Pools and Fluxes: A snapshot of nitrogen dynamics in Australian soils

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Abstract
Nitrogen (N) uptake by plants has been researched for well over a century, and continues to be of central importance both from an agricultural productivity and an environmental pollution perspective. Due to its prevalence as a fertilizer, mineral N is usually the only form routinely quantified in soil fertility assessments, despite significant quantities of dissolved organic N (DON) often being present. In the present study, we collected 358 topsoil samples from 89 sites under 13 different land uses. We quantified a wide range of soil N properties including pools and fluxes of organic and inorganic N to develop a better understanding of N cycling in Australian soils. Though nitrate dominated in some land uses, DON and free amino acid-N (FAA-N) were present in significant quantities in most land uses. Rates of N cycling were rapid in most soils, with only very nutrient-poor arid zone soils having particularly low N flux rates. Further research is required to better understand the availability of DON and its accessibility to plants.

Key Words
Dissolved organic nitrogen, DON, Proteolysis, Potentially mineralisable nitrogen, Land use, Microbial biomass nitrogen

Introduction

Despite well over a century of research, our understanding of the soil nitrogen (N) cycle is still evolving, driven by multiple needs and opportunities. These research needs include the requirement to increase nutrient use efficiency (NUE) in agricultural systems from a global average of only ca. 50% of applied N (Galloway *et al.* 2004), and in many areas a need to better understand the consequences of excess N on natural ecosystem processes (Vitousek *et al.* 1997). Research opportunities include our ever expanding knowledge of the mechanisms involved in N cycling, including improved tools to quantify and characterize both pools and fluxes of N (Warren 2014).

Nitrogen availability in soils is often defined as the pool of mineral N that can be extracted with a salt extract, and in agronomic systems it is this metric which is often used to inform N fertilization rates. However, this fails to take into consideration the dissolved organic N (DON) pool, which can be large relative to mineral N even in agricultural systems (Murphy *et al.* 2000; Macdonald *et al.* 2016), and a portion of which can be accessed by crops (Robinson *et al.* 2011; Hill *et al.* 2011). This is surprising, given work conducted at the turn of the 20th century which demonstrated the ability of plants to acquire organic N (Hutchinson and Miller 1912). Of the DON species present in soil, free amino acids (FAAs) are the most studied. Despite their generally small pool size (ca. 10 µM in the soil solution), both they and their oligopeptides cycle rapidly (Farrell *et al.* 2013), and as a consequence we know that pool size alone can be a poor measure of importance.

There is a lack of quantitative information available on the concentrations and flux rates of organic N in Australian soils, and contrasts in organic N status between land uses are poorly understood. Consequently, our aim was to characterise soils from a rage of land uses across Australia, with a particular focus on their organic N pools and fluxes. We hypothesized that despite expected high mineral N pools in some of the more intensive land uses, there would be a significant pool of organic N that cycled rapidly.

Methods

Site selection and sampling
We sampled topsoil (0-10 cm) from 89 Australian sites across 13 land uses in the austral autumn of 2015. At each site, four replicate samples (>1 kg) were collected using a shovel. Samples were immediately refrigerated (<4˚C) before processing and analysis within one week as detailed below.

Soil characterisation
Upon arrival in the laboratory, samples were immediately processed to reduce the amount of change occurring as a result of sample storage. To estimate plant available N pools, a 0.5 M K_2SO_4 extract was performed on the fresh soil from each sample (358 samples in total for the study). Mineral N was quantified on the extract by colorimentry, FAA-N by fluorimetry, and total dissolved N (TDN) by high temperature combustion. Dissolved organic N was calculated by subtracting mineral- and FAA-N from TDN. Dissolved organic carbon (DOC) was quantified on the same extract by high temperature combustion. $CHCl_3$-fumigated subsamples were also extracted with a 0.5 M K_2SO_4 and C and N quantified by high temperature combustion to estimate microbial biomass C and N (Voroney *et al.* 2008). After air-drying at 40˚C, electrical conductivity (EC) and pH were quantified in 1:2 w/v soil:solution slurries of 18.2 MΩ water or 0.01 M $CaCl_2$ respectively using standard electrodes. Total organic C and total N were quantified on finely ground air-dried soil by Leco dry combustion. Mid infra-red analysis (MIR) was used to both spectrally characterise the air-dried finely ground soil, and also to isolate carbonate-bearing samples (Baldock *et al.* 2013a). These were then pre-treated with H_2SO_3 to remove carbonates before re-analysis by Leco as above (Baldock *et al.* 2013b).

