a number of other studies (Di et al., 2009, 2010; Barton et al., 2013; Banning et al., 2015) who also suggest that AOB are likely driving topsoil nitrification.

In addition, we found that NH4<sup>+</sup> concentrations in the field were significantly greater in the topsoil than at depth, which we ascribe to its higher organic matter and cation exchange capacity. This likely favours AOB over AOA with previous studies showing that AOA may only have a competitive advantage at low ammonium concentrations due to their greater substrate affinity (Martens-Habbena et al., 2009) or due to greater sensitivity to growth inhibition at high ammonium concentrations (Prosser and Nicol, 2012). Soil pH is often described as having a significant influence on AOA and AOB abundance, although reports are not consistent. Some studies have reported AOB to be more sensitive than AOA to pH changes (Nicol et al., 2008; Yao et al., 2011). For example Yao et al. (2011) observed that AOB were more abundant in neutral and alkaline conditions than in acidic conditions, whereas there was no correlation between pH and AOA abundance. In contrast, Pereira e Silva et al. (2012) found soil pH did not influence AOB abundance but did increase AOA abundance; while Nicol et al. (2008) reported only AOA abundance and not AOB, was influenced by pH. In the current study pH did not change with depth and thus the increased abundance of AOB in the surface is not likely related to pH in this study.

It should also be noted that although there may be a significant reserve of nutrients at depth, these may be also physically or chemically protected, especially in well structured subsoils. It is therefore important for future studies to consider not only the size of the nutrient pool, but also the gross flux through this pool and its bio-accessibility.

#### 4.4. Implications of N cycle variations with depth for root uptake

Deeper rooting may promote the more efficient use of nutrients such as N and P (Lynch and Wojciechowski, 2015). However, we hypothesized that roots at the surface would be involved more in nutrient uptake than those at depth. The greater root length observed in this study corresponds with the areas of greater microbial activity, N concentrations and turnover rates. Therefore, it is likely that much more N is taken up by surface roots than those at depth. The surface soil is also likely to be the area where microbial N demand is greatest. Greater root length in topsoils would therefore allow for greater competition with microbes. The greater bulk density at depth may also suppress root growth making the access of nutrients more difficult (Salome et al., 2010).

Water uptake is as important a function of plant roots as nutrient uptake. The uptake rate of water is often proportional to root length density (Hinsinger et al., 2009; Hodge et al., 2009). During drought, soil surfaces dry, limiting both water and, potentially, nutrient uptake in roots near the surface. This can lead to near-surface roots dying and greater root growth at depth (Smucker et al., 1991). In dry conditions, deeper roots could become vital for maintaining plant nutrient uptake. In addition, more roots at depth could lead to a greater input of exudates which would increase microbial activity and decrease nutrient loss (Fisk et al., 2015). It may also promote the microbial priming of subsoil SOM and the loss of stable C from soil (Fontaine et al., 2007).

Contrary to expectation, the subsoil appeared to retain less water than the topsoil. We ascribe this to its lower SOM content which is known to aid water retention and promote soil structure (Rawls et al., 2003). Further, the subsoil dried out and rewet at a similar rate to the topsoil. This does not support the hypothesis that soil moisture becomes proportionally more available in subsoil as the soil progressively dries out due to evapotranspiration losses. Our results suggest that irrespective of root length density, water is removed evenly throughout the soil profile to balance plant demand, or less likely, that plant-mediated hydraulic lift is redistributing water from deeper soil layers to the surface. This suggests that drying out of the soil profile does not induce spatial niche partitioning in N availability.

