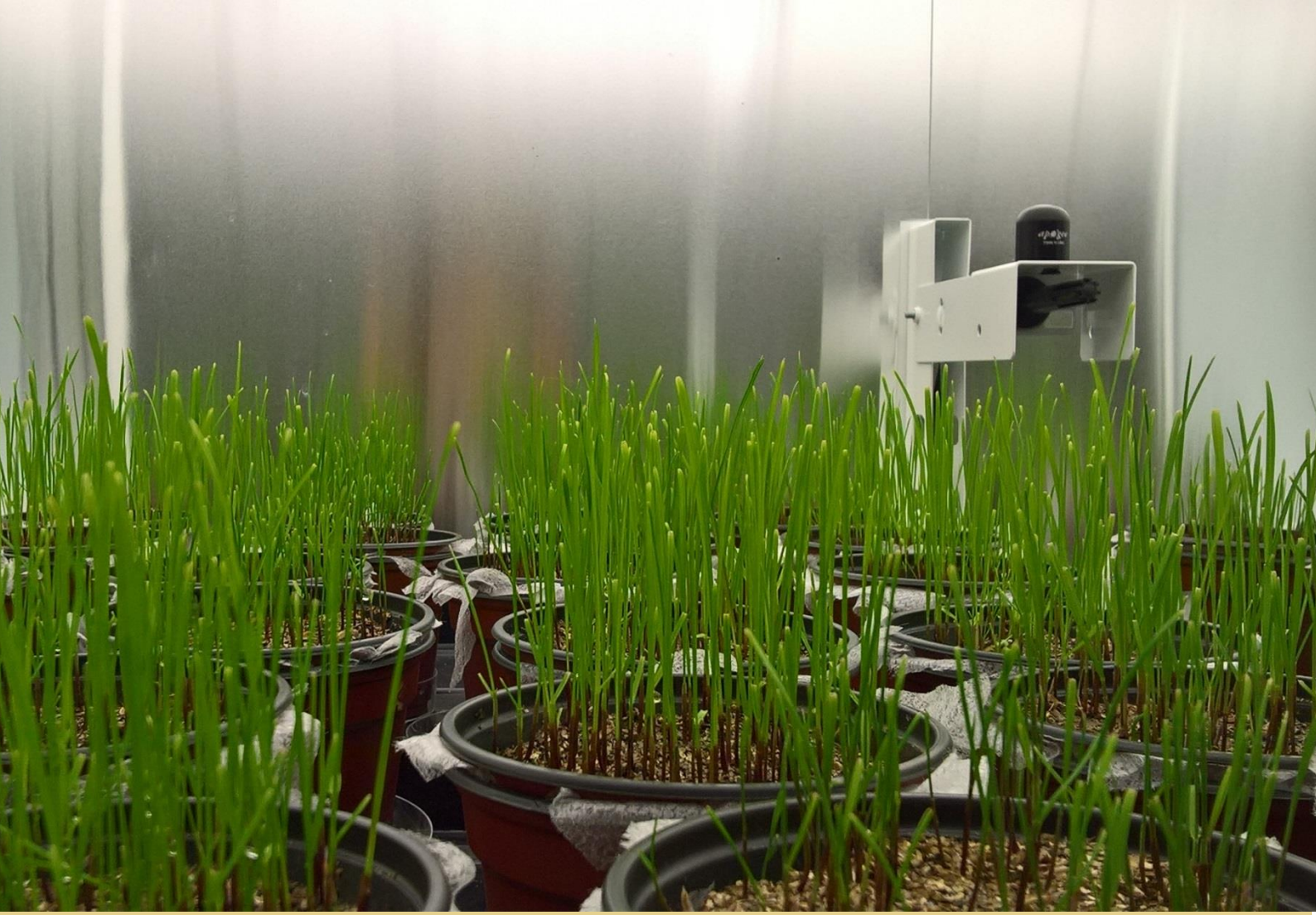

Norah Johanna Efosa

Nitrogen use efficiency of ryegrass and analysis of functional microbial genes for proteolysis in differently managed arable soils and under future projected rainfall variability

Tese de Mestrado em Ecologia, orientada por Andreas Gattinger (FiBL – Research Institute of Organic Agriculture) and José Paulo Sousa (Universidade de Coimbra) e apresentada ao Departamento de Ciências da Vida da Faculdade de Ciências e Tecnologia.

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Nitrogen use efficiency of ryegrass and analysis of functional microbial genes for proteolysis in differently managed arable soils and under future projected rainfall variability

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ABSTRACT

Future projected climate change and resource scarcity will challenge food and fodder production on a global scale. Organic farming systems resign from synthetic fertilizers and pesticides in order to promote sustainable production and to support ecosystem health. It has previously been shown, that organically farmed soils entail more abundant, more active and more diverse microbial communities compared to conventionally farmed soils and thus might potentially be more resilient when facing upcoming climate change. Despite the fundamental importance of microbial mediated nutrient cycling in soils, our understanding of the microbial performance in differently managed farming systems under future projected rainfall variability is still fragmentary. This study reports on proteolysis and mineralization of organically bound nitrogen and the subsequent nitrogen uptake by ryegrass, in a plant growth experiment using soils from the DOK long-term field experiment. ^{15}N isotope enriched lupine litter was used as a tracer for nitrogen fluxes whereas molecular genetic techniques were applied as a proxy for the soil microbial proteolytic potential combined with traditional geochemical parameters to monitor soil nitrogen pools. Our key finding is that under drought stress, ryegrass grown on organically managed soil assimilates significantly more ^{15}N in their total biomass derived from the labeled lupine litter compared to ryegrass grown in conventionally managed soil. This provides novel evidence for an enhanced microbial mediated nitrogen mineralization potential followed by enhanced nitrogen provisioning for plants in organically managed soil under future projected drought scenarios. No differences in ^{15}N accumulation between plants grown on the different soils were identified in optimal wet conditions. The quantification of functional genes involved in proteolysis, the initial and thus rate limiting step in nitrogen mineralization, could not fully explain our findings. The abundances of functional genes might serve as a proxy for certain soil functions but sampling timing is crucial and might not have been optimal in the current study. Furthermore, not only abundance of proteolytic microbes but also activity and diversity is essential and thus more in depths microbial investigations are required to fully understand the observed phenomena.

Keywords: Organic farming – Climate change – Proteolysis – Nitrogen use efficiency – Stable isotopes – Rainfall variability

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INTRODUCTION

Nitrogen (N) is regarded as one of the most important plant nutrients limiting crop production in terrestrial agricultural systems (Kraiser et al., 2011). Industrial N fertilizers work as an effective agent to increase yields, yet are detrimental for the environment and unsustainable (Seufert & Ramankutty, 2017).
The human population is predicted to reach 9.7 billion people by 2050 (UN, 2015) and alongside, an increase in global crop demand of 100-110% is projected for the same period (Tilman et al., 2011). Although, food and fodder shortage also reasons from several other factors like ineffective product storing and immense food waste (Smith, 2013), agricultural systems producing high and stable yields and without further threatening and exploiting Earth's limited resources are highly needed.

It is foresaid that besides global warming which will lead to increasing water losses due to enhanced evapotranspiration, stronger climatic events such as heavy droughts and strong rainfall events, will be encountered in the near future. It is likely that we will face a downturn in soil moisture in most regions of the planet already within this century with decreases up to 25% in subtropical and Mediterranean regions (Borken & Matzner, 2009). Environmental stresses and disturbances, such as drying and rewetting, can adversely impact the microbial physiology, community composition and thus functioning in terrestrial ecosystems (e.g. Schimel et al., 2007).

A knowledge gap regarding the performance of organic agriculture with respect to different climatic conditions, management practices and soil types, is present (Seufert et al., 2017).

Recent work identified a higher net proteolysis in organically managed soils under optimal wet but also drought scenarios compared to conventionally managed systems in a soil-microcosm incubation experiment (Lori et al., in preparation). Whether or not, the observed effects will translate into enhanced nutrient availability and uptake by plants is still unclear.

1.1 Towards sustainable agriculture in the 21st century

A central aim of sustainable farming is to mitigate the negative impacts on the environment such as loss of biodiversity, extensive land conversion and soil erosion, which arise from intensive agriculture worldwide (Seufert et al., 2017; Tilman et al., 2002). Overall, an organic farming system (FS) aims to enhance and maintain the physical, chemical and biological soil quality over time. Furthermore, diverse crop rotations including N-fixing crops like legumes and multi-annual clover-grass meadows promote biodiversity and support the soil structure (Fliessbach et al., 2007). Besides nutrient cycling, a healthy soil in its biotic and abiotic entirety provides many important ecosystem services: water purification, soil structuring, pest and erosion control and promotion of biodiversity (Bommarco et al., 2013).

At present, yields are on average 19-25% lower in organic FS compared to conventional ones (Seufert et al., 2012). This is due to nutrient limitation (e.g. during transition from conventional to organic farming), pest damage emerging in absence of pesticides and pressure by weeds (Tu et al., 2006). However, in 2011, the expenses for N losses from fertilizer were estimated to range around €70-320 billion annually in Europe (Sutton, 2011). Peak oil, the point at which global oil supplies will climax and then decline over the following decades, will be reached soon and the production of synthetic mineral fertilizer depends on crude oil and will be largely impacted by oil scarcity and increasing oil prices in the future (Neff et al., 2011). Further, surplus N from fertilization is subjected to be lost via volatilization and denitrification, likely producing harmful greenhouse gas emissions (IPCC, 2013). N leaching and runoff can evoke the eutrophication of water bodies and pollution of drinking water (Seufert et al., 2017).

