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Nitrogen mineralization in organically and conventionally managed soils under contrasting rainfall variability with focus on functional genes and proteolytic activity

Introduction

Future projected population growth and climate changes will challenge our food and farming system, and thus agricultural systems with a high potential to deal with extreme weather scenarios whilst minimizing negative environmental impacts, are required. One option is eco-functional intensification through organic farming, an approach based on exploiting internal natural resources and processes for securing and improving agricultural productivity whilst minimizing negative environmental impacts. In this concept an active soil microbiota plays an important role.

A previously conducted meta-study revealed i) higher microbial abundance ii) higher microbial activity iii) higher nitrogen mineralization potential and iv) a different microbial community structure in organic systems compared to conventional systems (Lori et al. in preparation).

The present study aims to analyse, whether the observed microbial community differences translate into functional differences in regard to nitrogen mineralization processes.

Hypotheses

1.) Organically manged soils possess a higher proteolytic potential than conventionally managed soils in "optimum wet" but also "extreme drought" conditions.

2.) Organically and conventionally managed soils, harboring diverse microbial communities, possess functional differences in regard to nitrogen cycling genes, especially proteolytic genes, in "optimum wet" but also dry conditions.

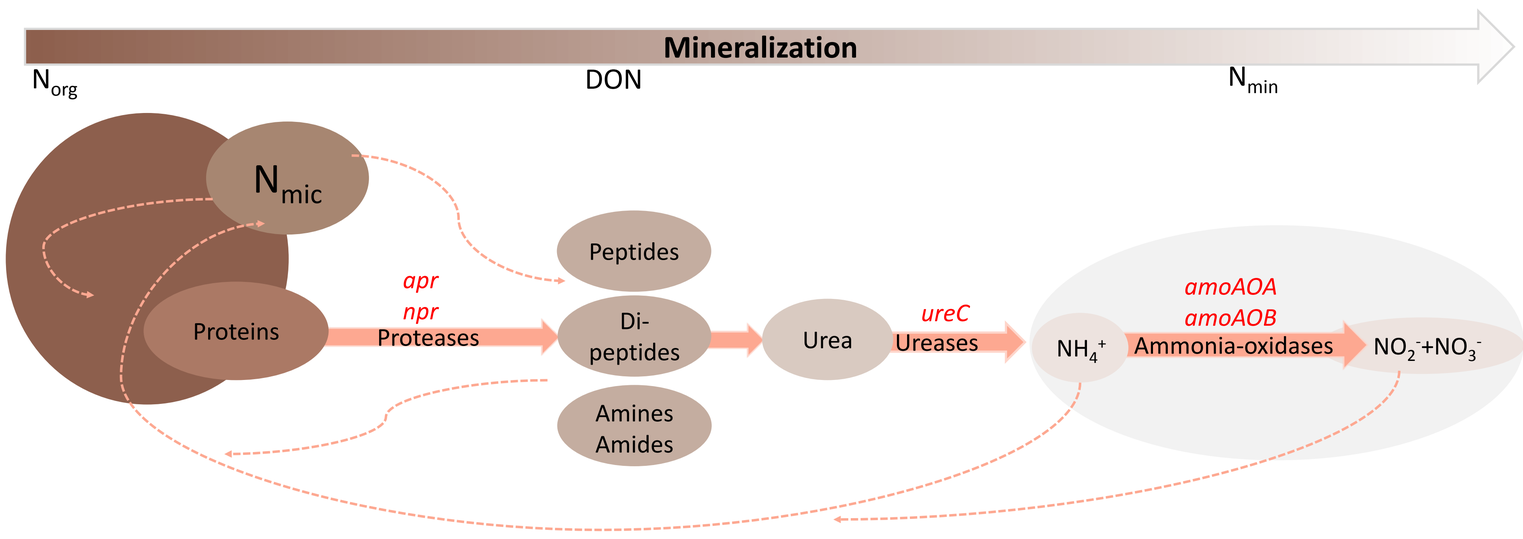

Figure 1: Simplified scheme of soil microbial mediated nitrogen mineralization. Organic nitrogen (N_{org}) gets mineralized into mineral nitrogen (N_{min}) via different steps and enzymes. Functional genes encoding for the respective enzymes are highlighted in red and abbreviated as alkaline-metalloprotease (*apr*), neutral-metalloprotease (*npr*), urease (*ureC*), bacterial ammonia-oxidase (*amoAOB*), archaeal ammonia-oxidase (*amoAOA*). Proteolysis, where proteins get cleaved into peptides and di-peptides is the initial and often rate limiting step of nitrogen mineralization. The bold orange arrows indicate the direction of mineralization whereas the fine dotted arrows indicate other (re)-cycling pathways. Nitrogen losses via plant uptake, leaching or N_2O production were prevented in our experimental set up and hence also not considered in this scheme. Microbially bound nitrogen (N_{mic}), dissolved organic nitrogen (DON) and N_{min} represent the labile N pool.