#### 4.5. Conclusions

In terms of plant-microbial nutrient cycling, subsoils remain understudied in comparison to topsoils. In addition to providing water to plants, however, recent reviews have suggested that subsoils may represent an important store of nutrients and have the potential to sequester large amounts of C (Torres-Sallan et al., 2017). Consequently, there is a growing view that subsoils should be actively managed to optimise their functioning (e.g. by mechanical or plant-based interventions; Kell, 2011; Tang et al., 2011; Alcantara et al., 2016, 2017). The results presented here suggest that although the subsoil has a low and slightly different microbial community than the topsoil, in terms of C cycling, the subsoil microbial community rapidly responds to new inputs of organic C and N. This suggests that the use of deeper rooting plants may not enhance long-term C storage in subsoils, especially if they destabilize subsoil SOM through rhizosphere priming. Our results also show that, as expected, root proliferation is greatest in the region of the soil profile where nutrient cycling is greatest. At present, the routine sampling of agricultural subsoils is costly and problematic. Further, subsoils can be expected to have higher spatial heterogeneity than topsoils. Combined, this makes it difficult to make informed decisions for active subsoil management. We conclude that the potential future importance of subsoils in sustainable agriculture may have been overstated.

#### Acknowledgements

This research was funded by the UK Natural Environment Research Council (NE/I012303/1), the Sêr Cymru LCEE-NRN project, Climate-Smart Grass and the Australian Research Council Future Fellowship Scheme (FT110100246) awarded to DVM.

#### References

- Abaas, E., Hill, P.W., Roberts, P., Murphy, D.V., Jones, D.L., 2012. Microbial activity differentially regulates the vertical mobility of nitrogen compounds in soil. Soil Biology & Biochemistry 53, 120–123.
- Agostini, F., Gregory, A.S., Richter, G.M., 2015. Carbon sequestration by perennial energy crops: is the jury still out? Bioenergy Research 8, 1057–1080.
- AHDB, 2015. Wheat Growth Guide. AHDB Cereals & Oilseeds, Agriculture and Horticulture Development Board, Kenilworth, Warwickshire, UK
- Alcantara, V., Don, A., Well, R., Nieder, R., 2016. Deep ploughing increases agricultural soil organic matter stocks. Global Change Biology 22, 2939–2956.
- Alcantara, V., Don, A., Vesterdal, L., Well, R., Nieder, R., 2017. Stability of buried carbon in deep-ploughed forest and cropland soils - implications for carbon stocks. Scientific Reports 7, 5511.
- Banning, N.C., Maccarone, L.D., Fisk, L.M., Murphy, D.V., 2015. Ammonia-oxidising bacteria not archaea dominate nitrification activity in semi-arid agricultural soil. Scientific Reports 5, 11146.

