Calculation of fungal and bacterial inorganic nitrogen immobilization rates in soil

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Abstract

Microbial inorganic nitrogen (N) immobilization constitutes an important mechanism for soil N retention. However, the immobilization rates of inorganic N by fungi and bacteria, the dominant microorganisms in soil, are still unavailable due to the high diversity and complexity of soil microorganisms. Here we propose a mathematical framework, based on measurable gross inorganic N immobilization rate and proxies of fungal and bacterial inorganic N immobilization rates, to quantify the respective immobilization rates of inorganic N by fungal and bacterial communities in soil. Our approach may help to further unravel retention mechanisms for bioavailable nitrogen in soils.

Keywords:

Amino sugar; ^{15}N; Stable isotope probing; microbial inorganic N immobilization; Fungi; Bacteria

Microbial immobilization of inorganic nitrogen (N) is of importance to control the size of soil inorganic N pool and to constitute an important mechanism of N retention in ecosystems (Davidson et al., 1992; Stark and Hart, 1997; Zogg et al., 2000; Zhang et al., 2013). Through inorganic N immobilization, inorganic N is converted to microbial biomass N and subsequently re-mineralized or converted to stable organic N, eventually reducing the risk of N losses from soil (Recous et al., 1990; Tahovská et al., 2013; Zhang et al., 2019). As the dominant microorganisms in soil, fungi and bacteria are

involved in inorganic N immobilization (Myrold and Posavatz, 2007; Boyle et al., 2008; Bottomley et al., 2012). Given the distinct physiologies, morphologies and lifestyles of these two microbial groups (Six et al., 2006; Lauber et al., 2008; Rousk and Bååth, 2011; Waring et al., 2013), the role of bacterial and fungal communities in soil inorganic N immobilization is different (Myrold and Posavatz, 2007; Bottomley et al., 2012; Li et al., 2019). However, due to the high diversity and complexity of soil microorganisms, quantifying the relative importance of fungi and bacteria in soil inorganic N immobilization is challenging (Fierer, 2017; Li et al., 2019).

Based on the microbial source specificity of amino sugars (AS) (Parsons, 1981; Zhang and Amelung, 1996; Amelung, 2001), amino sugar-based stable isotope probing (^{15}N-AS-SIP) has been developed to disentangle the immobilization processes of inorganic N by fungi and bacteria in soil (He et al., 2006; Liang and Balser, 2010; He et al., 2011a; He et al., 2011b; Reay et al., 2019a; Reay et al., 2019b). This approach was recently extended to indicate the inorganic N immobilization rates of fungal and bacterial communities in soils (Li et al., 2019). More specifically, given the persistence of AS in soils (Turnover time >6 years) (Glaser et al., 2006; Derrien and Amelung, 2011; Liu et al., 2016; Gunina et al., 2017), the accumulative amounts of newly formed ^{15}N-labeled AS after a certain period of incubation can be used as proxies of fungal and bacterial inorganic N immobilization rates (Li et al., 2019). However, because the respective contents of fungal glucosamine (GlcN) and bacterial muramic acid (MurN) in fungal and bacterial biomass are difficult to obtain (Glaser et al., 2004; Appuhn and Joergensen,

2006; Engelking et al., 2007; Joergensen and Wichern, 2008; Joergensen, 2018), ^{15}N-AS-SIP cannot get the actual inorganic N immobilization rates of fungi and bacteria in soil (Li et al., 2019).

Gross inorganic N immobilization rate in soil can be measured using ^{15}N isotope techniques (e.g., ^{15}N pool dilution method) (Murphy et al., 2003; Cheng et al., 2017). Combining gross inorganic N immobilization rate with the proxies of the respective inorganic N immobilization rates of fungi and bacteria, we propose a mathematical framework to quantify the conversion coefficients between fungal and bacterial inorganic N immobilization rates and their respective proxies, thereby obtaining the respective immobilization rates of inorganic N by fungal and bacterial communities in soil.

