Soil Biology and Biochemistry 117 (2018) 16–26

Contents lists available at ScienceDirect

Soil Biology and Biochemistry

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

Full ^{15}N tracer accounting to revisit major assumptions of ^{15}N isotope pool dilution approaches for gross nitrogen mineralization

Judith Braun[a], Maria Mooshammer[a], Wolfgang Wanek[a,*], Judith Prommer[a], Tom W.N. Walker[a,1], Tobias Rütting[b], Andreas Richter[a]

[a] Department of Microbiology and Ecosystem Science, Research Network "Chemistry Meets Microbiology", University of Vienna, Althanstrasse 14, 1090 Vienna, Austria
[b] Department of Earth Sciences, University of Gothenburg, Box 460, 405 30 Gothenburg, Sweden

ARTICLE INFO

Keywords:
Isotope pool dilution
Gross N mineralization
Gross ammonium consumption
Abiotic N fixation
Tracer reflux
Soil N cycle

ABSTRACT

The ^{15}N isotope pool dilution (IPD) technique is the only available method for measuring gross ammonium (NH_4^+) production and consumption rates. Rapid consumption of the added ^{15}N-NH_4^+ tracer is commonly observed, but the processes responsible for this consumption are not well understood. The primary objectives of this study were to determine the relative roles of biotic and abiotic processes in ^{15}N-NH_4^+ consumption and to investigate the validity of one of the main assumptions of IPD experiments, i.e., that no reflux of the consumed ^{15}N tracer occurs during the course of the experiments. We added a ^{15}N-NH_4^+ tracer to live and sterile (autoclaved) soil using mineral topsoil from a beech forest and a grassland in Austria that differed in NH_4^+ concentrations and NH_4^+ consumption kinetics. We quantified both biotic tracer consumption (i.e. changes in the concentrations and ^{15}N enrichments of NH_4^+, dissolved organic N (DON), NO_3^- and the microbial N pool) and abiotic tracer consumption (i.e., fixation by clay and/or humic substances). We achieved full recovery of the ^{15}N tracer in both soils over the course of the 48 h incubation. For the forest soil, we found no rapid consumption of the ^{15}N tracer, and the majority of tracer (78%) remained unconsumed at the end of the incubation period. In contrast, the grassland soil showed rapid ^{15}N-NH_4^+ consumption immediately after tracer addition, which was largely due to both abiotic fixation (24%) and biotic processes, largely uptake by soil microbes (10%) and nitrification (13%). We found no evidence for reflux of ^{15}N-NH_4^+ over the 48 h incubation period in either soil. Our study therefore shows that ^{15}N tracer reflux during IPD experiments is negligible for incubation times of up to 48 h, even when rapid NH_4^+ consumption occurs. Such experiments are thus robust to the assumption that immobilized labeled N is not re–mobilized during the experimental period and does not impact calculations of gross N mineralization.

1. Introduction

Nitrogen (N), in its inorganic forms ammonium (NH_4^+) and nitrate (NO_3^-), is often considered to be the limiting nutrient for plants in terrestrial ecosystems (Falkowski et al., 2008). Primary production, nitrification and denitrification are controlled by the rates at which inorganic N is both produced *via* mineralization of organic N and biological N fixation and consumed by biotic and abiotic processes. The understanding of this continuous cycling between organic and inorganic nitrogen forms is therefore of fundamental importance for estimating plant-available N in agricultural and natural soil systems (Hadas et al., 1992; Vitousek et al., 2002; Ward, 2012). A powerful tool for the determination of soil N transformation processes is the isotope pool dilution (IPD) technique (Barraclough, 1991; Di et al., 2000; Kirkham and Bartholomew, 1954; Wanek et al., 2010), which allows to estimate both rates of gross production and gross consumption of major plant nutrients in soil. This technique has been used across a wide range of natural and agricultural systems to study N transformation rates in soil (e.g., Booth et al., 2005, 2006; Hart et al., 1994; Murphy et al., 2003), and is particularly recognized as the recommended method to obtain estimates on soil N dynamics (Hart et al., 1994). Depending on tracer application approaches e.g. to intact soil-plant systems in situ or to sieved soils, plant mediated processes are included such as root uptake of inorganic N or tracer dynamics only reflect microbial processes such as in sieved soils (Murphy et al., 2003; Rütting et al., 2011).