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

- Barton, L., Gleeson, D.B., Maccarone, L.D., Zúñiga, L.P., Murphy, D.V., 2013. Is liming soil a strategy for mitigating nitrous oxide emissions from semi-arid soils? Soil Biology & Biochemistry 62, 28–35.
- Brauer, T., Grootes, P.M., Nadeau, M.J., 2013. Origin of subsoil carbon in a Chinese paddy soil chronosequence. Radiocarbon 55, 1058–1070.
- Buyer, J.S., Sasser, M., 2012. High throughput phospholipid fatty acid analysis of soils. Soil Biology & Biochemistry 61, 127–130.
- Chen, M.P., Sun, F., Shindo, J., 2016. China's agricultural nitrogen flows in 2011: environmental assessment and management scenarios. Resources Conservation and Recycling 111, 10–27.
- Coody, P.N., Sommers, L.E., Nelson, D.W., 1986. Kinetics of glucose-uptake by soil-microorganisms. Soil Biology & Biochemistry 18, 283–289.
- Cui, T.T., Li, Z.H., Wang, S.J., 2017. Effects of in-situ straw decomposition on composition of humus and structure of humic acid at different soil depths. Journal of Soils and Sediments 17, 2391–2399.
- De Vries, F.T., Shade, A., 2013. Controls on soil microbial community stability under climate change. Frontiers in Microbiology 4, 265.
- Di, H.J., Cameron, K.C., Shen, J.P., He, J.Z., Winefield, C.S., 2009. A lysimeter study of nitrate leaching from grazed grassland as affected by a nitrification inhibitor, dicyandiamide, and relationships with ammonia oxidizing bacteria and archaea. Soil Use and Management 25, 454–461.
- Di, H.J., Cameron, K.C., Shen, J.P., Winefield, C.S., O'Callaghan, M., Bowatte, S., He, J.Z., 2010. Ammonia-oxidizing bacteria and archaea grow under contrasting soil nitrogen conditions. FEMS Microbiology Ecology 72, 386–394.
- Doran, J.W., 1987. Microbial biomass and mineralization nitrogen distributions in notillage and plowed soils. Biology and Fertility of Soils 5, 68–75.
- DuPont, S., Beniston, J., Glover, J.D., Hodson, A., Culman, S.W., Lal, R., Ferris, H., 2014. Root traits and soil properties in harvested perennial grassland, annual wheat, and never-tilled annual wheat. Plant and Soil 381, 405–420.
- Fang, Y., Du, Y.L., Wang, J., Wu, A.J., Qiao, S., Xu, B.C., Zhang, S.Q., Siddique, K.H.M., Chen, Y.L., 2017. Moderate drought stress affected root growth and grain yield in old, modern and newly released cultivars of winter wheat. Frontiers in Plant Science 8, 672.
- Farrell, M., Hill, P.W., Wanniarachchi, S.D., Farrar, J.F., Bardgett, R.D., Jones, D.L., 2011. Rapid peptide metabolism: a major component of soil nitrogen cycling? Global Biogechemical Cycles GB3014.
- Farrell, M., Hill, P.W., Farrar, J., Deluca, T.H., Roberts, P., Kielland, K., Dahlgren, R., Murphy, D.V., Hobbs, P.J., Jones, D.L., 2013. Oligopeptides represent a preferred source of organic N uptake: a global phenomenon? Ecosystems 16, 133–145.
- Federle, T.W., Dobbins, D.C., Thornton-Manning, R.T., Jones, D.D., 1986. Microbial biomass, activity and community structure in subsurface soil. Ground Water 24, 365–374.