Resigning from the application of synthetic fertilizers, as done in organic farming, requires the provision of sufficient alternative N sources for the crop plants such as farmyard manure, slurry or green manures (Fliessbach et al., 2007) and the successful transformation of such inputs. Soil microbes dominate the soil nutrient cycling via mineralization in organic farming (Wardle, 1992) and can, moreover, act as a transient nutrient sink for N (Dalal, 1998). The application of organic fertilizers such as green manures has been shown to improve the microbial activity in the soil on the long term (e.g. Langmeier et al., 2002).

Slow nutrient release rates from organic fertilizers lower the amounts of nutrients lost via leaching and overall reduce environmental impacts of fertilization (Benincasa et al., 2011, Rosen & Allan, 2007). Soil quality in general is improved and moreover, organic fertilization promotes soil organic matter (SOM) build up and maintenance resulting in higher carbon (C) sequestration potentials and an overall better soil structure for drainage and infiltration as well as optimized habitat conditions for the biotic soil community (Rosen & Allan, 2007).

Recently, Lori et al. observed enhanced microbial abundance and activity in organically compared to conventionally managed soils in a global meta-analysis. Furthermore, by using a semi-quantitative approach, differences in microbial community structures between organic and conventional FS were identified in nineteen out of twenty-one comparisons as well (Lori et al., 2017).

Whether or not organically managed systems generally have a higher resistance and resilience to extreme weather events such as droughts or heavy rainfall is still rarely examined and much more case studies and primary research are required.

The analysis of long-term FS trials enables to study and investigate the microbial community on a functional level as affected by the FS. The well-known DOK long-term trial in Therwil, Switzerland, serves as an optimal platform where many studies have been performed to investigate the effect of farming practices on various soil parameters over the last 40 years (Mäder et al., 2002). In the organically managed plots of the DOK, soil microbial biomass and dehydrogenase and protease activities were higher compared to the conventionally managed ones (Fliessbach et al., 2007). This gives evidence for a higher overall microbial activity and a greater potential for nutrient cycling by microorganisms (Mäder et al., 2002). Microbial functional diversity determined by the Shannon diversity index was notably higher in soils from organic FS than in soils from conventional FS and a higher microbial diversity might allow a better resource exploitation, which could lead to an enhanced capacity to adapt to new conditions such as climate change induced rainfall variability (Mäder et al., 2002). The structure and abundance of microbial communities in soils from the DOK was assessed to be predominantly influenced by the presence or absence of farmyard manure applications, crop type and FS (Esperschütz et al., 2007; Hartmann et al., 2006; Widmer et al., 2006).

Most likely, the high SOM content in soils receiving farmyard manure allow a greater microbial biomass production as it has been shown earlier (Mäder et al., 2002, Widmer et al., 2006). Alternatively, microbial communities may be introduced via farmyard manure (Hartmann et al., 2006) and other factors such as the use of pesticides and synthetic fertilizers can have an influence (Hartmann et al., 2014).

Bacteria and fungi represent about 90% of the soil microbial biomass (Six, 2012). Healthy and diverse soil-biota communities are contemplated to have a higher adaption and resilience potential for environmental fluctuations such as predicted extreme weather events, e.g. soil drying (Phillips et al., 2015). Nevertheless, more abundant and more active microbial communities do not directly translate into enhanced yield (Rocca et al., 2015) but could be adaptively favorable under stress conditions.

1.2 The global N cycle

Gaseous dinitrogen (N_2) molecules embrace a strong triple bond which cannot directly be broken down by most organisms. However, several pathways can integrate N from N_2 into the biological N-cycle. Biological N-fixation is performed by prokaryotic microorganisms, which predominantly live in symbiosis with plants and convert gaseous N_2 into ammonia (NH_3) and ammonium (NH_4). During nitrification- another microbial-mediated process, NH_3 and NH_4 are further reduced via nitrite (NO_2) to nitrate (NO_3). Alternatively, lightning and photochemical reactions can catalyze the reduction from N_2 into dissolved mineral forms of N (NH_3/NH_4^+ and NO_3). A third pathway is the anthropogenic industrial fixation of N_2 by the Haber-Bosch technique that was developed in the early 20th century (Dunn, 2001). The various mineral N (N_{min}) forms can enter organism's biosynthesis processes or are back-transformed into gaseous N, closing the global N cycle. In the transition towards N_2, during natural or anthropogenic-induced denitrification, nitrous oxide (N_2O), can be produced and only partly converted into N_2. NO_x molecules, being 300-times more inimical than CO_2 emissions, aggressively impact the ozone layer and fuel the greenhouse effect (IPCC, 2013)

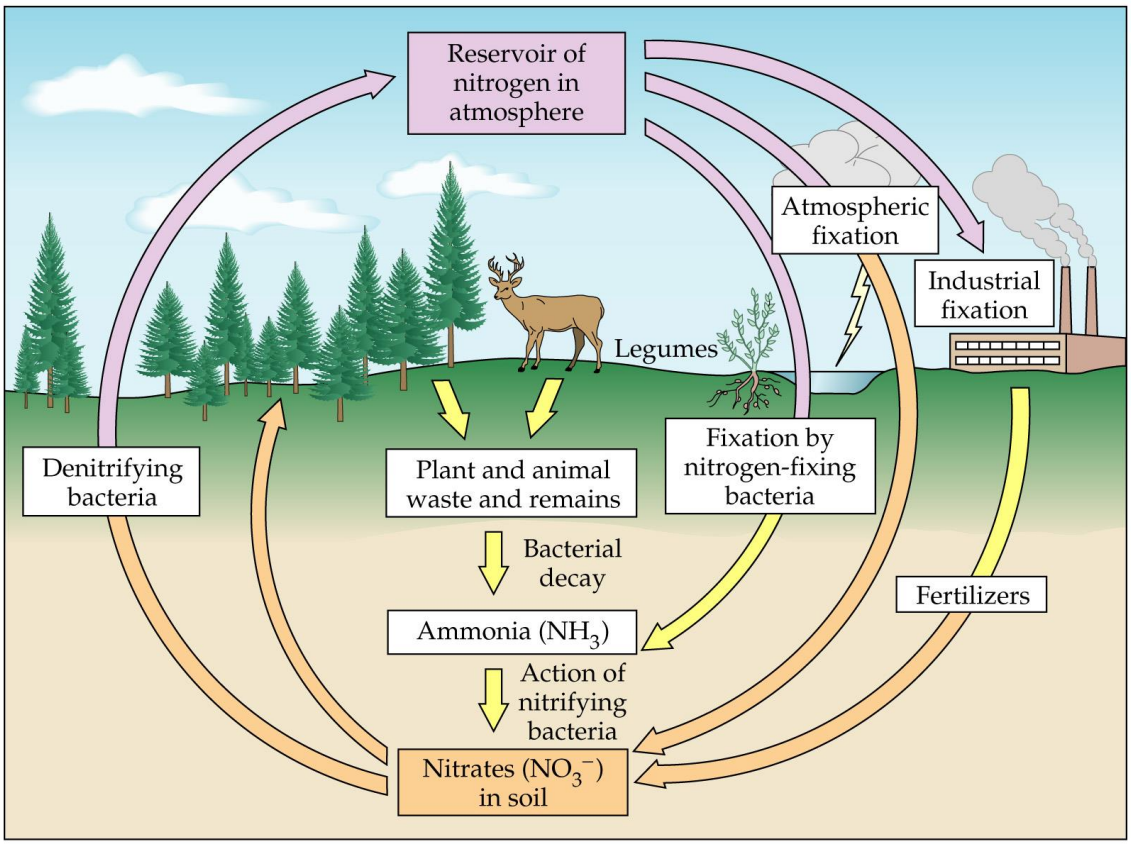

Figure 2 – Simplified scheme of the global N cycle (from: McMurry, 2010).

1.2.1 Proteolysis and N-mineralization

Most N in terrestrial ecosystems is bound in organic compounds. During N mineralization, organic N is converted into N_{min}, whereas N immobilization describes the opposite, the conversion of inorganic N into organic N.

N mineralization is besides N-fixation, the only source of N for plants in systems not receiving external N_{min} inputs (Gschwendter et al., 2010).

Proteolysis, the enzymatic hydrolysis of proteins into smaller peptides and amino acids, like most naturally occurring processes, is dependent on water and has been observed to decrease by 15-66% upon increasing soil drought (Sardans & Peñuelas, 2005). Proteolysis is the initial step of N mineralization and thus often referred to as "rate limiting" (Vranova et al., 2013).

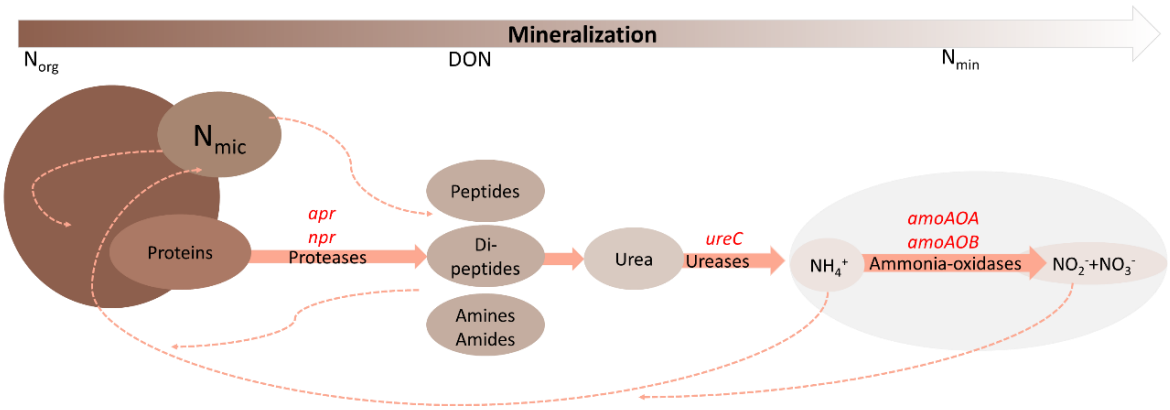

Figure 2 – Simplified scheme of soil microbial mediated nitrogen mineralization (Lori et al., in preparation). Organic nitrogen (N_{org}) gets mineralized into mineral nitrogen (N_{min}) via different steps and enzymes. Functional genes encoding for the respective enzymes are highlighted in red and abbreviated 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.

