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Soluble organic nitrogen in potentially mineralizable N assays: are we missing an important component?

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Abstract

We examined soluble organic nitrogen (SON) leached from long-term, sequentially leached, aerobic incubations. Leached SON, present in all depths (0-60 cm), ranged from 35% to 56% of total N. This unaccounted-for SON may have important implications in the estimation of plant available N and the potential for environmental N losses.

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Introduction

Sequential leaching of aerobically incubated soils has been the standard for the estimation of soil N mineralization potential for more than 40 years (Stanford and Smith 1972). In their classic work, Stanford and Smith (1972) proposed the measurement of nitrate and ammonium released in long-term, sequentially leached, aerobically incubated soils as a standard procedure to quantify potentially mineralizable N and its rate constant (Stanford and Smith 1972; Curtin and Campbell 2008). Widespread use of this method, with some variations (e.g., incubation length and temperature), have allowed researchers to better understand the process of soil N mineralization and its controlling factors (Dessureault-Rompré et al. 2016).

Mineral forms of N (i.e., NH_4^+ and NO_3^-) are the dominant sources of N in crop nutrition in agricultural systems and are also involved in processes which result in environmental impacts on air through emissions of NH_3 and N_2O and on water through groundwater NO_3^- contamination. As a result, mineral N has been the primary focus of attention in the study of N dynamics (van Groenigen et al. 2015). However, soluble organic N compounds also exist in soil, have environmental impact (Ghani et al. 2013), and often can be in greater concentration than the mineral N pool (Lee et al. 2018; Stamati et al. 2011).

Soluble organic N is typically not quantified in sequential leaching approaches to estimate N mineralization potential. Traditionally, the focus has been only on mineral forms of N which had “mineralized”, and therefore soluble organic forms of N were not considered (Schimel and Bennett 2004). However, awareness of the environmental impacts of soluble organic N (Ghani et al. 2013), recognition of the ability of plants to directly assimilate organic N compounds (Näsholm et al. 2009), and the wider availability of methods to analyse total soluble N in soil extracts, have increased interest in studying the relevance of this pool of N in the

environment. The potential significance of soluble organic N in the context of sequential leaching approaches to estimate potentially mineralizable N remains largely unexplored.

The objective of this study was therefore to quantify total soluble N leached from long-term, sequentially leached, aerobic incubations, in order to consider the importance of soluble organic N pools in assays to quantify soil potentially mineralizable N. A previous study quantified the depth distribution of potentially mineralizable N pools for six soils in western Canada (Dessureault-Rompré et al. 2016). This study includes the quantification of soluble organic N, in addition to previously published data on soil mineral N, for four contrasting soils at three soil depths.

Materials and Methods

The study used the Brandon, Carberry, Lacombe and Lethbridge sites of Dessureault-Rompré et al. (2016). All four sites are located in the Chernozemic soil zone and differed in their soil texture and organic matter content (Table 1). Each site included four replications. Soils were collected in spring prior to manure or fertilizer application. The soil was collected from 0-15, 15-30, and 30-60 cm depths. Information about site locations, soil classification, climate and crop management are presented in Dessureault-Rompre et al. (2016).

Mineralizable N pools were quantified using sequential extractions over a 22-week long-term aerobic incubation at 25 °C as described by Sharifi et al. (2007). The incubation used the original method of Stanford and Smith (1972) as modified by Curtin and Campbell (2008). Soil samples (30 g) were mixed with an equal amount of sand and packed into leaching tubes and wetted to approximately 55% of the soil water holding capacity. The soils were leached periodically (every 2 weeks for the first 10 weeks and every 4 weeks thereafter) with 0.01 M

CaCl₂ followed by a zero-N nutrient solution (Curtin and Campbell 2008). Leachates were analyzed using a Technicon AutoAnalyzer II system for NH₄⁺ using the phenate method (Technicon Industrial Method #98-70W), NO₂⁻ + NO₃⁻ using Cd reduction (Technicon Industrial Method #100-70W) and total soluble N (TSN; mineral N + soluble organic N) using persulfate digestion (AutoAnalyzer Application Method #G-086-93A). Soluble organic N (SON) was calculated as the difference between TSN and SMN. Percentage of N mineralized was calculated as the fraction of TSN leached during each leaching event that was recovered as mineral N (NH₄⁺ + NO₃⁻).

