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Recovery of individual soil nitrogen forms after sieving and extraction

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ABSTRACT

Plant biomass production and species composition is largely regulated by the availability of soil nitrogen (N). Detailed knowledge about the concentrations and composition of soil N pools are crucial for better understanding plant N nutrition. One commonly applied method to characterize soil N pools is the extraction of soil with water or salt solutions. The apparent problem with this sampling technique is the disruption of the soil matrix and the natural equilibrium of soil N pools during sampling and sample handling. Sieving and homogenizing soils as well as the extraction procedure itself are known to alter soil N composition through transformations, losses and contamination. Until now, however, information on the impact of soil extraction on individual N forms is scarce. This study therefore aimed at estimating the effect of sieving and extraction with water or KCl on NH_4^+, NO_3^- and individual amino acids. Nine different soils including boreal forest, agricultural and grassland soils were used for a series of experiments. In detail, after initial estimation of inorganic N and amino acid pools in extracts, in two separate experiments a small amount of standard solution containing NH_4^+, NO_3^- and amino acids was added either directly to the extractant or to the soils before sieving and extraction and, subsequently, the recovery of standard added was determined. I found that a significant proportion of amino acids were not recovered in any of the treatments and, conversely, there was a significant increase of inorganic N. Sieving and extracting generally led to a lower recovery of amino acids and a stronger increase of inorganic N than extraction only. The recovery of individual N forms strongly depended on soil type, extractant and N form, indicating that a comparison of results from soil extractions between different soils should be done with care. Still, soil extraction can provide valuable information on total plant-available N, as the sum of N added could largely be recovered in all soils and treatments. Future studies investigating the availability of individual N forms in soil for plant uptake should be aware of possible errors introduced during sample handling to avoid misinterpretation.

1. Introduction

The availability of soil nitrogen (N) is one of the most important factors determining soil fertility and plant biomass production. A large proportion (>80%) of soil N is present in organic form (e.g., Schulten and Schnitzer, 1997) and is thus a key player in the terrestrial N cycle. Accordingly, during the last decade the role of organic N for plant nutrition has received increasing attention (Jones and Kielland, 2002; Jones et al., 2005; Chen and Xu, 2008; Näsholm et al., 2009; Rothstein, 2009; Gärdenäs et al., 2010; Warren and Taranto, 2010; Warren, 2013a, 2013b). One finding significantly advancing our view on the importance of organic N in soils was that vascular plants are capable of taking up significant quantities of organic N (Kielland, 1994; Näsholm et al., 1998; Lipson and Näsholm, 2001; Persson and Näsholm, 2001; Jämtgård et al., 2008; Paungfoo-Lonhienne et al., 2008), thereby bypassing the supposed bottleneck of N mineralization (Chapin et al., 1993; Jones and Darrah, 1993; Warren, 2006). Despite this capacity of plants to directly utilize organic N, research on plant N nutrition has traditionally focused on inorganic N, while organic N has received comparably little attention (reviewed by Paungfoo-Lonhienne et al., 2012). The majority of studies examining organic N as plant nutrient source commonly addressed either total dissolved organic N (DON) or one specific group of organic N. Amino acids, in particular, have been studied intensively due to their importance in total soil N turnover (Jones and Kielland, 2002), their relatively small size and the comparably rapid uptake rates by plants (cf. Näsholm et al., 2009; Svennerstam et al., 2011). The pool of amino acids in soil is highly dynamic as it is affected by a variety of processes, such as uptake by plants and immobilization by soil microbes, mineralization to inorganic N, depolymerization of high molecular-weight N compounds into amino acids, or input via root exudation and during turn-over and decomposition of litter, roots or soil microbes (Abuarghub and Read, 1988; Schimel et al.,

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2004; Jones et al., 2005; Weintraub and Schimel, 2005; Warren and Taranto, 2010). Accordingly, many amino acids have been shown to turn over within only a few hours (Jones, 1999; Lipson et al., 2001; Owen and Jones, 2001; Jones and Kielland, 2002). Besides amino acids soils contain a huge diversity of other organic N forms (Warren, 2013a, 2013b) which vary significantly between soils, soil microsites and along temporal scales. Estimating and comprehending this heterogeneity and diversity of plant available N in soil is fundamental for a better understanding of the N cycle at the soil–plant interface. However, scientists are facing a challenge that until now remains hard to overcome: To choose the most suitable method for estimating soil N concentrations.

Traditionally, soil N pools were, and still are, estimated by removing soil from the field, subsequently extracting soil with water or salt solutions and analyzing these extracts. One apparent problem with this method is the disruption of the natural soil structure during sampling and sample preparation, thereby introducing a range of errors, not least the significant alteration of the natural equilibrium of the soil N composition as a consequence of transformations, losses and contamination (Miro and Frenzel, 2011). Most commonly, soil samples have to undergo additional treatment (e.g., sieving and homogenizing, filtration, pH buffering, freezing and thawing, derivatization, drying, grinding) before chemical analyses. These handling procedures increase the risk of introducing additional errors resulting in reduced reliability of the analytical results (Jones and Willett, 2006; Miro and Hansen, 2006; Warren and Taranto, 2010; Carillo-Gonzalez et al., 2013; Makarov et al., 2013). For example, continuous production and decomposition processes during sampling and sample handling might lead to an over- or underestimation of available nutrient pools (Jämtgård et al., 2010; Rousk and Jones, 2010). Further, extracting soil with salt solutions have been shown to produce different results depending on sieving procedure, extractant strength, soil-to-extractant ratio and extraction time (Stevens and Laughlin, 1995; Reemtsma et al., 1999; Jones and Willett, 2006; Rousk and Jones, 2010; Warren and Taranto, 2010; Carrillo-Gonzales et al., 2013; Chen and Williams, 2013; Makarov et al., 2013). To allow comparison of results from soil extraction among different studies Jones and Willett (2006) recommended using a standardized extraction method. However, recent evidence has shown that different soils respond differently to soil extraction (Chantigny, 2003; Willett et al., 2004; Jones and Willett, 2006). These problems are well known and it is increasingly recognized that the results from disruptive soil sampling (especially the removal of soil from its natural environment and subsequent soil homogenization) preceding soil analyses may only marginally reflect nutrient availabilities and dynamics in situ (Miro and Frenzel, 2011; Inselsbacher and Näsholm, 2012; Hobbie and Hobbie, 2013). In the case of amino acids, Hobbie and Hobbie (2012) inferred that destructive soil sampling followed by processing introduces artefacts such that the size and composition of the amino acid pool deviate from those in situ.