Extracellular soil proteases are ubiquitous and secreted of distinct origins varying from plants, fungi and bacteria and can be stabilized over time in the soil (Fuka et al., 2008, Sakurai et al., 2007). However, using selective inhibition of certain microbial groups the majority of microbial mediated proteolytic processes in soils is considered to be performed by bacteria (Hayano & Watanabe, 1993).

Bach and Munch identified Pseudomonas fluorescens, Bacillus cereus, Bacillus mycoides and Flavobacterium cytophaga species to be the numerically dominant proteolytic bacteria in a broad range of different soils using culturing methods – all of the latter named species secrete extracellular metalloproteases (Bach et al., 2001). The major dominant bacterial metalloproteases in soils are the alkaline- (*apr*) and the neutral metalloprotease (*npr*) (Fuka et al., 2008). Degenerated oligonucleotide sets targeting conserved regions of *apr* and *npr*, published 2001 by Bach and co-workers, allow in depths analyses of abundance, expression and phylogeny of proteolytic microbial communities in response to various factors such as FS, climatic changes and many more (Bach et al., 2001).

Some plants can excrete proteolytic exudates and absorb peptides and proteins of low molecular mass, yet, adding only minor important contributions to the overall soil proteolytic activity (Paungfoo-Lonhienne et al., 2008).

The "Birch-effect"

Rewetting after drought resembles osmotic stress for microbes due to a rising water potential in the soil. Microorganisms lyse upon osmotic stress induced by drying and rewetting or- in some cases, can resist (Griffiths et al., 2003) or enter the dormancy state until the conditions become favorable again (Schimel et al., 2007). Borken & Matzner found microbial activity to rapidly boost upon rewetting in response to freshly released nutrients (Borken & Matzner, 2009). This effect is known as the "Birch"-effect and the microbial activity is sometimes stimulated to outreach the activity in a permanently moist soil for up to a few days after rewetting, due to the high nutrient availability (Birch, 1958). Moreover, recalcitrant nutrients stored in SOM micro- and macroaggregates can become available for assimilation due to the breakdown of aggregates (Fierer & Schimel., 2003).

1.2.2 Plant-microbe competition for N

In N-limited ecosystems such as agroecosystems, a delicate balance of N mineralization and N immobilization exists and N scarcity e.g. due to stressors like drought, can potentially induce shifts towards an enhanced competition for N between microbes and plants (Kaye & Hart, 1997).

Competition with soil microbes, besides the availability of inorganic N, is one of the most critical factors affecting the plant N uptake from the soil (Kaye & Hart, 1997). Previous studies have identified microbes to be the stronger competitors upon introducing new N sources, at first. However, microbial biomass population was observed to reach a steady state within the first days of inorganic N fertilizer application, most likely due to C-limitation. From that point on, plants take up the most N from fertilizer (Hodge et al., 2000; Inselbacher et al., 2010). For agriculture, it should be favorable to avoid conditions in which immobilization increases beyond the point of providing a sufficiently performing N cycling community. Several methodologies can be applied to examine the state of the plant-microbe competition for N. Inselbacher et al. measured the recoveries

of ^{15}N from fertilizer in plants and in the microbial biomass (Inselbacher et al., 2010). Kyle and Hart correlated total plant N with N stored in microbial biomass- a negative relationship in this case, would indicate a stronger competition (Kyle & Hart, 1997).

1.3 N use efficiency

The overall goal of calculating N use efficiencies is to optimize the nutrient uptake from fertilizer to minimize losses and to maximize plant growth and yields (Fixen et al., 2015; IAEA, 2001). N use efficiency is a function of the N uptake from natural and fertilizer-borne N sources, N assimilation and plant physiology specific rates of translocating N into (harvestable) biomass (Dawson et al., 2008; Ladha et al., 2005). Crop N use efficiency is strongly determined by environmental factors such as soil pH, SOM content, soil texture, bulk density and the depth of soil sampling, since most readily available nutrients are stored in the surface soil (Ladha et al., 2005). Other factors influencing the N use efficiency in the field are crop management (crop variety, density, spatial arrangement of plants), fertilization rate and methods of application (place & timing) and water management (Fixen et al., 2015).

Depending on the study focus and complexity of the studied system, the N use efficiency can be a simple formula or very complex and expanded with environmental variables and soil geochemical measurements (IAEA, 2001). Trends in N use efficiency calculation methods vary globally across regions and the most appropriate method is determined by the research question and the data (Fixen et al., 2015).

The N recovery efficiency is one of the many ways to calculate the N use efficiency (Ladha et al., 2005). It refers to the uptake of a nutrient from the fertilizer by a plant relative to the amount that was added as fertilizer (IAEA, 2001). Thus, the latter is suitable for the study in hand where^{15}N labelled lupine litter representing the fertilizer input, was used. The use of ^{15}N labeled fertilizer allows to distinguish whether freshly mineralized nitrogen (^{15}N) or initial soil-borne N, which can be different in distinct FS, is taken up by the plant.

Isotopes are a useful tool in agricultural studies, as they enable to trace a particular element which behaves similar to its non-isotopic analogue, through various pathways and to obtain quantitative measurements.

The ^{15}N isotope is a stable isotope and occurs naturally with an abundance of 0.3663% and mass spectrometry is the most common and precise method to measure stable isotopes (IAEA, 2001).

For experiments with data from labelled N fertilizer, the direct method is usually the first method of choice (Hauck & Bremner, 1976; IAEA, 2001). It is commonly applied in studies on fertilizer N use efficiency but also N-fixation or animal nutrition studies, among others (Fixen et al., 2015; IAEA, 2001). Whereas other methods focus on the yield per unit of fertilizer or on the overall N balance (Ladha et al., 2005).

1.4 Research objectives

The study in hand investigates the performance of proteolytic processes and subsequent N availabilities and N uptake efficiencies by ryegrass in differently managed soils (organic, conventional) while simultaneously considering the effects of future projected climate change-induced rainfall variabilities (drought, drying and rewetting). Soils were amendment with ^{15}N isotope-labelled plant litter input to allow tracing of the amounts of freshly (microbial mediated) proteolyzed and mineralized N that is released from organic macromolecules and taken up by plants. Several studies have already investigated the recovery of ^{15}N from organic fertilizers but to date, could not detect impacts of different farming management practices (Bosshard et al., 2009; Langmeier et al., 2002; Glendining et al., 1997).

This study is part of the European joint project "Sustainable provisioning of multiple ecosystem services in agricultural landscapes" (ECO-SERVE), that targets the adaptation potential of various agricultural systems to increased rainfall variability and drought scenarios across Europe. ECO-SERVE is funded by the BiodivERsA/FACCE-JPI and the Swiss National Science Foundation (SNF) National Research Program (NRP 68) «Soil as a resource».

Main research questions

1) Are there differences in the provisioning of plant available N from fresh litter between distinct FS and different rainfall scenarios?
2) How do farming management and rainfall variability influence the recovery of N from fresh plant litter by ryegrass?
3) Can potential differences in microbial mediated proteolysis and subsequent N uptake by ryegrass be explained by the abundances of functional microbial genes for proteolysis?

Hypotheses

H1) Organic FSs perform better in the provisioning of plant available N from fresh litter when subjected to drought and drying and rewetting scenarios compared to conventional FSs.

H2) The recovery of N from fresh litter by ryegrass is higher in organic FSs, especially under drought and drying and rewetting scenarios compared to conventional FSs.

H3) The abundances of functional microbial genes for proteolysis can explain the patterns in provisioning of plant available N and the subsequent N uptake by ryegrass.

Additional research question

Does drought stress induce a shift from N-mineralization to pronounced N-immobilization, potentially inducing a strong competition for N between plants and microbes and if so, are there differences between FS?

2 METHODOLOGY AND MATERIALS

2.1 *Lolium multiflorum*

The Italian ryegrass (*Lolium multiflorum*, Lam.) is a tetraploid annual or biennial grass (Poaceae) and was cultivated in the present study. *L. multiflorum* is historically widely used for ley farming and cattle grazing (Beddows, 1973).

2.2 Soils from the DOK-trial field site

The DOK-trial (D: bio-dynamic, O: bio-organic, K: ‘‘konventionell”/”conventional”) is a long-term agroecosystem experiment that was initiated in 1978 by the Research Institute of Organic Agriculture (FiBL, Frick) and the Agroscope Reckenholz Tänikon Research Station (ART Zürich Reckenholz) (e.g. Fliessbach et al., 2007, Mäder et al., 2002). With almost 40 years of research history, the DOK-trial is a rare and precious basin for acquiring scientific knowledge about the effects of different FSs.