- Findlay, R.H., 2004. Determination of Microbial Community Structure Using Phospholipids Fatty Acid Profiles. Molecular Microbial Ecology Manual Volume: 4.08. Kluwer Academic Publishers, Netherlands, pp. 983–1004.
- Fischer, D., Uksa, M., Tischler, W., Kautz, T., Kopke, U., Schloter, M., 2013. Abundance of ammonia oxidizing microbes and denitrifiers in different soil horizons of an agricultural soil in relation to the cultivated crops. Biology and Fertility of Soils 49, 1243–1246.
- Fisk, L.M., Barton, L., Jones, D.L., Glanville, H.C., Murphy, D.V., 2015. Root exudate carbon was more effective than soil organic carbon at decreasing the risk of nitrogen loss in a semi-arid soil. Soil Biology & Biochemistry 88, 380–389.
- Fontaine, S., Barot, S., Barré, P., Bdioui, N., Mary, B., Rumpel, C., 2007. Stability of organic carbon in deep soil layers controlled by fresh carbon supply. Nature 450, 277–280.
- Frostegård, Å., Bååth, E., Tunlio, A., 1993. Shifts in the structure of soil microbial communities in limed forests as revealed by phospholipid fatty acid analysis. Soil Biology & Biochemistry 25, 723–730.
- Glanville, H.C., Hill, P.W., Maccarone, L.D., Golyshin, P.N., Murphy, D.V., Jones, D.L., 2012. Temperature and water controls on vegetation emergence, microbial dynamics, and soil carbon and nitrogen fluxes in a high Arctic tundra ecosystem. Functional Ecology 26, 1366–1380.
- Gocke, M.I., Huguet, A., Derenne, S., Kolb, S., Dippold, M.A., Wiesenberg, G.L.B., 2017. Disentangling interactions between microbial communities and roots in deep subsoil. Science of the Total Environment 575, 135–145.
- Hart, S.C., Stark, J., Davidson, E., Firestone, M.K., 1994. Nitrogen, mineralization, immobilization, and nitrification. In: Weaver, R., Angle, S., Bottomley, P. (Eds.), Methods of Soil Analysis. Part 2. Microbiology and Biochemical Properties. SSSA Book Series No 5. Soil Science Society of America, Inc., pp. 985–1018.
- He, J.Z., Hu, H.W., Zhang, L.M., 2012. Current insights into the autotrophic thaumarchaeal ammonia oxidation in acidic soils. Soil Biology & Biochemistry 55, 146–154.
- Herkelrath, W.N., Miller, E.E., Gardner, W.R., 1977. Water uptake by plants: I. Divided root experiments. Soil Science Society of America Journal 41, 1033–1038.
- Hill, P.W., Marshall, C., Williams, G.G., Blum, H., Harmens, H., Jones, D.L., Farrar, J.F., 2007. The fate of photosynthetically-fixed carbon *in Lolium perenne* L. grassland as modified by elevated CO<sub>2</sub> and sward management. New Phytologist 173, 766–777. Hinsinger, P., Bengough, A.G., Vetterlein, D., Young, I.M., 2009. Rhizosphere: biophysics,
- biogeochemistry and ecological relevance. Plant and Soil 32, 117–152. Hoang, D.T.T., Pausch, J., Razavi, B.S., Kuzyakova, I., Banfield, C.C., Kuzyakov, Y., 2016. Hotspots of microbial activity induced by earthworm burrows, old root channels, and their combination in subsoil. Biology and Fertility of Soils 52, 1105–1119.
- Hobley, E., Baldock, J., Hua, Q., Wilson, B., 2017. Land-use contrasts reveal instability of subsoil organic carbon. Global Change Biology 23, 955–965.

