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Seasonal variation in soluble soil carbon and nitrogen across a grassland productivity gradient

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ABSTRACT

Understanding the fate and turnover of the pools that comprise dissolved organic nitrogen (DON) in soil is key to determining its role in ecosystem functioning. We investigated seasonal changes of dissolved organic carbon (DOC) and nitrogen (DON) concentrations within four molecular weight (MW) size fractions across an altitudinal gradient (from lowland to montane systems), and quantified individual amino acids and amino acid constituents of oligopeptidic-N, as well as nitrate and ammonium. We tested two ideas: first, that DON is more abundant than DIN in low-productivity relative to high-productivity grassland ecosystems; and second, that the abundance of peptides and amino acids is likewise greater in low- than high-productivity grassland. The most productive site had a history of inorganic fertiliser application, and hence in this site alone DIN was more abundant than DON. Plant productivity varied 3-fold between the other sites, and DON was generally at higher concentrations in the sites of lower productivity both in absolute terms as well as relative to DIN, with a large increase observed in spring. The fraction containing the highest concentration of the DON had a MW of >100 kDa, and in summer and autumn this fraction was more abundant at the lowest productivity site. We conclude that relationships between the abundance of DON relative to DIN and ecosystem productivity is dependent on season, and hence more complex than previously suggested, and that peptides are a dynamic and potentially nutritionally significant component of DON.

1. Introduction

An ever-increasing body of evidence from many ecosystems shows that a small fraction of dissolved organic nitrogen (DON) in soils: free amino acids (FAAs), may be directly assimilated by plants (Kielland, 1994; Näsholm et al., 1998; Nordin et al., 2001; Weigelt et al., 2003, 2005), circumventing the microbially mediated and potentially rate-limiting mineralisation step. Whilst DON is a large fraction of total dissolved nitrogen (TDN) in most agricultural soils, it still represents only ca. 30% of the TDN pool (Christou et al., 2005). However, in nutrient limited soils, DON can make up the bulk of TDN, and is therefore the N species of greatest interest in natural ecosystems in terms of N cycling and potential plant uptake.

DON is not one pool, but a heterogeneous mixture of compounds, the most studied of which are the free amino acids (FAAs) (Kielland, 1995; Bardgett et al., 2003; Jones et al., 2004, 2009; Näsholm et al., 2009; Geisseler et al., 2010), which are generally < 5% of total DON (Jan et al., 2009; Jones et al., 2009). Of the remaining un-characterised DON, ^{15}N NMR studies have indicated that the majority is peptidic or proteinaceous moieties (Kögel-Knaber, 2006; Nannipieri and Eldor, 2009). Jämtgard et al. (2010) quantified soil-solution proteins to be in the order of 50 times the concentration of soil-solution amino acids in agricultural soils, forming a much larger reservoir of potentially utilisable N. However, Jan et al. (2009) observed protein mineralisation rates in an agricultural soil to be 50-times slower than those of an amino acid mixture – further emphasising the need to focus on purely directly-assimilatable N to ascertain a complete picture of immediate N availability. As the high rate of turnover of FAAs (Jones et al., 2009) mirrors turnover rates of organic acids such as oxalate and citrate, it has been suggested that a split of the DON pool between LMW (<1 kDa) and HMW (>1 kDa) compounds be used when investigating the rate of turnover and ecological role of the DON pool (Jones et al., 2004). The LMW fraction would therefore be limited to peptide chain lengths of ca. 2–12 amino acids.

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Direct utilisation of peptides by microbes may be limited by their Stoke's radius (the effective size of the molecule), with a cutoff roughly equivalent to 650 Da (Payne, 1980; Weiss et al., 1991). A 1 kDa filter may therefore select peptides that could potentially be assimilated intact, rather than considering the hydrolysable-DON pool as a whole. The ericoid mycorrhizae are able to directly mobilise and assimilate oligopeptides intact and translocate this N to plant roots (Bajwa and Read, 1985). It is therefore clear that in order to increase our understanding of the nutritive function of the LMW DON pool, quantification of oligopeptidic-N is required. Whilst several authors have quantified combined amino acids as peptides or proteins using acid hydrolysis followed by HPLC or GC detection (Yu et al., 2002; Amelung et al., 2006; Roberts and Jones, 2008; Jämtgard et al., 2010), to our knowledge only Isnor and Warman (1990) did molecular weight (MW) fractionation of extracted peptides to <5 kDa prior to hydrolysis. Smolander and Kitunen (2002) investigated size fractionation of DON in a podzol in a forest ecosystem, and found the fraction with the highest concentration to be <1 kDa, with most DOC in the 10–100 kDa fraction. This appears to be the only study that has quantified size fractionation of DON, albeit in this case in a water extract.

There are very few studies quantifying individual amino acid pools and their changes either between soil types or over time, and those that do exist are on radically different timescales: either hours in laboratory studies (Jones et al., 2009) or months for field work (Bardgett et al., 2002), and we cannot yet causally link these timescales. Basic, neutral, and acidic amino acids diffuse through the soil at different rates (Owen and Jones, 2001), and plants take up individual amino acids at different rates (Persson and Näsholm, 2001; Weigelt et al., 2005; Harrison et al., 2007). Jones et al. (2009) demonstrate that rates of bulk FAA turnover are conserved across a global latitudinal gradient when water and temperature restraints are removed, whereas Warren and Taranto (2010) argue that questions regarding the role of amino acids in ecosystem N cycling require amino acids to be considered individually. Amino acid concentrations may vary seasonally (Kielland, 1995; Weintraub and Schimel, 2005) or remain relatively constant (Werdin-Pfisterer et al., 2009). Whilst Warren and Taranto (2010) found large variations in DIN and FAA concentrations between months in sub-alpine grasslands; no overall seasonal patterns emerged.