The DOK long-term experiment entails four different FSs. The organic bio-dynamic (BIODYN) and bio-organic (BIOORG) FSs are supplied with organic fertilizers and the conventional systems receive mineral fertilizer (CONMIN) and mineral fertilizer and farmyard manure (CONFYM). In addition, an unfertilized control FS is included (NOFERT). Farmyard manure and slurry corresponding to a livestock density of 1.4 DGVE/ha are regularly applied to the BIOORG system while the CONMIN system receives mineral NPK-fertilizer exclusively (e.g. Fliessbach et al., 2007). Distinct fertilization and plant protection schemes are applied to each FS, while crop rotation is identical for all systems. The entire experiment is replicated four times and fully randomized. The DOK-trial site (7°33´E, 47°30´N, Therwil, Switzerland) is characterized by a mild and rather dry climate with an annual precipitation of 972 mm, a mean temperature of 9.5°C and a soil composed of 16% clay, 70% silt and 14% sand (Fliessbach et al., 2007).

Soil for this study was extracted from the bioorganic (BIOORG) and the conventional mineral (CONMIN) FS in summer 2015 shortly after winter wheat harvest with soybean as a pre-crop. Soils were sieved at 2mm and stored at 4°C until the experimental start.

2.3 Experiment setup and handling

The experiment was set up in a full factorial design (n=36) comparing two FS and three water regimes (WRs) (Table 1). Each pot (⌀ = 9 cm) received a soil mixture composed of 200 g dry weight (DW) equivalents soil from the DOK-site and 1.5 g ^{15}N-labelled lupine litter (1005 ± 97 µg ^{15}N pot^{-1}). The ^{15}N labelled lupine litter was gently and homogenously worked into the soil. 4 g of ryegrass seeds (Italian ryegrass, 4n, var. Gemini) were uniformly distributed on top of the soil and covered with a thin layer of vermiculite to prevent moisture loss during germination.

Table 1 - Experimental design. A multi-factorial mesocosm experiment was set up with soils from two distinct farming systems (FSs) and three water regimes (WRs): dry, intermittent and wet, with respect to the soil's maximum water holding capacity (mWHC).

FS	WR	Replicates
Organic	Dry = 20% mWHC	6
	Intermittent = 20% mWHC, 80% mWHC	6
	Wet = 80% mWHC	6
Conventional	Dry = 20% mWHC	6
	Intermittent = 20% mWHC, 80% mWHC	6
	Wet = 80% mWHC	6

After three days, the seeds germinated homogenously and from day three, the respective WR treatment was applied. The experiment lasted for 56 days and the rewetting of the dry soil in the intermittent treatment occurred at halftime (day 28). The plants were incubated in a climate chamber with a day/night cycle of 10/14h, the temperature ranging between 20-22°C and the humidity around 60%. The pots were weighed and watered daily according to water evaporation losses and WR treatment, throughout the entire experiment.

The ^{15}N labelled lupine litter (*Lupinus alba*) was produced beforehand by supplying lupine seedlings with a ^{15}N amended modified Hoagland solution (Lori et al., in preparation). To account for the natural ^{15}N abundance in soil and plant seeds (IAEA, 2001), a side experiment was set up in a similar manner but lacking the input of ^{15}N-labelled litter. Only two WR (dry and wet) were applied per FS (n=24). The measured background abundance of ^{15}N (0.3681%±0.0003%) is in line with the standard value of 0.3663%, used in the literature (IAEA, 2001).

2.4 Sampling and harvest

The aboveground shoot biomass was cut for the first time after 28 days and a second time at the final sampling after 56 days. The plant root system was recovered at the final sampling. Soil samples of each pot were taken at the start and the end of the experiment. Subsamples were aliquoted and directly analyzed for microbial biomass, air dried for pH estimation, oven dried for C/N analysis and frozen at -80°C for molecular analysis until further processing.

2.5 Measurements

2.5.1 Geochemical analyses

Soil microbial biomass carbon (C_{mic}) and soil microbial biomass N (N_{mic}) were measured following the protocol of Joergensen (Joergensen, 1996). About 24.5 g soil (20g DW) were portioned for extraction with a 0.5 M K_2SO_4-solution (Fluka, Switzerland) and shaken at 250 rpm for 90 min to be finally filtered (filter paper type 615¼ Ø185mm, Macherey-Nagel, Düren, Germany) and analyzed (TOC/TNb-analyzer, multi N/C® 2100S, Analytik Jena AG, Jena, Germany). Chloroform-fumigation was applied to the same quantities of soil of each sample and samples were treated likewise non-fumigated samples.

Soil pH was measured using a digital pH-meter (inoLab pH Level 1, WTW GmbH, Germany) in air dried soil suspended in demineralized water (1:2.5, w:v).

N_{min}, i.e. NH_4, NO_2 and NO_3 as well as dissolved organic N (DON) contents (all the later N forms together = $N_{available}$), were extracted from 30 g soil with a water content of 20 ± 8 % and diluted in 120 ml 0.01 M calcium chloride solution. After shaking at 150 rpm for 60 min at room temperature, extracts were filtered through 185 mm pore size filters (type 619¼ Ø185mm filter paper, Macherey-Nagel, Düren, Deutschland) and analyzed (SAN-plus Segmented Flow Analyzer, Skalar Analytical B.V., Breda, Netherlands). Total soil carbon (C_{tot}) and soil N (N_{tot}) were measured via dry combustion (Vario EL III Element Analyzer, Elementar Analysensysteme GmbH, Langenselbold, Germany). For each sample, 50 mg oven dried (24 h, 40 °C) soil was weighed into tin capsules (Tin capsules for solids, 5x9mm, SÄNTIS Analytical AG, Teufen, Switzerland).

2.5.2 ^{15}N stable nitrogen isotope analysis

Samples of the plant root and shoot tissue were oven dried at 55 °C for 24 h and homogenized for 30 s at a frequency of 30 1 s^{-1} (Retsch Mixer mill MM 200, RETSCH GmbH, Hahn, Germany). 1.5 - 2 mg plant tissue was transferred into tin capsules (Tin capsules for solids, 3.2x4mm, SÄNTIS Analytical AG, Teufen, Switzerland). ^{15}N abundance was determined with an Isotope Ratio Mass Spectrometer/IRMS (delta V Advantage, Thermo Fisher, Dreieich, Germany) coupled to an Elemental Analyzer (Euro EA, Eurovector, Milano, Italy) at the Helmholtz Zentrum München, Munich, Germany.

2.5.3 Molecular Analyses

DNA-Extraction

The DNA was extracted from 450 mg frozen fresh weighed soil using the Fast DNA™ Spin Kit for Soil (MP Biomedicals, Solon, OH, USA) and eluted in 50µl DES (DNase/Pyrogen-Free Water, DNA elution solution, FastDNA Spin Kit for Soil, MP Biomedicals) following the manufacturer's instructions. DNA extraction efficiency was assessed by spiking each DNA sample with a known concentration (5.64E+08 copy numbers) of the APA 9 plasmid (vector pUC19 with an insert of the cassava mosaic virus, GenBank accession number: AJ427910) (von Felten et al., 2010; Thonar et al., 2011). After DNA extraction, the amount of recovered spike plasmid was assessed using qPCR and the recovery factor was calculated.

Quantitative real-time PCR (qPCR)

The abundances of the functional microbial genes *apr*, *npr* and the 16S rRNA subunit, as a proxy for microbial community size, were quantified (CFX96 TouchTM Real-Time PCR Detection System, Bio-Rad, California, US) using qPCR (Kapa SYBR® Fast qPCR Kit Master Mix, Kapa Biosystems, Wilmington, MA). One qPCR reaction comprised of each, 1.5 µl forward and reverse primer (Microsynth AG, Switzerland) at a final concentration of 10 nmol, 7.5 µl SYBR, 3.0 µl ultrapure water and 1.5 µl DNA template (Appendix 1). Three technical replicates per biological replicate were measured at gene specific cycling conditions (Appendix 2). Serial dilutions of linearized plasmid standards containing the gene of interest were included in each run to calculate standard curves and reaction efficiencies.

Copy numbers of the standard dilution were measured using fluorometric quantification (Qubit Fluorometer, Invitrogen, California, US). Gene abundance was corrected with the recovery factor and the water content of the extracted soil.

2.5.4 Plant nitrogen (recovery) use efficiency

Working under controlled conditions in a mesocosm, no N is added to the system via watering and no N is lost from leaching. A previous incubation experiment with the same soil and similar treatments showed that gaseous N losses in the form of N_2O were negligible. Thus, the formula for the recovery efficiency of ^{15}N (*REC*) from lupine litter is a function of ^{15}N applied and ^{15}N assimilated into total plant biomass.

The total ^{15}N recovery from lupine litter was calculated as:

$$\%\, REC = \frac{a-b}{c-d}\, x\, 100 \qquad (1)$$

Where a = % ^{15}N in plant tissues, b = % ^{15}N background abundance in plant tissues, c = % ^{15}N in the fertilizer (lupine litter) and d = % ^{15}N background abundance in the fertilizer (modified from Hauck & Bremner, 1976; IAEA, 2001).

2.5.5 Statistical analysis

Statistical analyses were performed using the JMP software version 11.2.1 (SAS Institute Inc. Cray, NC, 2014). The distribution of the residuals was tested by a Shapiro-Wilk Goodness-of-Fit Test. The data of the 16S rRNA showed in-homogenously distributed variances and was thus transformed (square root). The effects of FS, WR and the interaction among the latter two factors (FS x WR), were assessed by a two-way ANOVA. Contrast analyses were conducted to test for significant differences between FSs within the same WR. Variables were investigated for correlations using a Pairwise comparison (Pearson's correlation) or- in case of nonparametric data, Spearman's correlation. The data that was collected at the start of the experiment was investigated for FS effects via Student's t-test or Wilcoxon signed-rank test. Graphs were created with SigmaPlot ® version 12.5 (Systat Software Inc., Chicago, IL, 2003).