Differences among sites and depths were evaluated using Analysis of Variance (ANOVA) where the site and depth were both fixed effects, followed by the post-hoc Tukey's Honestly-Significant-Difference Test [SYSTAT 13.0 (SYSTAT Software Inc., Richmond, CA)].

Results and Discussion

The first leaching event at the start of the incubation (T₀) represents the N removed during the rewetting of air-dried soils, and therefore presumably reflects the N compounds present in the air-dry soil. The TSN in the first leaching event generally decreased with depth for each of the four soils (Fig. 1). In comparison, the soil mineral N (SMN) at T₀ was low and no depth effect was observed. Across sites and depths, the average TSN and SMN at T₀ were 13.9 and 4.5 mg kg⁻¹, respectively. Thus, about two-thirds of the TSN pool was composed of SON. The osmotic shock and immediate extraction associated with the leaching event may result in the release of intracellular organic solutes (primarily amino acids), the desorption of organic matter sorbed to soil surfaces, or the physical release of protected organic matter, resulting in increased soluble organic N (SON) in the first leaching event (Wu and Brookes 2005).

After two weeks of incubation (T_2), production of TSN increased sharply together with production of SMN, in what is commonly referred to as the flush of N upon rewetting where the microbes had the time to utilize the newly available substrates (Wu and Brookes, 2005). The increase in TSN production was observed for each site and declined systematically with depth. The same trend was observed for SMN. Across sites and depths, the average TSN and SMN at T_2 was 25.3 and 18.8 mg kg⁻¹, respectively, indicating that about one-quarter of the TSN pool was composed of SON.

Calculation of potentially mineralizable N normally excludes the T_0 and T_2 leaching events and considers the cumulative leaching of SMN from the remaining leaching events. For the bi-weekly leaching events from week four (T_4) to week ten (T_{10}), the production of TSN generally decreased over time and with depth for all sites. The only exception was for the Lethbridge site at 30-60 cm depth which increased from T_4 to T_{10} . The reason for this exception is not apparent. The same trend was observed for SMN. Across sites and depths, the average TSN and SMN for T_4 to T_{10} was 8.1 and 5.0 mg kg⁻¹, respectively. On average, SON represented about 40% of the TSN pool during that incubation period.

In comparison, the TSN for the trends in monthly leaching events from week 14 (T_{14}) to week 22 (T_{22}) were more variable among sites, however a systematic decrease with depth was observed for all sites. During the final half of the incubation period, TSN and SMN production in general decreased slightly with occasional increases being observed in each soil/depth. Across sites and depths, the average TSN and mineral N data for the monthly leaching events was 8.2 and 5.5 mg kg⁻¹, respectively. Therefore, across sites and depths, about one-third of the TSN pool was composed of SON.

Over the course of the long-term experiment, the proportion of TSN that was mineralized ranged from 13 to 81% (average 44%) for Carberry, 27 to 87% (average 57%) for Brandon, 27 to 94% (average 61%) for Lethbridge and 22 to 95% for Lacombe (average 65%), across all depths. The presence of SON in the leachate increased numerically with depth for Carberry and Brandon sites, however, the difference observed between the 0-15 cm depth layer and the 15-30 and 30-60 cm depth layers was significant only for Carberry site (Fig. 2). The C:N ratio of the Brandon, Carberry and Lethbridge soils narrowed with depth, but the change was not as great at the Lacombe site (Table 1). The Carberry site was also characterized by a greater proportion of SON compared to the three other sites which had a quite similar average proportion of SON (~35-40%). The Carberry site had a coarser texture (sandy-loam) than the other three sites (silt-loam to clay-loam) and was the only site subject to tillage (Dessureault-Rompré et al. 2016).