Methodology and Set up

Sieved soil from a farming system comparison in Switzerland (long-term trial DOK) was incubated pairwise (organic vs. conventional) for 56 days with the input of lupine litter (~135 kg N ha^{-1}) under "optimal wet" and "extreme drought" conditions in microcosms (without vegetation). The different soil nitrogen pools, total mitrogen (N_{tot}), mineral nitrogen (N_{min}), microbial nitrogen (N_{mic}), total nitrogen (N_{tot}) and dissolved organic nitrogen (DON), were measured at the start and the end of the incubation period. CO_2 and N_2O production were monitored regularly using gas chromatography. Proteolysed nitrogen added to the labile nitrogen pool (N_{labile}= N_{mic} +N_{min}+DON) was calculated as the difference between $N_{labile\text{-}end}$ and $N_{labile\text{-}start}$. Net fluxes of the other nitrogen pools were calculated similarly. The abundance of functional genes involved in the nitrogen mineralization cascade was measured using qPCR.

Results

- Larger N_{tot}, N_{min}, N_{mic} and N_{labile} pools in organically managed soils under dry and wet conditions (Table 1)
- Enhanced proteolysis (ΔN_{labile}) in organically managed soil under dry and wet conditions (Table 1)
- No statistical difference between farming systems in regard to gene abundance of proteolytic functional genes *apr* and *npr* (Table 1)
- Functional genes encoding for extracellular proteases (*apr, npr*), which are involved in the initial and often rate limiting step of nitrogen mineralization, correlate with ΔN_{labile} (proteolysis), as well as with ΔN_{min} (net mineralization) and with the more downstream fluxes of ΔNO_2^-+NO_3^- (net nitrification) (Table 2)
- *amoAOA* correlates with net nitrification but also with linked upstream fluxes of proteolysis and net mineralization (Table 2)
- *amoAOB* is very low abundant and not correlating with nitrogen fluxes (Table 2)

Table 1: Effects of farming system (FS) and water regime (WR) on nitrogen pools, nitrogen fluxes and functional genes after 56 days of incubation. Two way ANOVAs were used to asses effects of farming system and water regimes over the entire data set and contrast analyses to detect differences between farming system within a water regime (n=4). p≤ *0.05, **0.01, ***0.001, n.s=non-significant.