- Hodge, A., Berta, G., Doussan, C., Merchan, F., Crespi, M., 2009. Plant root growth, architecture and function. Plant and Soil 321, 153–187.
- Hoyle, F.C., Murphy, D.V., 2011. Synchrony of nitrogen supply: influence of organic residues and soil disturbance on microbial processes altering plant available nitrogen. Plant and Soil 347, 53–64.
- Jan, M.T., Roberts, P., Tonheim, S.K., Jones, D.L., 2009. Protein breakdown represents a major bottleneck in nitrogen cycling in grassland soils. Soil Biology & Biochemistry 41, 2272–2282.
- Jarvis, N., Forkman, J., Koestel, J., Katterer, T., Larsbo, M., Taylor, A., 2017. Long-term effects of grass-clover leys on the structure of a silt loam soil in a cold climate. Agriculture Ecosystems & Environment 247, 319–328.
- Jones, D.L., Willett, V.B., 2006. Experimental evaluation of methods to quantify dissolved organic nitrogen (DON) and dissolved organic carbon (DOC) in soil. Soil Biology & Biochemistry 38, 991–999.
- Jones, D.L., Owen, A.G., Farrar, J.F., 2002. Simple method to enable the high resolution determination of total free amino acids in soil solutions and soil extracts. Soil Biology & Biochemistry 34, 1893–1902.
- Jones, D.L., Kemmitt, S.J., Wright, D., Cuttle, S.P., Bol, R., Edwards, A.C., 2005. Rapid intrinsic rates of amino acid biodegradation in soils are unaffected by agricultural management strategy. Soil Biology & Biochemistry 37, 1267–1275.
- Jones, D.L., Clode, P.L., Kilburn, M.R., Stockdale, E.A., Murphy, D.V., 2013. Competition between plant and bacterial cells at the microscale regulates the dynamics of nitrogen acquisition in wheat (*Triticum aestivum*). New Phytologist 200, 796–807.
- Kaiser, C., Kilburn, M.R., Clode, P.L., Cliff, J.B., Solaiman, Z.M., Koranda, M., Fuchslueger, L., Murphy, D.V., 2015. Exploring the transfer of recent plant photosynthates to soil microbes: mycorrhizal pathway versus direct root exudation. New Phytologist 205, 1537–1551.
- Kell, D.B., 2011. Breeding crop plants with deep roots: their role in sustainable carbon, nutrient and water sequestration. Annals of Botany 108, 407–418.
- Kemmitt, S.J., Wright, D., Goulding, K.W.T., Jones, D.L., 2006. pH regulation of carbon and nitrogen dynamics in two agricultural soils. Soil Biology & Biochemistry 38, 898–911.
- Kinoshita, R., Schindelbeck, R.R., van Es, H.M., 2017. Quantitative soil profile-scale assessment of the sustainability of long-term maize residue and tillage management. Soil & Tillage Research 174, 34–44.
- Kinyangi, J., Solomon, D., Liang, B., Lerotic, M., Wirick, S., Lehmann, J., 2006. Nanoscale biogeocomplexity of the organomineral assemblage in soil. Soil Science Society of America Journal 70, 1708–1718.
- Kramer, C., Gleixner, G., 2008. Soil organic matter in soil depth profiles: distinct carbon preferences of microbial groups during carbon transformation. Soil Biology & Biochemistry 40, 425–433.
- Kramer, S., Marhan, S., Haslwimmer, H., Ruess, L., Kandeler, E., 2013. Temporal variation in surface and subsoil abundance and function of the soil microbial community in an arable soil. Soil Biology & Biochemistry 61, 76–85.
- Kresović, M.M., Antić-Mladenović, B.S., Ličina, D.V., 2005. Aerobic and anaerobic incubation biological indexes of soil nitrogen availability. Proceeding of the National Academy of Sciences. Matica Srpska Novi Sad 109, 45–57.
- Large, E.C., 1954. Growth stages in cereals: illustration of the Feekes scale. Plant Pathology 3, 128–129.
- Lassaletta, L., Billen, G., Grizzetti, B., Anglade, J., Garnier, J., 2014. 50 year trends in nitrogen use efficiency of world cropping systems: the relationship between yield and nitrogen input to cropland. Environmental Research Letters 9, 105011.
- Lavania, U.C., Lavania, S., 2009. Sequestration of atmospheric carbon into subsoil horizons through deep-rooted grasses - vetiver grass model. Current Science 97, 617–618.
- Li, C.H., Yan, K., Tang, L.S., Jia, Z.J., Li, Y., 2014. Change in deep soil microbial communities due to long-term fertilization. Soil Biology & Biochemistry 75, 264–272.
- Li, X.Y., Simunek, J., Shi, H.B., Yan, J.W., Peng, Z.Y., Gong, X.W., 2017. Spatial distribution of soil water, soil temperature, and plant roots in a drip-irrigated intercropping field with plastic mulch. European Journal of Agronomy 83, 47–56.
- Liu, C., Wang, K., Zheng, X., 2013. Effect of nitrification inhibitors (DCD and DMPP) on nitrous oxide emission, crop yield and nitrogen uptake in a wheat-maize cropping system. Biogeosciences 10, 2427–2437.
- Liu, J., Zhang, Q.C., Li, Y., Di, H.J., Xu, J.M., Li, J.Y., Guan, X.M., Xu, X.Y., Pan, H., 2016. Effects of pasture management on soil fertility and microbial communities in the semi-arid grasslands of Inner Mongolia. Journal of Soils and Sediments 16, 235–242.
- Loeppmann, S., Biagodatskaya, E., Pausch, J., Kuzyakov, Y., 2016. Enzyme properties down the soil profile - a matter of substrate quality in rhizosphere and detritusphere. Soil Biology & Biochemistry 103, 274–283.
- Lynch, J.P., Wojciechowski, T., 2015. Opportunities and challenges in the subsoil: pathways to deeper rooted crops. Journal of Experimental Botany 66, 2199–2210.
- Martens-Habbena, W., Berube, P.M., Urakawa, H., José, R., Stahl, D.A., 2009. Ammonia oxidation kinetics determine niche separation of nitrifying Archaea and Bacteria. Nature 461, 976–979.
- Matus, F., Rumpel, C., Neculman, R., Panichini, M., Mora, M.L., 2014. Soil carbon storage and stabilisation in andic soils: a review. Catena 120, 102–110.
- Menichetti, L., Ekblad, A., Katterer, T., 2015. Contribution of roots and amendments to soil carbon accumulation within the soil profile in a long-term field experiment in Sweden. Agriculture Ecosystems & Environment 200, 79–87.
- Miranda, K.M., Espey, M.G., Wink, D.A., 2001. A rapid, simple spectrophotometric method for simultaneous detection of nitrate and nitrite. Nitric Oxide Biology and Chemistry 5, 62–71.
- Muller, K., Kramer, S., Haslwimmer, H., Marhan, S., Scheunemann, N., Butenschon, O., Scheu, S., Kandeler, E., 2016. Carbon transfer from maize roots and litter into bacteria and fungi depends on soil depth and time. Soil Biology & Biochemistry 93, 79–89.