3 RESULTS

3.1 Soil geochemical parameters

Soil geochemical parameters were quantified at the start of the experiment (Appendix 3) and after 56 days of incubation in soils from two different FSs, subjected to three different WRs (Table 2).

N_{min} contents decreased gradually from the wet WR over the intermittent and to the dry WR (Figure 3). After 56 days of soil drying, N_{min} was significantly lower in the conventional FS compared to the organic FS ($p \leq 0.0025$) under drought. No FS effect on N_{min} occurred in the other WR. N_{min} was lowered by almost 75% in the conventional FS of the dry WR compared to the wet WR. Whereas, in the dry WR of the organic FS, N_{min} values were lowered by only 50% compared to the wet WR.

No gradual decrease with WR, as observed in N_{min}, was measured for N_{mic} (Figure 3). N_{mic} in the conventional FS, was significantly lower in the wet WR ($p \leq 0.0024$) and in the dry WR ($p \leq 0.002$) compared to the organic FS. However, N_{mic} was not affected by FS in the intermittent WR.

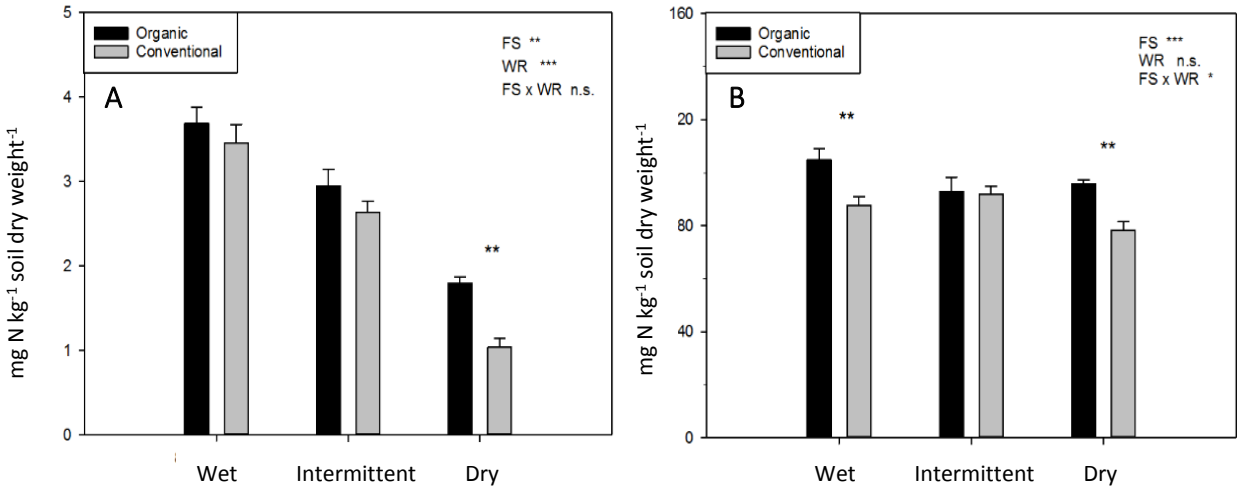

Figure 3 – Soil mineral nitrogen (N_{min}, A) and microbial nitrogen (N_{mic}, B) after 56 days of incubation.
Three water regimes (WRs): wet = 80% maximum water holding capacity (mWHC), intermittent = 20% mWHC (days 0-28), 80% mWHC (days 28-56) and dry = 20% mWHC, were applied to soils derived from an organic farming system (FS) and a conventional FS. The effects of WR, FS and their interaction (FSxWR) were tested by a full-factorial ANOVA. Results of the comparison between treatments were obtained with a Contrast Analysis based on the ANOVA. Significance levels: * $p<0.05$, ** $p<0.01$ and *** $p<0.001$, n.s.=not significant, vertical bars indicate the standard error (n=6).

A highly significant WR effect ($p \leq 0.0001$) was detected on the C_{mic}:N_{mic} ratio, being elevated in the dry WR compared to the other two WRs. (Table 2) No significant C_{mic}:N_{mic} ratio-differences occurred between FSs.

3.2 ^{15}N recovery efficiency

At the end of the growth period after 56 days, the highest ^{15}N recovery from lupine litter, *REC*=9.80%±0.2% (in the total plant biomass), was reached in the organic FS under the wet WR and not significantly less by plants grown in conventional soil (*REC*=9.26%±0.3%) (Figure 4). *REC* was 8.93.0%±0.3% in the organic soil of the intermittent WR and showed a highly significant FS effect when compared to the conventional soil where *REC* was only 7.41%±0.3%. Under dry soil conditions, *REC* declined further and is lowest in the conventional soil (*REC*=4.87%±0.1%) and highly significantly distinct (*p<0.0000*) from the organic soil (*REC*=6.36%±0.1%).

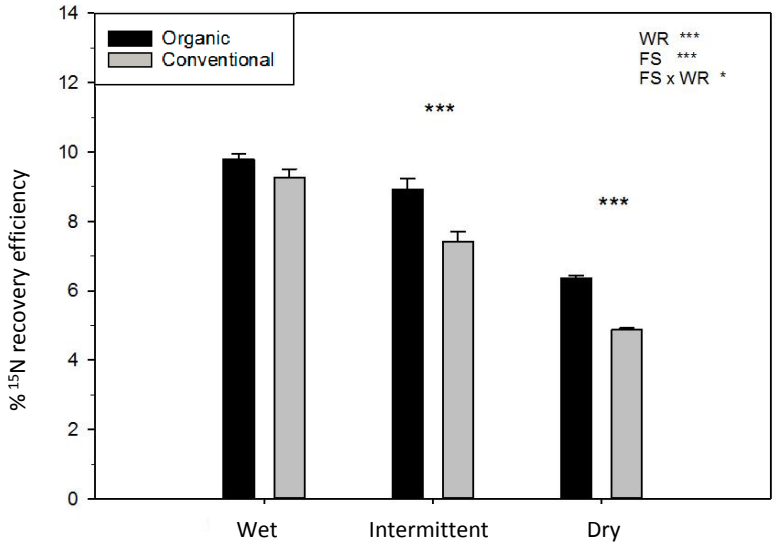

Figure 4 – ^{15}N recovery efficiency measured in the total ryegrass plant biomass.

Three water regimes (WRs): wet = 80% maximum water holding capacity (mWHC), intermittent = 20% mWHC (days 0-28), 80% mWHC (days 28-56) and dry = 20% mWHC, were applied to soils derived from an organic farming system (FS) and a conventional FS. The effects of WR, FS and their interaction (FSxWR) were tested by a full-factorial ANOVA. Results of the comparison between treatments were obtained with a Contrast Analysis based on the ANOVA. Significance levels: * p<0.05, ** p<0.01 and *** p<0.001, n.s.=not significant, vertical bars indicate the standard error (n=6).

3.3 Total plant biomass, plant N and ^{15}N contents

Ryegrass accumulated between 16.2 mg and 10.2 mg N in the total plant biomass. Strong differences are present between WRs when comparing sampling data from shoots cut after 28 and 56 days (Figure 5). After 28 days, the lowest N values were measured in the intermittent WR. Vice versa, the intermittent treatment revealed the highest N values in shoots cut after 56 days. When comparing the shoot biomass and also when combining the total plant biomass, tissue N contents in the intermittent WR were in the same range as the values from the wet WR.

Plants grown in organic and conventional soil produced on average 1.49 g ± 0.02 g and 1.40 g ± 0.04 g total dry plant biomass, respectively, when subjected to the wet WR (Figure 5). The corresponding values for the other treatments were 1.36 g ± 0.04g in the organic and 1.19 g ± 0.05 g in the conventional FS of the intermittent treatment and 0.96 g ± 0.02 g in the organic and 0.78 g ± 0.02 g in the conventional FS in the dry WR. In both of the latter WRs, the organic FS had a significantly higher productivity (intermittent: $p \leq 0.0007$, dry: $p \leq 0.0002$) in terms of plant biomass compared to the conventional FS (Figure 5).

The amounts of ^{15}N in the total plant tissue ranges from the highest value of 0.064mg mg^{-1} dry weight^{-1} in the wet WR of the organic FS to the lowest value of 0.031mg mg^{-1} dry weight^{-1} in the conventional FS of the dry WR (Figure 5). In the wet WR, no significant differences in the plant ^{15}N contents between FSs were found. ^{15}N contents in shoots cut after 28 days were twice as high ($p \leq 0.0056$) in the organic FS (0.02 mg ± 0.00 mg) when compared to the conventional FS (0.001 mg ± 0.01 mg) within the intermittent WR and higher by one third in the dry WR ($p \leq 0.0054$). This FS effect on ^{15}N contents was consistent for all tissue parts of the intermittent and dry WR, except for the shoots cut after 56 days, where no significant FS effect occurred in any WR.