The IPD approach relies on labeling the target pool, i.e. the product pool of the reaction to be measured, which in the case of N mineralization is the NH_4^+ pool, with ^{15}N-enriched tracer (^{15}N-NH_4^+). The

* Corresponding author.
E-mail address: wolfgang.wanek@univie.ac.at (W. Wanek).
[1] Present address: Laboratory of Ecological Systems (ECOS), EPFL-ENAC-IIE, Bâtiment GR, Station 2, CH-1015 Lausanne, Switzerland.

https://doi.org/10.1016/j.soilbio.2017.11.005
Received 26 January 2017; Received in revised form 3 November 2017; Accepted 5 November 2017

isotopic tracer is then diluted as a consequence of mineralization of unlabeled organic N to NH_4^+. Gross N mineralization (i.e., NH_4^+ production or influx) and gross NH_4^+ consumption (i.e., NH_4^+ efflux) are then calculated from the change in size of the total NH_4^+ pool ($^{14}N + {}^{15}N$), and from the decline in the ^{15}N enrichment above natural abundance over time (Barraclough, 1991; Hart et al., 1994; Kirkham and Bartholomew, 1954; Murphy et al., 2003). Kirkham and Bartholomew (1954) stated that the following assumptions need to be met in order to convert the measured quantities and isotope ratios to absolute rates: (i) the isotopically heavy (tracer) and the lighter molecules (tracee) behave in the same way in a soil; (ii) mineralization and immobilization rates remain constant during the interval between successive measurements; (iii) the ratio of tracer to tracee in the efflux is in proportion to that of the labeled pool, and (iv) immobilized labeled N is not remobilized during the experimental period.

The last of these key assumptions – no recycling of tracer consumed during the experiment – could be violated during IPD experiments if rapid consumption of the tracer takes place. It is well known that such a reflux of ^{15}N tracer into the NH_4^+ pool could lead to a substantial error in calculations, resulting in an underestimation of gross mineralization and consumption rates (Barraclough and Puri, 1995; Bjarnason, 1988; Davidson et al., 1991). Rapid consumption of ^{15}N-NH_4^+ has been reported by several studies (e.g. > 50% tracer loss within minutes), but it is not clear which consumption processes are involved or whether remobilization of the ^{15}N tracer is likely (Davidson et al., 1991; Kowalenko and Cameron, 1978; Morier et al., 2008). We here define all processes removing NH_4^+ from the available NH_4^+ pool as consumption processes, following the accepted terminology (Booth et al., 2005; Murphy et al., 2003), which can be further distinguished into biotic NH_4^+ consumption (i.e., microbial uptake and nitrification; hereafter "immobilization") and abiotic NH_4^+ consumption (i.e., fixation by the mineral or organic soil fraction; hereafter "fixation"). Biotic processes are often assumed to be the dominant consumptive processes in IPD experiments lasting for a few days (Monaghan and Barraclough, 1995; Morier et al., 2008; Trehan, 1996). Indeed, several authors have reported microbial uptake of inorganic and organic compounds within minutes and even seconds after tracer addition (Farrell et al., 2011; Hill et al., 2012; Jones et al., 2013; Tahovská et al., 2013; Wilkinson et al., 2014). Nevertheless, others have suggested abiotic fixation to be the main mechanism explaining rapid NH_4^+ consumption (Davidson et al., 1991; Johnson et al., 2000; Trehan, 1996). In fact, NH_4^+ fixation by clay minerals is known to occur within h after NH_4^+ addition (Cavalli et al., 2015; Nieder et al., 2011; Nõmmik and Vahtras, 1982). Physical sorption or chemisorption to organic matter might also be responsible for the removal of ^{15}N-NH_4^+ from the extractable N pool (Mortland and Wolcott, 1965; Nieder et al., 2011; Nõmmik and Vahtras, 1982). However, despite the potential for biotic and abiotic processes to rapidly consume ^{15}N-NH_4^+ during IPD experiments, the sinks involved have not as yet been clearly quantified.

The objective of this study was to determine the fate of added ^{15}N-NH_4^+ during the duration that ^{15}N-IPD experiments usually last (i.e., < 48 h) in two sieved soils that differ in their NH_4^+ consumption rates. We considered all possible sources of tracer reflux to evaluate whether the requirement that consumed labeled N is not remobilized during the experimental duration of normal IPD experiments is valid. Additionally we investigated the constancy of transformation rates over time. We hypothesized that rapidly consumed ^{15}N tracer is mainly subjected to biotic (microbial) immobilization processes, that the ^{15}N tracer can therefore be remineralized or released during the incubation period, and that such reflux causes an underestimation of gross N mineralization fluxes in soils that exhibit rapid ^{15}N-NH_4^+ consumption.