Mulvaney, R.L., 1996. Nitrogen- inorganic forms. In: Sparks, D.L. (Ed.), Methods of Soil Analysis. Part3. Soil Society of America Inc., Madison, WI, pp. 1123–1184.

- Murphy, D.V., Sparling, G.P., Fillery, I.R.P., 1998. Stratification of microbial biomass C and N and gross mineralization with soil depth in two contrasting western Australian agriculture soils. Australian Journal of Soil Research 36, 45–55.
- Nicol, G.W., Leininger, S., Schleper, C., Prosser, J.I., 2008. The influence of soil pH on the diversity, abundance and transcriptional activity of ammonia oxidizing archaea and bacteria. Environmental Microbiology 10, 2966–2978.
- Pereira e Silva, M.C., Poly, F., Guillaumaud, N., van Elsas, J.D., Salles, J.F., 2012. Fluctuations in ammonia oxidizing communities across agricultural soils are driven by soil structure and pH. Frontiers in Microbiology 3, 77.
- Pierret, A., Maeght, J.L., Clement, C., Montoroi, J.P., Hartmann, C., Gonkhamdee, S., 2016. Understanding deep roots and their functions in ecosystems: an advocacy for more unconventional research. Annals of Botany 118, 621–635.
- Pinggera, J., Geisseler, D., Piepho, H.P., Joergensen, R.G., Ludwig, B., 2015. Effect of substrate quality on the N uptake routes of soil microorganisms in different soil depths. Pedobiologia 58, 211–218.
- Prosser, J.I., Nicol, G.W., 2012. Archaeal and bacterial ammonia-oxidisers in soil: the quest for niche specialisation and differentiation. Trends in Microbiology 20, 523–531.
- Rawls, W.J., Pachepsky, Y.A., Ritchie, J.C., Sobecki, T.M., Bloodworth, H., 2003. Effect of soil organic carbon on soil water retention. Geoderma 116, 61–76.
- Rumpel, C., Kogel-Knabner, I., 2011. Deep soil organic matter-a key but poorly understood component of terrestrial C cycle. Plant and Soil 338, 143–158.
- Salome, C., Nunan, N., Pouteau, V., Lerch, T.Z., Chenu, C., 2010. Carbon dynamics in topsoil and subsoil may be controlled by different regulatory mechanisms. Global Change Biology 16, 416–426.
- Sanaullah, M., Chabbi, A., Maron, P.A., Baumann, K., Tardy, V., Blagodatskaya, E., Kuzyakov, Y., Rumpel, C., 2016. How do microbial communities in top-and subsoil respond to root litter addition under field conditions? Soil Biology & Biochemistry 103, 28–38.
- Sartain, B.J., Obrezai, A.T., 2010. Improving nitrogen and phosphorus fertilizer use efficiency of Florida's horticultural crops. Hort Technology 20, 23–33.
- Schrumpf, M., Kaiser, K., Guggenberger, G., Persson, T., Kogel-Knabner, I., Schulze, E.D., 2013. Storage and stability of organic carbon in soils as related to depth, occlusion within aggregates, and attachment to minerals. Biogeosciences 10, 1675–1691.
- Smucker, A.J.M., Nunez-Barrios, A., Ritchie, J.T., 1991. Root dynamics in drying soil environment. Belowground Ecology 1, 1–5.
- Soudi, B., Sbai, A., Chiang, C.N., 1990. Nitrogen mineralization in semiarid soils of Morocco: rate constant variation with depth. Soil Science Society of America Journal 54, 61–756.