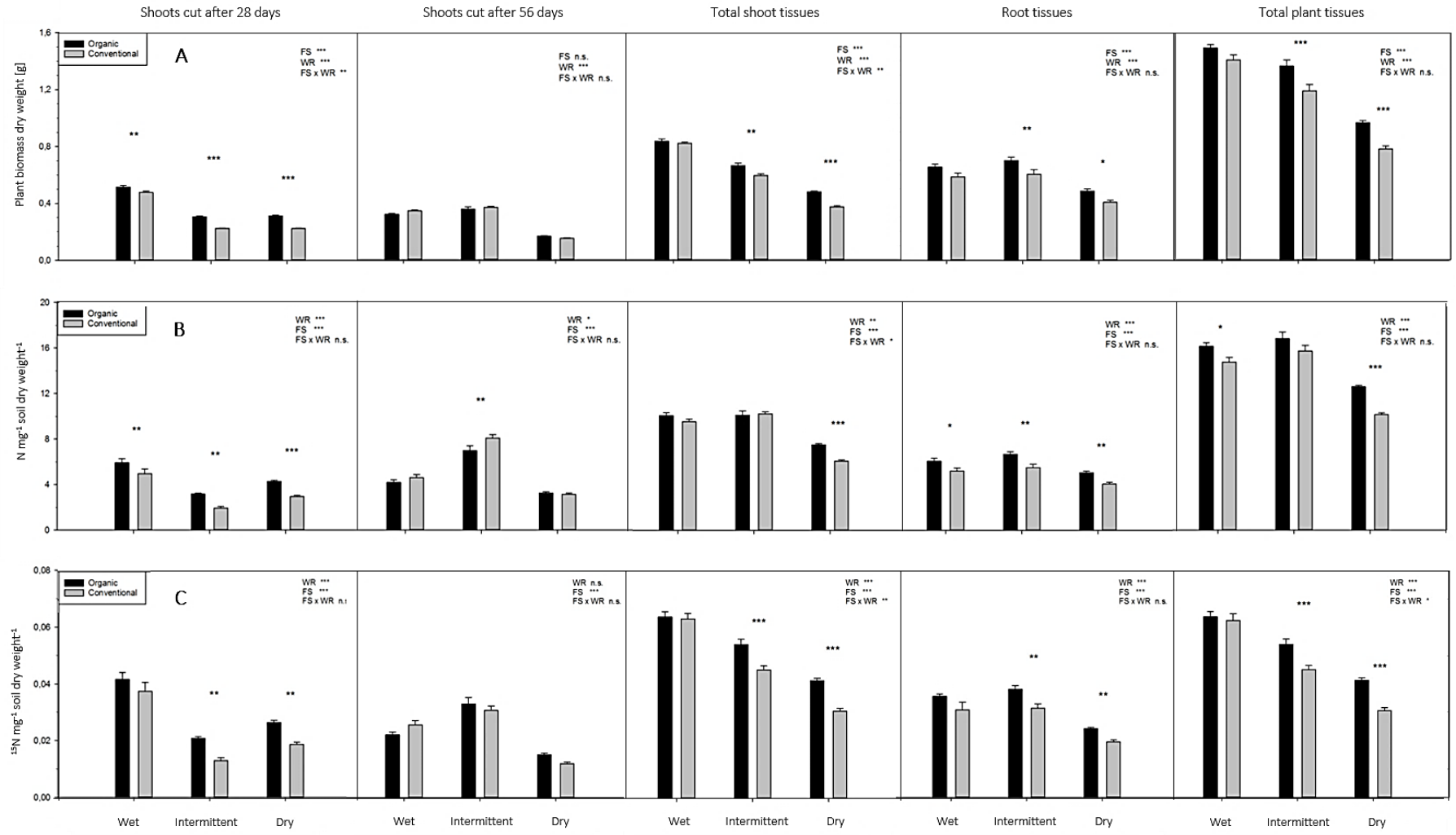

Figure 5 – Plant biomass, plant tissue nitrogen contents and plant tissue ^{15}N nitrogen isotope contents.

Panels A-C show the dry weight in g (A), N contents in mg (B) and ^{15}N contents in mg (C) in ryegrass tissues of shoots harvested after 28 days, 56 days, the total shoot biomass, the total root biomass and the total plant biomass. The effects of water regime (WR), farming system (FS) and their interaction (FS x WR) were tested by a full-factorial ANOVA. Results of the comparison between WR treatments (wet = 80% of the soil's maximum water holding capacity (mWHC), intermittent = 20% mWHC (days 0-28), 80% mWHC (days 28-56) and dry = 20% mWHC) and FS treatments (organic and conventional), were obtained by a Contrast Analysis based on the ANOVA. Significance levels: * $p<0.05$, ** $p<0.01$ and *** $p<0.001$, vertical bars indicate the standard error (n=6).

Table 2 – Soil geochemical parameters and abundances of functional microbial genes for proteolysis after 56 days of incubation. Three water regimes (WRs): wet = 80% maximum water holding capacity (mWHC), intermittent = 20% mWHC (days 0-28), 80% mWHC (days 28-56) and dry = 20% mWHC, were applied to soils derived from an organic farming system (FS) and a conventional FS. The effects of WR, FS and their interaction (FSxWR) were tested by a full-factorial ANOVA. Results of the comparison between treatments were obtained with a Contrast Analysis based on the ANOVA. Significance levels: * $p<0.05$, ** $p<0.01$ and *** $p<0.001$, n.s.=not significant, vertical bars indicate the standard error (n=6).

		Moist WR					Intermittent WR					Dry WR							
		Organic FS		Conventional FS			Organic FS		Conventional FS			Organic FS		Conventional FS			ANOVA effects		
Parameter	Unit	mean	SE	mean	SE	Contrast analysis	mean	SE	mean	SE	Contrast analysis	mean	SE	mean	SE	Contrast analysis	FS	WR	FSxWR
N_{min}	mg N/kg DW soil	3.7	0.2	3.5	0.2	n.s.	2.9	0.2	2.6	0.1	n.s.	1.8	0.1	1.0	0.1	**	**	***	n.s.
NO_2, NO_3	mg N/kg DW soil	2.0	0.2	1.4	0.1	**	2.0	0.1	1.4	0.1	**	1.9	0.1	1.3	0.1	**	***	n.s.	n.s.
NH_4	mg N/kg DW soil	1.9	0.1	2.3	0.2	n.s.	1.3	0.1	1.5	0.2	n.s.	0.2	0.1	0.2	0.1	n.s.	n.s.	***	n.s.
C_{mic}	mg C/kg DW soil	597	17	500	19	**	533	32	523	18	n.s.	702	7	552	20	***	***	***	**
N_{mic}	mg N/kg DW soil	105	4	88	3	**	93	5	92	3	n.s.	96	2	78	3	**	***	n.s.	*
C_{mic}:N_{mic}	ratio	5.71	0.11	5.70	0.06	n.s.	5.75	0.05	5.70	0.07	n.s.	7.33	0.07	7.08	0.15	n.s.	n.s.	***	n.s.
DON	mg N/kg DW soil	1.1E-03	5.3E-05	8.8E-04	8.5E-05	-	6.4E-04	4.3E-05	5.7E-04	2.5E-05	-	1.9E-04	1.0E-04	1.5E-04	5.6E-05	-	-	-	-
$N_{available}$	mg N/kg DW soil	109	4	91	3	**	96	5	94	3	n.s.	98	2	79	3	**	***	*	*
C_{total}	mg C/200g soil	3199	13	3114	12	-	3236	10	3128	18	-	3326	18	3188	18	-	-	-	-
N_{total}	mg N/200g soil	350	1	346	1	n.s.	354	1	350	3	n.s.	371	2	351	2	***	***	***	***
pH	-	6.2	0.0	6.2	0.0	n.s.	6.2	0.0	6.2	0.0	n.s.	6.1	0.0	6.0	0.0	n.s.	n.s.	***	n.s.
apr	copy numbers	1.01E+07	3.28E+06	6.99E+06	2.08E+06	n.s.	1.71E+07	6.22E+06	9.19E+06	3.73E+06	n.s.	1.04E+07	2.78E+06	8.24E+06	2.31E+06	n.s.	n.s.	n.s.	n.s.
npr	copy numbers	1.71E+07	4.12E+06	1.80E+07	2.74E+06	n.s.	2.83E+07	4.23E+06	1.50E+07	4.75E+06	*	1.24E+07	2.29E+06	1.82E+07	2.71E+06	n.s.	n.s.	n.s.	*
16S	copy numbers	1.58E+10	3.00E+09	1.80E+10	2.86E+09	n.s.	1.96E+10	2.88E+09	2.14E+10	3.77E+09	n.s.	1.78E+10	2.32E+09	2.37E+10	4.24E+09	n.s.	n.s.	n.s.	n.s.

3.4 Plant-microbe competition for N

To determine whether a strong competition for N between the plants and microbes could have influenced the fate of the organic fertilizer (lupine litter), in this experiment, the relationships of N_{min} and N_{mic} with total N assimilated into plant biomass were studied. A strong competition state indicating enhanced N immobilization was expected to be manifested by a negative relationship of the total plant N contents decreasing with increasing N_{mic} values.

We found highly significant positive relationships of both, N_{mic} and N_{min} with the total plant N in the dry WR (Table 3). No significant correlations between N_{min} and total plant N were obtained in the intermittent WR and in the wet WR, though N_{mic} showed a positive relationship with total plant N in the wet treatment.

Table 3 – Relationships between soil microbial nitrogen (N_{mic}) and mineral nitrogen (N_{min}) and with N assimilated into plant biomass (N_{tot}) after 56 days of incubation. The data was separated by water regimes (WRs): wet = 80% maximum water holding capacity (mWHC), intermittent = 20% mWHC (days 0-28), 80% mWHC (days 28-56) and dry = 20% mWHC. The relationships were analyzed with a Pairwise comparison. Significance levels: * p<0.05, ** p<0.01 and *** p<0.001, n.s.=not significant, vertical bars indicate the standard error (n=12).

		N_{mic}			N_{min}		
	WR	r	p		r	p	
N_{tot}	Dry	0.8479	0.0005	***	0.9046	0.0001	***
	Intermittent	0.2468	0.4644	n.s.	0.5542	0.0769	n.s.
	Wet	0.6202	0.0418	*	0.0005	0.9987	n.s.