Algorithm

Our proposed calculation is based on the assumption that fungi and bacteria are involved in soil microbial inorganic N immobilization. Gross inorganic N immobilization rate (*T*) and proxies of inorganic N immobilization rates of fungi (*F*) and bacteria (*B*) in soils can be adequately measured.

The measured variables:

$F=\begin{bmatrix} F_1 \\ F_2 \\ \vdots \\ F_n \end{bmatrix}$: The fungal-derived ^{15}N-GluN synthesis rates of n samples (mg N kg^{-1} d^{-1});

$B=\begin{bmatrix} B_1 \\ B_2 \\ \vdots \\ B_n \end{bmatrix}$: The bacterial-derived ^{15}N-MurN synthesis rates of n samples (mg N kg^{-1} d^{-1});

$T=\begin{bmatrix} T_1 \\ T_2 \\ \vdots \\ T_n \end{bmatrix}$: Gross microbial inorganic N immobilization rates of n samples (mg N kg^{-1} d^{-1}).

Two parameters for estimation:

K_F: Conversion coefficient between fungal-derived ^{15}N-GluN synthesis rate and fungal inorganic N immobilization rate;

K_B: Conversion coefficient between bacterial-derived ^{15}N-MurN synthesis rate and bacterial inorganic N immobilization rate.

The estimated values:

The estimated fungal inorganic N immobilization rate (mg N kg^{-1} d^{-1}): $R_F = K_F \times F$

The estimated bacterial inorganic N immobilization rate (mg N kg^{-1} d^{-1}): $R_B = K_B \times B$

Thus, the estimated sum of fungal and bacterial inorganic N immobilization rate (mg N kg^{-1} d^{-1}): $\hat{T} = R_F + R_B = K_F \times F + K_B \times B$

The estimation error: $e = T - \hat{T}$

Thus, $T = \hat{T} + e = K_F \times F + K_B \times B + e$

the formula can be rewritten as matrix format:

$$T = \begin{bmatrix} F & B \end{bmatrix} \begin{bmatrix} K_F \\ K_B \end{bmatrix} + \mathrm{e}$$

Alternatively,

$$\begin{bmatrix} T_1 \\ T_2 \\ \vdots \\ T_n \end{bmatrix} = \begin{bmatrix} F_1 & B_1 \\ F_2 & B_2 \\ \vdots & \vdots \\ F_n & B_n \end{bmatrix} \begin{bmatrix} K_F \\ K_B \end{bmatrix} + \begin{bmatrix} e_1 \\ e_2 \\ \vdots \\ e_n \end{bmatrix}$$

Let $K = \begin{bmatrix} K_F \\ K_B \end{bmatrix}$, $X = \begin{bmatrix} F_1 & B_1 \\ F_2 & B_2 \\ \vdots & \vdots \\ F_n & B_n \end{bmatrix}$, we get:

$$T = X\,K + e$$

The least squares estimators that minimize the sum of squared residuals are given below (see appendix for the detailed derivation) (Wackerly et al., 2014):

$$\widehat{K} = (X'X)^{-1}X'T$$

To show how the approach might work, we calculated soil nitrate (NO_3^-) immobilization rates of fungi and bacteria from gross NO_3^- immobilization rates reported by (Zhang et al., 2013) and ^{15}N-AS-SIP data reported by (Li et al., 2019). Both studies focused on the effect of land conversion from forest to agriculture on soil NO_3^- immobilization in subtropical zones of China.

The results showed that the NO_3^- immobilization rates of fungi in woodland and agricultural soils were approximately 8.4 and 4 times that of bacteria, which indicates that fungi are likely to dominate the microbial NO_3^- immobilization in the studied soil (Table 1). Moreover, agricultural utilization led to decreases in fungal and bacterial NO_3^- immobilization rates by 0.34 and 0.03 mg N kg^{-1} d^{-1} respectively, which inferred

that the decrease in fungal NO_3^- immobilization was likely to dominate the total decline in soil microbial NO_3^- immobilization caused by agricultural use.