These observations do not support the assumption that SON is negligible in sequential leaching experiments. In the four soils studied, a significant portion of the N leached from the soil in sequential leaching was as SON. This is consistent with studies of forest ecosystems where repeated soil leaching continued to yield SON compounds (Lee et al., 2018). These findings suggest that the current sequential leaching method may greatly underestimate the total quantity of N solubilized during the experiment. There are two major implications for this observation. First, if labile, this SON represents a substantial quantity of N which is not considered in assessing potentially mineralizable N, and therefore in the estimation of plant available N. For example, the spring SMN tests in the Canadian Prairies (which is comparable to the T_0 leaching event) and short-term incubation assays (e.g., Pool I of Sharifi et al. (2007) based on the T_2 leaching event), commonly used to adjust crop fertilizer N recommendations, may be greatly underestimating soil N supply.

Second, the potential for environmental impact of N contained in soil leachates needs to be considered. The movement of solutes to tile-drain water or other surface water bodies is common. This study suggests that there is a large proportion of N which may be leached from the soil in organic form. This may result in significant loss of nitrate (if the SON is labile), or of SON compounds (if not labile), to surface water bodies. This has the potential to contribute to significant indirect GHG emissions, and to have a significant impact on the nutrient availability in surface waters.

A better understanding of the nature of the SON recovered from sequential leaching experiments is needed. It is not clear whether these organic N compounds are recalcitrant (Jones et al. 2012), would become sorbed to surfaces lower in the profile (Mariano et al. 2016; Wagai et al. 2013) or would eventually be mineralized (Ghani et al. 2013). Consideration of the kinetics of the solubilization of organic N compounds and their subsequent mineralization will help clarify the factors controlling the availability of N to plants.

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Table 1: The influence of location and sampling depth (cm) on sand, silt, clay, soil organic carbon (SOC), soil total nitrogen (STN), all in (g kg^{-1}), and C:N ratio of soils for each depth and location. Reproduced from Dessureault-Rompere et al. 2016.

Location	Depth	Sand	Silt	Clay	SOC	STN	C:N
Brandon	0-15	332 ^{ab}	575 ^b	93	32.1 ^{de}	32.3 ^{cd}	9.9
	15-30	338 ^{ab}	624 ^c	38	12.9 ^{abc}	1.49 ^{ab}	8.6
	30-60	369 ^{ab}	613 ^c	18	3.8 ^a	0.62 ^a	6.3
Carberry	0-15	717 ^c	155 ^a	129	14.2 ^{abc}	1.35 ^{ab}	10.4
	15-30	549 ^{bc}	327 ^{ab}	124	13.9 ^{abc}	1.41 ^{ab}	9.8
	30-60	397 ^{ab}	573 ^b	30	8.4 ^{abc}	1.13 ^{ab}	7.4
Lacombe	0-15	314 ^a	532 ^b	154	51.6 ^f	4.77 ^e	10.9
	15-30	295 ^a	613 ^c	92	42.3 ^e	4.06 ^d	10.4
	30-60	342 ^{ab}	570 ^b	88	20.9 ^{cd}	2.2 ^{bc}	9.3
Lethbridge	0-15	385 ^{ab}	512 ^b	103	16.1 ^{abc}	1.87 ^{ab}	8.6
	15-30	337 ^{ab}	595 ^b	69	10.7 ^{abc}	1.51 ^{ab}	7.1
	30-60	352 ^{ab}	630 ^c	18	6.7 ^{ab}	1.03 ^{ab}	6.5

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Figure 1. Total Soluble N (TSN; organic N + mineral N) and SMN leached (mg N kg⁻¹) on a given date over the course of the 22-week leaching experiment. Data are presented for four soil types and for three soil depths.

Figure 2. Percentage of total soluble N (TSN) as soluble organic N by soil type and depth. The * indicates a significant difference ($p < 0.05$, Tukey's Honestly-Significant-Difference post-hoc Test) between the 0-15 cm and 15-30 cm depths and between the 0-15 cm and 30-60 cm depths for the Carberry site.