3.5 Abundances of microbial proteolytic genes

The abundances of functional microbial genes involved in proteolysis showed high variations within treatments after 56 days of incubation and were exclusively significantly different between the FS for *npr* being elevated in organic FS subjected to intermittent WR ($p \leq 0.0142$) (Figure 6). Average *npr* gene copy numbers per g soil DW ($\bar{x}$ =1.82E+07) were by trend higher than *apr* copy numbers ($\bar{x}$ =1.04E+07), throughout treatments.

No significant effects of WR and/or FS on the abundance of 16S rRNA transcript copy numbers were measured (Table 2).

Both, *apr* (R^2=0.6566, $p \leq 0.001$) and *npr* (R^2=0.6001, $p \leq 0.001$) gene abundances correlated significantly positive with the 16S rRNA abundance, the data explained 66% and 60% of the data, respectively (Appendix 4).

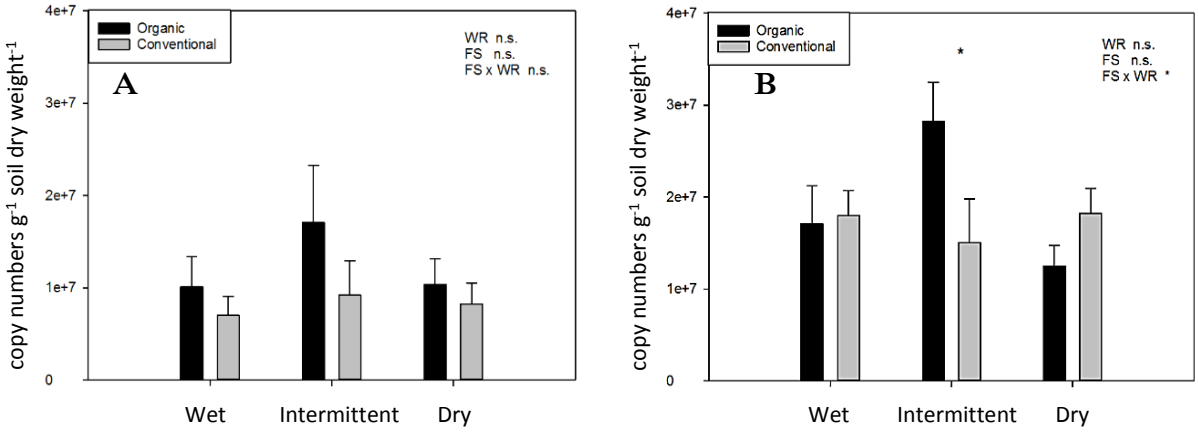

Figure 6 - Abundances of the bacterial alkaline metalloprotease (*apr*) (A) and the bacterial neutral metalloprotease (*npr*) (B). Three water regimes (WR): wet = 80% maximum water holding capacity (mWHC), intermittent = 20% mWHC (days 0-28), 80% mWHC (days 28-56) and dry = 20% mWHC, were applied to soils derived from an organic farming system (FS) and a conventional FS. The effects of water regime (WR), farming system (FS) and their interaction (FSxWR) were tested by a full-factorial ANOVA. Results of the comparison between treatments were obtained with a Contrast Analysis based on the ANOVA. Significance levels: * p<0.05, ** p<0.01 and *** p<0.001, n.s.=not significant, vertical bars indicate the standard error (n=6).

4 DISCUSSION

The aim of the present study was to assess the effects of future projected rainfall variabilities on microbial mediated proteolysis of fresh organic litter input (*Lupinus alba*) and the subsequent N recovery by ryegrass (*Lolium multiflorum*) cultivated on soil with different farming histories (conventional vs. organic). To our knowledge, this is the first study simultaneously investigating the effect of different FS and climate change induced rainfall variability on microbial mediated N transformations followed by plant N provisioning and uptake.

Geochemical parameters

After 56 days of incubation, N_{min} showed strong WR effects with decreasing N_{min} values from the wet over the intermittent to the dry WR. A remarkably larger pool of N_{min} was observed in the organic FS of the dry WR compared to the conventional FS. No FS effect was detected within the other WR. The organic FS was expected to perform better regarding the provisioning of plant available N from litter than the conventional FS under drought stress and thus the first hypothesis was confirmed under drought conditions, by the measurements, using N_{min} as a proxy for plant available N.

N_{mic} was influenced by FS, WR and the interaction between FS and WR, but not in a similar matter as N_{min}. While N_{min} decreased gradually with increasing drought stress, N_{mic} was higher in the wet WR. No significant differences occurred between the intermittent WR and the organic FS of the dry WR. This contradicts the findings of other studies, where N_{mic} decreased upon drying and rewetting (e.g. Gordon et al., 2008). However, in the wet and dry WR, the organic FS harbored more N_{mic} than the conventional FS but no such differences were observable in the intermittent treatments. The intermittent WR was the only treatment where no FS effect on N_{mic} occurred. This could be due to a higher degree of cell death via lysis upon rewetting in the conventional FS and a larger pool of newly released nutrients from dead cells (Birch, 1958) which could have led to enhanced growth in the conventional FS. This hints towards distinct behaviors of the microbial community in organic versus conventional FS and could potentially reason from differences in the structure of the microbial community.

15N recovery efficiency

At the start of the experiment, soils were amended with ^{15}N labelled lupine litter aimed to be proteolyzed by microbes and further transformed into mineral N and assimilated by the ryegrass. Using ^{15}N stable isotopes we could calculate the fraction of N which derived from litter and was subsequently taken up by the plant, in soils from organic and conventional FS under rainfall variability.

The experiment was set up as a closed system. Losses via volatilization were assessed to be negligible in a previous experiment, no leaching occurred and the natural abundance of ^{15}N was accounted for. We can therefore assume that the estimations of the *REC* are accurate and the amount of ^{15}N which was not taken up by ryegrass remains stored in the soil's N pools (SOM, N_{mic}, N_{min}).

Plants grown on organically managed soil took up significantly higher amounts of ^{15}N compared to plants grown on conventionally managed soil when subjected to intermitted and dry WRs. No such FS effect was observable under optimal wet conditions.

Therefore, the second hypothesis is confirmed as well and we indeed could demonstrate an enhanced microbial mediated N cycling potential and N plant provisioning in organically compared to conventionally FS in drought stress scenarios. It has to be noted that our findings are based on a strictly controlled incubation experiment and further testing of the observed phenomena in the field is required.

When comparing our results to field studies, one should keep in mind that the soil structure was homogenized via sieving prior to this experiment. The N use efficiency varies strongly depending on the plant's development status and environmental factors such as soil structure (e.g. Fixen et al., 2015). Therefore, a field experiment measuring the ^{15}N recovery from litter must be designed carefully to avoid high variability due to the heterogeneity of soil patches in a field and contamination by external N inputs. They should further include various environmental parameters, for example the deposition of N from the atmosphere as done in a three-years field experiment by Bosshard et al. in a study about the fate of N from slurry, manure and mineral fertilizer (Bossard et al., 2009).

The study of Bosshard et al. did not find higher N recoveries in organically farmed soils compared to conventionally farmed soils, although the microbial community was assessed to be larger and more active beforehand (Bosshard et al., 2009, Mäder et al., 2002). Other studies on ryegrass (Langmeier et al., 2002) and on spring barley

(Glendining et al., 1997) could neither asses an impact of the FS on the recovery of ^{15}N from organic fertilizers. However, these studies did not focus on the influence of drought and as we found the effects between FS to be pronounced only when subjected to drought stress, future field- and laboratory studies on the ^{15}N recovery should entail measurements from soils with different water status.

The findings of this laboratory study provide rare evidence of organic FS to perform better than conventional FS regarding the proteolysis of organic plant litter and interestingly, the effects only occur under water stress conditions. This hints towards a higher potential of organic FSs to function well under future projected rainfall variability due to climate change and may serve as an argument to promote organic farming in the present and in the future.

Drying and rewetting

Increased amounts of N contents in the shoots cut after rewetting was observed in the intermittent WR, with the conventional FS having higher N contents than the organic FS. However, the ^{15}N recovery did not show the same pattern when comparing between FS. In fact, another relationship was observed for the ^{15}N recovery in the intermittent WR, being higher in the organic FS compared to the conventional FS. Accordingly, the organic FS performed better in terms of proteolysis of lupine litter and subsequent N uptake by the plant. The elevated N contents could reason from a different origin than the labeled litter. Most likely, the Birch effect (Birch, 1958), the rapid release of nutrients due to cell lysis and organic matter aggregate breakdown upon rewetting, was more prominent in the conventional FS. This means that the elevated tissue N contents in the conventional FS could be due to plant N uptake from other sources than the labeled fertilizers i.e. large quantities of nutrients that origin from large quantities of lysed microbial cells in the conventional FS, which populated the soil already at the start of the experiment (initial N_{mic} contents). This is in agreement with the literature where a transient boost effect due to freshly released nutrients upon rewetting has led to a transient growth-boost effect (Birch, 1958; Borken & Matzner, 2009).

When combining shoot biomass from cutting after 28 days and 56 days, no FS effect on the tissue N contents is observable in the intermittent WR.

For the biomass dry weight and also the tissue ^{15}N contents of the combined shoot biomass in the intermittent WR, higher values were measured for the organic FS compared to the conventional FS. This further hints towards organic FS to perform better than conventional FS in terms of litter-proteolysis and subsequent ^{15}N uptake by ryegrass.