It is therefore clear that DON is not a single component of soil N, that FAAs are not the only ecologically significant part of DON, and that even FAAs should possibly be considered individually. Here, our main objective was to determine seasonal variation in the size and form of DON and DOC, and in particular to test the notion that the availability (and hence potential plant use) of DON (in the form of peptides and amino acids) relative to DIN is greatest in low productivity ecosystems. This was tested using an altitudinal gradient along which primary productivity decreased with increased altitude and vegetation changed from *Lolium perenne* dominated pasture at 15 m above sea level (asl) to *Festuca ovina* and *Tricophorum cespitosum* dominated upland grassland at 710 m asl. We hypothesise, following Smolander and Kitunen (2002), that the bulk of DON will be <10 kDa, whereas the bulk of DOC will be >10 kDa. Further, we predict distinct seasonal changes due to seasonal plant growth and climatic patterns, and the direct and indirect effects these have on the soil. Given the differing information about seasonal variations in the soil AA pool (Werdin-Pfisterer et al., 2009; Warren and Taranto, 2010), we hypothesise that FAAs and peptidic-AAs vary between sites and seasons due to differences in DON turnover rates and plant inputs. Due to the fact that peptides may be broken down to FAAs extracellularly by soil microbes, we predict correlation between the FAA- and oligopeptidic-AA pools, in that patterns in FAAs remain conserved to those observed in the constituent peptidic-AAs.

2. Materials and methods

2.1. Site characterisation

An altitudinal gradient was established on grassland up a north-facing slope above Abergwyngregyn, Gwynedd, UK (53°14'N; 4°10'W); above-ground net primary productivity (ANPP) decreased with increased elevation. Five sites were selected with different soil characteristics along this gradient, with four randomly positioned replicate plots (10 m^2) at each site. Although our study is based on a single elevation gradient, it was selected to include a broad range of vegetation, soil and climatic conditions representative of wet, lowland, sub-montane, and montane regions of western Britain (Rodwell, 1992). The soils along this gradient cover 90% of the total area of Wales (Rudeforth et al., 1984). In order to minimise the sources of error associated with the use of a single gradient, we used multiple, randomly-located sites at each position along the elevation gradient. Mean annual temperature ranged from 9.8 °C at Site 1 to 6.5 °C at Site 5, with annual rainfall ranging from 800 mm at Site 1 to 2300 mm at Site 5. Vegetation was recorded as percentage cover using 1 m^2 quadrats at each plot, and ranged from *L. perenne* L./*Trifolium repens* L. dominated pasture at Sites 1 and 2 to *Agrostsis canina* L./*Agrostis capillaris* L./*Anthoxanthum odoratum* L./*Potentilla erecta* (L.) Rauschel grassland at Site 3, to *F. ovina* L./*Juncus effusus* L. dominated moorland at Site 4, with *F. ovina*/*T. cespitosum* (L.) Hartman dominated moorland at Site 5 (see Supplementary Information S1 for species list). Above-ground standing biomass and net annual primary productivity (ANPP) were determined between April and October 2009 according to Vile et al. (2006). Briefly, above-ground vegetation was harvested at the start of the growing season, and at the end of the growing season from an adjacent area from which large grazers were excluded by cages. The difference was expressed as a mean growth rate in g dry matter m^{-2} d^{-1}. In order to account for anthropogenic inputs of atmospheric N, which at the higher sites could confound interpretation, we also estimated atmospheric N deposition at each of the sites by interpolation from the maps in NEGTAP (2001), with a 23% increase to account for organic N deposition (Gonzalez Benitez et al., 2009) not quantified in the NEGTAP survey.

2.2. Soil sampling and analysis

Soil samples were taken from the top 15 cm of each plot in December 2008, April 2009, July 2009 and October 2009, sealed in sample bags and refrigerated at 4 °C until analysis. Soil pH and electrical conductivity (EC; 1:2 v/v soil:distilled water) were determined with standard electrodes. Moisture content was determined after drying at 80 °C for 72 h, and organic matter as loss on ignition at 450 °C for 16 h. Bulk density was determined using 100 cm^3 cores (Rowell, 1994). Total C and N of both soil and vegetation were determined using a Carlo Erba NA 1500 Elemental Analyzer (Thermo Fisher Scientific, Milan, Italy). Extractable potassium (K) and phosphorus (P) were extracted using a 0.5 M acetic acid (1:5 w/v) shaken for 1 h, then centrifuged for 10 min at 3220 *g* before passing through a Whatman 42 filter (Quevauviller, 1998). Potassium was analysed by flame emission spectroscopy (Sherwood 410 flame photometer: Sherwood Scientific, Cambridge, UK) and P by the colourimetric method of Murphy and Riley (1962).

Soil solution was extracted within 6 h of collection using the centrifugal-drainage method of Giesler and Lundström (1993). Briefly, ca. 1 kg field-moist soil was centrifuged at 3220 *g* for 30 min, in containers drilled to facilitate drainage of the soil solution into a collection vessel below. This method extracts plant-available water, in contrast to other extraction methods which may overestimate pool sizes (Jones and Willett, 2006). Soil solutions were then sterile-filtered (0.2 μm Whatman GDX Sterile PES filters) into