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

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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_2-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

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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^{-3} and in the subsoil (30–60 cm) 1.63 ± 0.10 g cm^{-3}. 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^{-1}. 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^{-1} as ammonium nitrate, 80 kg K ha^{-1}, 28 kg P ha^{-1}) 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 m × 0.5 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_2 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 × 20 cm clear plastic plates and root length determined with WinRhizo® (Regent Instruments Inc., Ville de Québec, Canada).

Basal respiration was determined on field-moist soil (50 cm^3) 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 quasi-stabilized.