Table 1 An illustration of the approach for calculating soil fungal and bacterial NO_3^- immobilization rates under different land-use. Gross NO_3^- immobilization rates are from (Zhang et al., 2013). The production rates of fungal-derived ^{15}N-GluN and bacterial-derived ^{15}N-MurN are from (Li et al., 2019). Our values are presented as illustrative examples, rather than as reliable estimates.

Land-use type	T *	F #	B #	K_F †	K_B †	R_F ‡	R_B ‡
	mg N kg^{-1} d^{-1}					mg N kg^{-1} d^{-1}	
Woodland	0.47	0.0303	0.0022	13.85	23.83	0.42	0.05
Agriculture	0.10	0.0057	0.0009	13.85	23.83	0.08	0.02

* Soil microbial gross NO_3^- immobilization rate. The data used was taken from (Zhang et al., 2013).

F and *B* denote the production rates of fungal-derived ^{15}N-GluN and bacterial-derived ^{15}N-MurN, respectively. The data used was taken from (Li et al., 2019).

† K_F and K_B denote the conversion coefficients between *F*, *B* and NO_3^- immobilization rates of fungi and bacteria, respectively.

‡ R_F and R_B denote the inorganic N immobilization rates of fungi and bacteria, respectively.

Limitations and advantages

The approach relies on several simplifying assumptions. First, it assumes that only fungi and bacteria are involved in soil microbial inorganic N immobilization. This assumption may not quite hold true, because archaea may also contribute to inorganic N immobilization (Laughlin et al., 2009). Due to archaea contain GlcN, but not Mur (Joergensen, 2018), they cannot be identified through ^{15}N-AS-SIP. However, considering that archaea accounts for less than 1% of the soil microbial biomass (Fierer, 2017), errors caused by this assumption are probably minimal.

Moreover, as for the proxy of fungal inorganic N immobilization rate (measured by ^{15}N-AS-SIP), it should be noted that Fungal-derived ^{15}N-GlcN is calculated by subtracting the bacterial-derived ^{15}N-GlcN from the total ^{15}N-GlcN assuming that GlcN and Mur averagely occur at a molar ratio of 2:1 in bacterial cells (measured from selective cultured bacteria) (Engelking et al., 2007; Bodé et al., 2013; Li et al., 2019). We acknowledge that the ratio of GlcN and Mur in bacterial mass is variable due to the high variations for different species (Appuhn and Joergensen, 2006; Engelking et al., 2007; Joergensen, 2018), yet constitute an appropriate simplification in order to relate ^{15}N incorporation of GlcN to the role of fungi in N immobilization processing in soil.

Due to the high diversity and complexity of microbial communities, quantification of the process rates of different microbial groups has been a great challenge, especially in

soil (Stres and Tiedje, 2006; Bardgett and Van Der Putten, 2014; Fierer, 2017). Our approach provides an effective way to mathematically rather than mechanically quantify the relative importance of fungal and bacterial communities in soil inorganic N immobilization. Since the rationale and mathematical derivation are universal, it is expected that this method may also be applicable to other environmental systems, such as freshly colonized organic substates (Appuhn and Joergensen, 2006).

Conclusions

In conclusion, we first developed a mathematical approach, which combines mechanically accessible gross inorganic N immobilization rate and proxies of fungal and bacterial inorganic N immobilization rates, to quantify inorganic N immobilization rates of fungal and bacterial communities in soil. The approach we describe, while not without limitations, provides a promising way to reveal the actual contributions of fungi and bacteria to N-containing substrates immobilization in soil. Furthermore, our approach may help to quantitatively linking microbial community characteristics to N process rates of fungal and bacterial communities in soil under different scenarios.

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Appendix A. Supplementary data

Supplementary data related to this article can be found in the online version.

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