Not surprisingly, in recent years several alternative methods for estimating soil N pools emerged, including soil centrifugation, in-situ water perfusion and extraction, lysimeter or microdialysis approaches (Giesler and Lundström, 1993; Weihermüller et al., 2007; Inselsbacher et al., 2011; Inselsbacher and Näsholm, 2012; Chen and Williams, 2013). However, due to a number of obvious advantages extracting soils with aqueous solutions and subsequent analysis of soil extracts often remains the method of choice for estimating bulk soil N concentrations. Extracting soil is cheap, can be done in remote areas without access to advanced infrastructure, allows for studying dry soils and yields sufficiently large sample volumes often required for analyses.

In order to be able to properly interpret the results from soil extractions it is, therefore, important to get more detailed information on the influence of handling and extracting soil on individual N forms. In a previous study the loss of ^{14}C-labeled amino acids added to the extractant was shown to be substantial (Rousk and Jones, 2010). However, when using ^{14}C as a label it is not possible to trace the fate of lost amino acids into inorganic N compounds and it remains unclear how much of the inorganic N in the extract might have come from amino acid turn-over and subsequent nitrification during the extraction procedure. Further, depending on soil properties and the chemical nature of individual N forms (e.g. acidic versus neutral or basic amino acids) the effect of soil extraction may vary significantly between different N forms, but such information is still missing. Therefore, this study aimed at evaluating the influence of (1) water and salt extraction and of (2) sieving and subsequent extraction on the recovery of NH_4^+, NO_3^- and amino acids. To accomplish this, I studied nine different soils from boreal forests, agricultural fields and grassland in a set of experiments under controlled laboratory conditions.

2. Material and methods

2.1. Site description and soil properties

Soils were collected from 9 sites in the vicinity of Umeå, Sweden (63°49′ N, 20°17′ E). In detail, 3 soils were collected from a poor Scots pine heath forest at the Åheden research area within the Svartberget Experimental Forest, which has been described previously (Gundale et al., 2011; Inselsbacher and Näsholm, 2012). Briefly, the site is a c. 60-yr-old Scots pine (*Pinus sylvestris* L.) forest and the soil is classified as a sandy glacial till Haplic Podzol (FAO, 1998) with 2% silt, 97% sand and 1% gravel, an organic layer depth of 10–15 cm depth with a C to N ratio of 38.7 and a pH (H_2O) of 5.2. In 2003, a large-scale N-addition experiment was established and soils were taken from the control site receiving no N fertilizer (Forest soil 0 kg N), 50 kg N ha^{-1} yr^{-1} (Forest soil 50 kg N) and 100 kg N ha^{-1} yr^{-1} (Forest soil 100 kg N). Two additional forest soils were collected from two recently clear-cut and replanted Scots pine forest stands in the vicinity of Sävar, 70 km east of the Svartberget Experimental Forest (Inselsbacher and Näsholm, 2012). Briefly, the soil is classified as Eutric Cambisol (FAO, 1998), dominated by sand (98%) and an organic layer depth of just 0–2 cm. Both sites have been heavily disturbed and turned over during recent harvest. One site was clear-cut and replanted in 2007 (Forest soil 5 years) and the other in 1997 (Forest soil 15 years). The other 4 soils were collected from 2 agricultural and 2 grassland sites located at the Röbäcksdalen Research facilities on an estate belonging to the Swedish University of Agricultural Sciences in Umeå which have been used frequently in previous studies (e.g., Jämtgård et al., 2008; Carlsson et al., 2009; Sohlenius et al., 2011). The soil is a fine silty sand with low clay content (4% clay, 58% silt and 38% fine sand) and a C to N ratio in the uppermost 15 cm of 14.8 (Mulder et al., 2002). The agricultural sites were part of a 6-year crop rotation comprising barley (*Hordeum vulgare* L. cv. Agneta) with undersowing of ley, first year ley, second year ley, green-fodder rape, potato and ryegrass. The same crop rotation and fertilization regimes had been used since 1965 (Jämtgård et al., 2008). Soil samples were taken from one plot receiving chemical fertilizer at amounts of 40–15–25 kg ha^{-1} N–P–K (Agricultural soil 1) and from a plot receiving 30–5–0 kg ha^{-1} N–P–K from chemical fertilizer and about 10–10–45 kg ha^{-1} from stable manure (Agriculture soil 2). Soil samples were taken in May 2012 before fertilization and sowing. Grassland sites were in direct vicinity of the agricultural soils and have been