	dry			wet						
	Organic	Conventional		Organic	Conventional		ANOVA effect tests			
	mean ± SE		Contrast analysis	mean (± SE)		Contrast analysis	FS	WR	WRxFS	
N_{tot}	0.175 ± 0.001	0.167 ± 0.001	**	0.173 ± 0.001	0.168 ± 0.002	*	*	n.s	n.s	%
N_{min}	36.9 ± 5.3	15.4 ± 0.9	*	164.8 ± 9.3	149 ± 6.7	n.s	*	***	n.s	mg kg^{-1} DW $soil^{-1}$
N_{mic}	97.7 ± 0.6	75.5 ± 1.7	***	34.6 ± 11.5	11.5 ± 2.2	***	***	***	n.s	mg kg^{-1} DW $soil^{-1}$
DON	9.5 ± 0.7	14.7 ± 0.9	**	7.32 ± 0.9	3.81 ± 0.8	*	n.s	***	***	mg kg^{-1} DW $soil^{-1}$
N_{labile}	136.8 ± 5.7	99.7 ± 2.2	**	200.6 ± 7.9	159 ± 6.8	***	***	***	n.s	mg kg^{-1} DW $soil^{-1}$
ΔN_{min}	-32.3 ± 5.3	-50.9 ±0.9	n.s	95.6 ± 6.7	83.5 ± 9.3	***	***	***	**	mg kg^{-1} DW $soil^{-1}$
ΔN_{mic}	53.3 ± 0.6	35.6 ± 1.7	***	-9.8 ± 2.2	-28.5 ± 2.4	***	***	***	***	mg kg^{-1} DW $soil^{-1}$
ΔDON	-3.7 ± 0.7	-4.75 ± 0.9	n.s	-5.86 ± 0.9	-15.6 ±0.8	***	***	***	***	mg kg^{-1} DW $soil^{-1}$
ΔN_{labile}	2.7 ± 5.7	-18.8 ± 2.18	*	66.5 ± 7.9	40.5 ± 6.8	*	**	***	n.s	mg kg^{-1} DW $soil^{-1}$
apr	5.5E+06 ±6.2E+05	4.6E+06 ± 2.0E+05	n.s	7.6E+06 ± 1.1E+06	5.3E+06 ± 3.8E+05	n.s	n.s	*	n.s	copy numbers g^{-1} DW $soil^{-1}$
npr	7.3E+04 ± 7.4E+03	7.6E+04 ± 1.2E+04	n.s	1.2E+05 ± 2.2E+04	1.4E+05 ± 1.3E+04	n.s	n.s	**	n.s	copy numbers g^{-1} DW $soil^{-1}$
ureC	1.1E+09 ± 1.4E+08	7.6E+08 ± 1.1E+08	n.s	1.0E+09 ± 1.5E+08	9.2E+08 ± 7.5E+07	n.s	n.s	n.s	n.s	copy numbers g^{-1} DW $soil^{-1}$
amoAOA	2.4E+06 ± 4.2E+05	1.5E+06 ± 1.6E+05	n.s	5.7E+06 ± 7.9E+05	3.2E+06 ± 3.4E+05	**	**	***	n.s	copy numbers g^{-1} DW $soil^{-1}$
amoAOB	1.6E+05 ± 3.1E+04	2.3E+05 ± 4.1E+04	n.s	2.0E+05 ± 3.0E+04	2.6E+05 ± 5.0E+04	n.s	n.s	n.s	n.s	copy numbers g^{-1} DW $soil^{-1}$
16S	5.0E+07 ± 6.1E+06	4.2E+07 ± 6.9E+06	n.s	6.1E+07 ± 1.1E+07	5.0E+07 ± 6.7E+06	n.s	n.s	n.s	n.s	copy numbers g^{-1} DW $soil^{-1}$
CO_2 respiration	10072.4 ± 276.2	9020.1 ±566.6	n.s	14507.5 ± 401.7	16954.4 ± 760.5	**	n.s	***	**	g CO_2 L^{-1} produced within 56 d
qCO_2	14.0 ± 0.4	17.6 ± 1.3	n.s	27.9 ± 1.2	38.3 ± 2.1	***	***	***	*	g CO_2 L^{-1} C_{mic}^{-1} produced within 56 d

Table 2: Relationship between the abundance of functional genes and fluxes in nitrogen pools across all treatments after 56 days of incubation. Relationship assessed by correlation analysis using spearman's rho (n=16).

	Δ N_{labile} (Proteolysis)		Δ N_{min} (net mineralization)		Δ NO_2^-+NO_3^- (net nitrification)		Δ N_{mic} (net microbial change)	
	r	p	r	p	r	p	r	p
apr	**0.80**	**<0.001**	**0.75**	**0.001**	**0.76**	**0.001**	0.34	0.204
npr	**0.65**	**0.006**	**0.57**	**0.020**	**0.63**	**0.001**	-0.24	0.374
ureC	0.38	0.161	0.27	0.319	0.34	0.202	0.28	0.302
amoAOA	**0.93**	**<0.001**	**0.91**	**<0.001**	**0.90**	**<0.001**	0.30	0.256
amoAOB	0.15	0.572	0.09	0.845	0.15	0.587	-0.23	0.386
16S	**0.58**	**0.019**	0.48	0.062	**0.55**	**0.028**	0.21	0.437

Conclusion

1. *apr* and *npr* correlate well with proteolytic fluxes (ΔN_{labile}, ΔN_{min}) and may serve as indicators for proteolytic activity/capacity
2. Enhanced microbial mediated functional proteolytic activity/capacity of organically managed soil, as observable in nitrogen pools and fluxes, is not directly translated to differences in functional gene abundance of *apr* and *npr*

→ Gene abundance is a genetic potential but not a direct measure (DNA→RNA→Protein)

→ The enhanced proteolytic activity/capacity in the organically managed soil is not explainable by a higher abundance of *apr* and *npr* but might be explained by differences in the microbial communities inhabiting the soil

→ **Analysis of *apr* and *npr* community structure could reveal differences being responsible for the enhanced proteolytic potential of organically farmed soils (amplicon sequencing of *apr* and *npr* planned for 2017)**

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