Table 1
Selected soil characteristics of the top soil (0–10 cm) of the forest and the grassland soil (means ± 1 SE, n = 3).

Soil parameter		Forest	Grassland
Soil pH		4.0 ± 0.0	6.0 ± 0.0
Soil texture	Clay (%)	16.3 ± 0.1	26.2 ± 1.3
	Silt (%)	63.4 ± 0.5	56.1 ± 1.7
	Sand (%)	20.3 ± 0.6	17.7 ± 1.0
Soil C & N content	Total C (%)	3.4 ± 0.7	2.9 ± 0.5
	Total N (%)	0.2 ± 0.0	0.3 ± 0.1
Soil C:N ratio		13.4 ± 0.2	10.1 ± 0.2
Soil NH_4^+ concentration	(μg N g^{-1} d.w.)	29.2 ± 0.7	1.3 ± 0.1
^{15}N-NH_4^+ tracer recovery	(%; after 15 min)	99 ± 0.2	41 ± 2.8

2. Materials and methods

2.1. Sampling site and soil description

Soils were collected from two sites in Austria differing in vegetation composition and soil pH (Table 1). Top soils were sampled from a beech (*Fagus sylvatica* L.) forest (N 48.228656°, E 16.260713°, 382 m a.s.l., Schottenwald, Vienna) and from a permanent grassland (N 48.049063°, E 16.197592°, 323 m a.s.l., Mödling, Lower Austria). The soils are hereafter referred to as "forest" and "grassland" soil, respectively. The forest soil is classified as a dystric Cambisol (Kaiser et al., 2010) and the grassland soil as a Cambisol (Nestroy et al., 2011). Samples were taken from the upper 10 cm of the mineral soil (A) horizon in October 2014. The soil was sieved to 2 mm and stored at 4°C until experiments were performed. Soil pH was measured in 10 mM $CaCl_2$. Total carbon (C) and N contents were measured in finely ground, oven dried (105°C, 24 h) soil using an elemental analyzer (EA1110, CE Instruments, Milan, IT) coupled to a continuous flow stable isotope ratio mass spectrometer (DeltaPLUS, Thermo Finnigan, Bremen, DE) (EA–IRMS). Soil ammonium contents were determined photometrically in 1 M KCl extracts [soil to extractant ratio of 1:7.5 (w:v)] based on the Berthelot reaction (Hood-Nowotny et al., 2010). Soil texture analysis was done based on a micropipette method modified from Miller and Miller (1987), by using 5% sodium hexametaphosphate as a dispergent.

The soils were selected because of their similarity in general soil properties, such as soil texture (silt loam) and C and N content, but they differed considerably in soil pH and available NH_4^+ content (Table 1). Additionally, the soils strongly differed in their consumption of added $^{15}NH_4^+$ as determined in a preliminary tracer recovery experiment, in which both soils were labeled with 10 atom% $(^{15}NH_4^+)_2SO_4$ solution (20% of the initial NH_4^+ pool) and after 15 min extracted with 0.5 M K_2SO_4 [soil to extractant ratio of 1:7.5 (w:v)]. We found that 99% of the added ^{15}N tracer could be recovered as NH_4^+ from the forest soil but only 41% from the grassland soil (Table 1).

2.2. Experimental design

The IPD assay was performed with two treatments, live (non-sterilized) and sterilized (autoclaved) soil, to distinguish between biotic immobilization processes and abiotic fixation (Fig. 1). Five consecutive measurements of concentrations and isotopic composition of NH_4^+, NO_3^-, microbial biomass N (N_{mic}), and dissolved organic N (DON) were taken over the course of 48 h. To obtain high-resolution time kinetics of measured processes, we stopped incubations within 2–3 min (0 h), 0.25 h, 3.5 h, 24 h, and 48 h after tracer addition. We thereby accommodated the standard experimental duration suggested by Murphy et al. (2003) (i.e. t_1: 4 h–24 h; t_2 48 h–144 h), with two additional early sampling points to track rapid consumptive processes. In addition, the contribution of abiotic fixation (i.e., fixation by clay and humic substances) was determined at two fixed time points (0 h and 24 h) in live soils (Fig. 1).