In addition to the state measurements outlined above, several quantitative rate assays were performed to understand N fluxes in these soils. Firstly, a traditional potentially mineralizable N (PMN) assay was perfumed using the anaerobic incubation assay of Curtin and Campbell (2008). Proteolysis rate, a measure of extracellular depolymerisation of proteins to amino acids was conducted according to Hofmockel *et al.* (2010). ^{14}C mineralization assays (Farrell *et al.* 2013; Macdonald *et al.* 2014; Farrell *et al.* 2014) were used to quantify the rate of mineralisation and partitioning of low molecular weight DON components (oligopeptides and FAAs).

Statistical analysis
Extractable N pools for each land use were ranked by TDN, and then 95% confidence intervals were calculated for each pool (DON, FAA-N, NH_4^+, NO_3^-) to illustrate differences. In order to understand overall differences and similarities in the samples across land uses, multivariate statistical analysis was employed using the Primer 7 software (Clarke 1993). Differences between the 358 samples were explored using non-metric multi-dimensional scaling (nMDS) on Euclidean distance for all variables measured, after the dataset transformed as appropriate (ln[x+1]) for most variables and normalised. Non-parametric multivariate analysis of similarities (ANOSIM) was used to identify differences between land use types across the whole dataset.

Results and Discussion
As was expected from the huge variety of soils and land uses in Australia, there was wide range of values across the samples. Organic C content ranged from 0.53 g/kg on a coastal heath soil from Queensland (Qld) to 450 g/kg in a rainforest soil also from Qld. Total N contents ranged from 0.03 g/kg in the same coastal heath soil to 16.2 g/kg in a wetland soil from Qld. C:N ratios also varied greatly, ranging from 5.3 in an arid zone sample from South Australia to 39 in a forest soil from New South Wales. Soils ranged in pH from 2.7 to 8.46, and in salinity (as electrical conductivity) from 13 µS/cm to 10 mS/cm. Consequently, it can be seen that we captured a wide variety of soils in this study.

Dissolved organic N as a proportion of TDN ranged from below limit of detection through to over 90% of the extractable pool, highlighting the wide range of dominance from mineral N through to DON. As can be seen from Figure 1, though nitrate dominated the extractable N pool in some of the higher TDN-bearing land uses (horticulture, cropping), forests, pastures and dairy land uses all had more balanced extractable N pool chemistries. It should be noted that all cropping-based systems (inclusive of sugar, cotton and rice) were sampled when fallow, so these pools may not represent values as observed during the growth season.

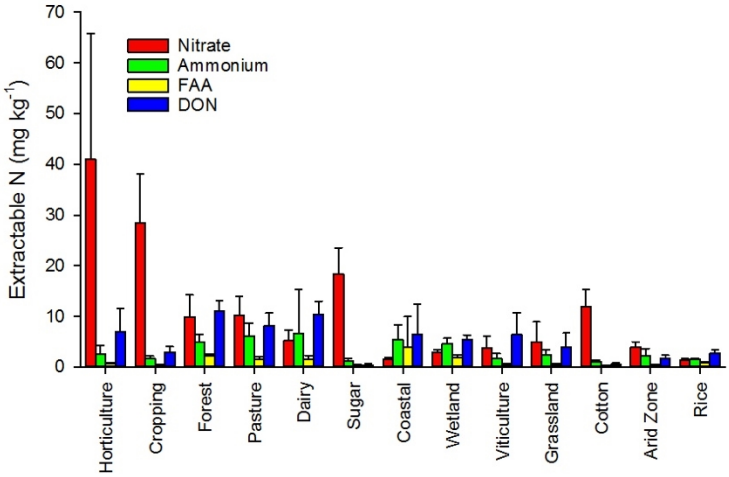

Figure 1. Extractable N pools in soils from the 13 land uses, ranked L-R by TDN. Values are means ($n \geq 8$) ± 95% confidence intervals. FAA = Free amino acids, DON = Dissolved organic nitrogen, minus FAAs.

While pool sizes provide some information on the N status of the soil, flux rates are also important to understand rates of production and consumption of different N forms in the soil. Hypothesized as the rate limiting step in soil N cycling (Jan *et al.* 2009), extracellular proteolysis represents an important metric to complement traditional measures of N mineralization such as PMN. In the present study, we observed rates of proteolysis ranging from 0.01 mg/kg/h in a desert dune sample from SA to 37 mg/kg/h in a Qld rainforest soil. The anaerobic PMN assay (Curtin and Campbell 2008) gave estimates of N mineralization ranging from 0.01 to 12.2 mg/kg/d. These rates are much lower than the rates of proteolysis estimated by the more targeted method of Hofmockel *et al.* (2010), indicating potential problems to directly comparing rates from these two metrics of available N production. Mineralization of FAA-N and peptide-N was universally rapid ($t_{1/2} \leq 1$ d) for the 10 µM spike added, with turnover rates much slower in the more arid systems. Partitioning of C from the labelled FAA or peptide ranged from 33-78% of the C being immobilized after the 7 d incubation.