- Stone, M.M., DeForest, J.L., Plante, A.F., 2014. Changes in extracellular enzyme activity and microbial community structure with soil depth at the Luquillo Critical Zone Observatory. Soil Biology & Biochemistry 75, 237–247.
- Tang, C.X., Conyers, M.K., Nuruzzaman, M., Poile, G.J., Liu, D.L., 2011. Biological amelioration of subsoil acidity through managing nitrate uptake by wheat crops. Plant and Soil 338, 383–397.
- Taylor, H.M., Klepper, B., 1975. Water uptake by cotton root system: an examination of the assumption in the single root model. Soil Science 120, 57–67.
- Torres-Sallan, G., Schulte, R.P.O., Lanigan, G.J., Byrne, K.A., Reidy, B., Simo, I., Six, J., Creamer, R.E., 2017. Clay illuviation provides a longterm sink for C sequestration in subsoils. Scientific Reports 7, 45635.
- Uksa, M., Fischer, D., Welzl, G., Kautz, T., Kopke, U., Schloter, M., 2014. Community structure of prokaryotes and their functional potential in subsoils is more affected by spatial heterogeneity than by temporal variations. Soil Biology & Biochemistry 75, 197–201.
- Uksa, M., Schloter, M., Kautz, T., Athmann, M., Kopke, U., Fischer, D., 2015. Spatial variability of hydrolytic and oxidative potential enzyme activities in different subsoil compartments. Biology and Fertility of Soils 51, 517–521.
- Van Egmond, N.D., Bresser, A.H.M., Bouman, A.F., 2002. The European nitrogen case. Ambio 31, 72–78.
- Wang, J.C., Zhang, L., Lu, Q., Raza, W., Huang, Q.W., Shen, Q.R., 2014. Ammonia oxidizer abundance in paddy soil profile with different fertilizer regimes. Applied Soil Ecology 84, 38–44.
- Ward, S.E., Smart, S.M., Quirk, H., Tallowin, J.R.B., Mortimer, S.R., Shiel, R.S., Wilby, A., Bardgett, R.D., 2016. Legacy effects of grassland management on soil carbon to depth. Global Change Biology 22, 2929–2938.
- Waring, S.A., Bremner, J.M., 1964. Ammonium production in soil under waterlogged conditions as an index of N availability. Nature 201, 951–952.
- Yao, H., Gao, Y., Nicol, G.W., Campbell, C.D., Prosser, J.I., Zhang, L., Han, W., Singh, B.K., 2011. Links between ammonia oxidizer community structure, abundance, and nitrification potential in acidic soils. Applied and Environmental Microbiology 77, 4618–4625.
- Zadoks, J.C., Chang, T.T., Konzak, C.F., 1974. A decimal code for the growth stages of cereals. Weed Research 14, 415–421.
- Zhang, L.M., Hu, H.W., Shen, J.P., He, J.Z., 2012. Ammonia-oxidizing archaea have more important role than ammonia-oxidizing bacteria in ammonia oxidation of strongly acidic soils. The ISME Journal 6, 1032–1045.
- Zhang, H.L., Lal, R., Zhao, X., Xue, J.F., Chen, F., 2014. Opportunities and challenges of soil carbon sequestration by conservation agriculture in China. Advances in Agronomy 124, 1–36.