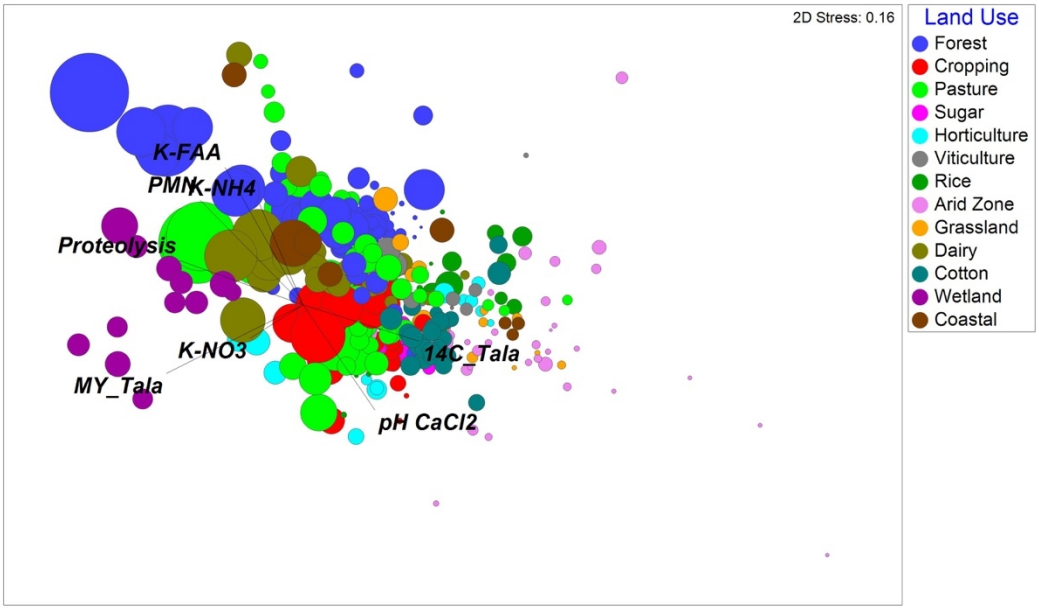

Figure 2. non-Metric Multi-Dimensional Scaling (nMDS) two-dimensional plot of a Euclidean distance matrix of all 18 variables, with "bubble size" proportional to proteolysis rate (larger bubbles = faster rate). A reduced number of variable vectors are shown for clarity. K-FAA = K_2SO_4 extractable free amino acids, K-NH4 = K_2SO_4 extractable ammonium, K-NO3 = K_2SO_4 extractable nitrate, PMN = potentially mineralizable nitrogen, MY_Tala = carbon use efficiency of carbon derived from peptides, 14C_Tala = half-time of peptides in soil solution where larger value (longer half time) = slower rate.

In order to visualise and understand wider patterns across all variable for the 13 land uses investigated here, a multivariate statistical approach was taken. ANOSIM, a multivariate equivalent to univariate ANOVA (Clarke 1993) demonstrated a significant effect (ρ=0.348, P<0.001) of land use on the combined dataset. A non-metric multidimensional scaling (nMDS) plot was constructed from a Euclidean distance matrix of the

dataset to illustrate how land uses differed in relation to each other, with "bubble size" showing relative proteolysis rates (Figure 2). Clear separation of many of the land uses was observed. In particular, arid zone samples had high pH, longer turnover times of peptides and amino acids (as assessed by ^{14}C mineralization assays), and low standing N pools. Though it is unsurprising that such a wide variety of regionally dispersed land uses and soil types would differ in their chemistries and N processing rates, it is interesting to see that DON-associated variables such as proteolysis, FAA-N concentration, and amino acid and peptide turnover and partitioning were such important drivers of this separation.

Conclusions and Next Steps

Mobile, potentially available N consists of more than just the mineral N pools routinely measured. Here, we have demonstrated that FAA-N and bulk DON can be significant extractable N pools in soils under many land uses, and that fluxes through these pools appear rapid, thus confirming our hypothesis. Notably, rates of proteolysis were high in all but the most N-poor samples. Given that it has been demonstrated that even crops in highly productive systems can access DON for nutrition (Robinson *et al.* 2011; Hill *et al.* 2011; Brackin *et al.* 2015), further research is required to better understand the availability of this resource, and its accessibility to plants. In order to better understand whether inclusion of DON pools and fluxes as indices of plant available N improves prediction of plant uptake, a follow-on experiment is planned to assess plant N uptake in relation to the quantified N pools in these soils.

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