The tissue N contents as well as of the total plant biomass of the combined shoot biomass in the intermittent WR were very similar to the respective measurements in the wet WR. This was surprising, as the Birch-effect appears to have led to quite large amounts of N from other sources than labeled litter, to be released and assimilated into plant biomass. Yet, it is unlikely that the observed enhancements translate into a higher cumulative net N mineralization, since from tracing the fate of organic litter, it was clearly visible from the data on the ^{15}N recovery and total plant tissue ^{15}N contents, that the wet WR outperformed the intermittent WR.

In this study, the N recovery was investigated using the ^{15}N isotope approach and thus, we could differentiate between N from litter and other sources. It is thereby advisable for future studies on the N recovery from organic fertilizers under drying and rewetting scenarios to use the isotopic determination method as well. Alternatively, using unlabeled organic N fertilizer, one must take into account and mention that the phenomena of the Birch-effect may influence the results on N contents in plant tissues and also N_{mic} contents in the soil.

The findings of this study could potentially have revealed more detailed information of the effect of drying and rewetting on microbial mediated proteolysis and subsequent N uptake by ryegrass if sampling had occurred twice, after 28 days and after rewetting (after 56 days). However, scientific experiments most often have limited access to large pools of replicates which would allow repetitive samplings. In our case, the limitation was given by the production of ^{15}N labelled lupine litter, which is extremely costly, financially and time-wise.

Plant-microbe competition for N

We assessed the potential of stress conditions (prolonged drought and drying and rewetting) to lead to an enhanced competition for N between plants and microbes, as microbes could shift from N mineralization to N immobilization under stress (Inselbacher et al., 2010; Kaye & Hart, 1997).

A strong negative relationship between N_{mic} and N assimilated into plant biomass could have indicated a strong competition (Kaye & Hart, 1997). However, throughout WR, no such negative relation was observed. In fact, the opposite took place. With increasing N_{mic} values, more N was assimilated into plant biomass. Therefore, it is highly unlikely that the results from this experiment were strongly influenced by competition for N and moreover, it was shown that both, microbial biomass and the provisioning of plant available N, could be maintained even under 56 days of drought at only 20% of the soil's mWHC. However, a correlation can represent a relationship- but should not be treated as a causality. To be certain about the procedure of N mineralization and N immobilization from litter in detail, in this experiment, it would be necessary to conduct analyses with measurements of the ^{15}N contents in the microbial biomass in relation to ^{15}N that was stored in plant biomass. These parameters could still be measured in a follow-up study, as the respective soil samples were taken. For novel studies, it could be interesting to sample and analyze at several points in time.

Abundance of functional microbial genes for proteolysis

The abundance of the 16S ribosomal subunit correlated positively with the abundance of *apr* and *npr* copy numbers. This is in line with other studies that used the 16S rRNA as an estimate of the size of the microbial population (e.g. Fuka et al., 2008). For *apr*, 66% and for *npr*, 60% of the data could be explained by the relationship with the 16S rRNA. However, the estimation strength is to be interpreted carefully, since the copy numbers of 16S rRNA, *apr* and *npr* can vary from cell to cell and between species depending on the proliferation patterns of bacteria: fast growing & fast responding organisms most often have a higher number of 16S rRNA copies than slow growing organisms (e.g. Fuka et al., 2008).

In this study, the abundance of *apr* and *npr* gene copy numbers served as a proxy for microbial mediated proteolysis and N mineralization (Bach et al., 2001; Gschwendtner et al., 2010). Several studies observed a higher proteolysis rate in organic soils due to a more active and larger (Lori et al., in preparation; Fliessbach et al., 2007) and organic soils harbor a more diverse (Hartmann et al., 2014) microbial community. However, the abundances of functional genes do not necessarily correspond to in situ mineralization.

The mRNA and DNA transcripts and/or enzymes might undergo posttranscriptional and -translational modifications and other factors, such as spatial and temporal heterogeneity in the habitat and fluctuating turnover rates play a role (Rocca et al., 2015).

Apr copy numbers were tendentially but not significantly elevated by one order of magnitude in organic soil compared to the conventional soil, throughout all WRs. *Apr* copy numbers were less abundant than *npr* copy numbers in the present study. This is surprising, since copy numbers for *apr* were higher in previous studies, than for *npr* (Gschwendtner et al., 2010).

Npr copy numbers showed a significant FS effect in the intermittent WR. This is in line with earlier studies which have observed increases in N mineralization upon rewetting of dry soil due to microbial cell lysis emerging from osmotic stress and accumulations of dead plant and microbial biomass (Borken & Matzner, 2009).

No further significant effects of FS and WR on *apr* abundances were determined. This is unexpected, as drought was observed to negatively impact the abundances of *apr* and *npr* in previous experiments (Lori et al., in preparation) and reduced soil moisture has previously resulted in decreased microbial activity due to low osmolarity and low motility of microbes (Borken & Matzner, 2009).

Recently, Lori et al. observed the abundances of *apr* and *npr* to decrease with a stronger negative WR effect on *npr* compared to *apr*, in response to water stress and positive relationships between the abundances of *apr*/*npr* with N_{min} and the total labile N pools (Lori et al., in preparation). However, the study of Lori et al. entailed degradable plant litter input but no plant, absorbing N from the soil and thereby strongly influencing the distribution of N, was present in the experimental setup of Lori et al. (in preparation). Furthermore, plants have been identified to affect microbial communities in the rhizosphere (Kowalchuk et al., 2002), yet, still poorly understood and further research needs to be conducted on the influence of a plant's presence on bacteria, archaea and fungi (Gschwendtner et al., 2010).

The effects in the present study might have been mimicked by sampling rather late, when the peak of microbial proteolytic activity had passed already. Future studies should therefore ideally include repetitive measurements of gene copy number, for example, after two, four and/or six weeks of incubation to shed light on the abundances of functional proteolytic genes in relation to the degradation of organic matter, over time.

However, using ^{15}N labeled litter, such repetitive samplings entail high financial expenditures to produce the labeled litter and may thus, be carried out with unlabeled litter inputs as well.

Not all genes might have been detectable with the degenerate primers in use. Rocca et al. suggested in their meta-study on functional gene abundance versus process function, to conduct metagenomic analyses including sequencing instead of qPCR for studies on particular biogeochemical processes (Rocca et al., 2015). In depth research on the composition of the respective microbial community is necessary and is currently assessed (in preparation). Further, caution is required, when comparing the abundance of functional microbial genes across studies with different soil and site characteristics (Fuka et al., 2008).

Fungal:Bacterial ratio

Fungi take part in the breakdown of proteinaceous organic matter (e.g. Fuka et al., 2008). Arbuscular mycorrhizal fungi support the nutrient uptake from the soil by the plant roots and improve the resistance of the root system to drought (Mäder et al., 2000). A shift in the fungal:bacterial ratio, indicated by an increase in the soil C:N ratio, can be induced by intense tillage, N fertilizer inputs and by climate change and comes along with changes in the community of organisms that feed on microorganisms and therefore can also affect ecosystem functioning (Six, 2012). Although, no differences in the fungal:bacterial ratio occurred in the wet and intermittent WRs in this study, an increase in the C:N ratio in the dry WR, hints towards a shift from bacteria to fungi dominated networks. Subsamples of the root networks from this experiment were obtained and will be analyzed for arbuscular mycorrhizal root colonization (in preparation). It was previously shown that root colonization by arbuscular mycorrhizae is 30-60% higher in soils from low-input farming compared to conventional soils (Mäder et al., 2000). Therefore, we expect elevated root colonization in the organic FS compared to the conventional FS, in this study and potentially, a reduced colonization under drought due to the dependence of biological processes, on water. Although, 56 days might not be sufficient to establish fully developed networks of generally slow-growing fungi, throughout treatments, patterns could be visible already. Studies with similar focus as the study in hand might consider measuring fungal, in particular mycorrhizal parameters in detail, in order to cover a broader specter of potential factors influencing proteolysis.

5 CONCLUSIONS

We observed a more efficient ^{15}N lupine litter recovery in organic FS compared to conventional FS when subjected to drought and drying and rewetting. This FS effect was not observable when plants have been grown under optimal wet water conditions. The enhanced performance of the organic FS under rainfall variability was supported by a higher biomass production of ryegrass grown in the organically farmed soil, under drought and drying and rewetting. A remarkably larger pool of mineral plant available N was measured in the organically farmed soil compared to the soil from conventional agriculture after a prolonged drought period. Although, the observed differences could not be explained by the abundances of functional microbial genes for proteolysis, we assume that the increased performance of the organic FS in this study, is due to a better performance of the microbial proteolytic community. Different proteolytic bacteria are known to vary in protease gene expression and activity and therefore, emphasis has to be laid on the community composition of proteolytic bacteria for overall soil proteolytic activity (Sakurai et al., 2007). Metagenomic analyses of the microbial community composition will thus be conducted in a follow-up experiment.

This study is, to our knowledge, the first study investigating the performance of organic compared conventional FS while simultaneously taking into account future projected climate change induced rainfall variability. Multi-stage and multi-factorial studies, like the present one, should be encouraged in the future in order to investigate the full potential of organic FSs to potentially better adapt to environmental fluctuations compared to conventional FSs. Conventional FS receiving mineral fertilizers are not as dependent on N provisioning via microbial mediated proteolysis and biological N_2-fixation, as organic FS are. However, the upcoming challenges of peak oil and the globally rising negative impacts of industrial N fertilizers will require conventional systems to rely more and more on organic fertilizers and thus, require healthy soil functioning. Moreover, a high adaption potential together with fewer and less costly inputs could balance out the yield gap between organic and conventional farming on the long-term, considering the challenges of a changing environment, rising pollution, scarcity of resources and climate change. Organic farming should be strongly encouraged, as it supports the natural soil nutrient cycles, improves the water holding capacity and positively impacts biodiversity with a higher potential for resilience an adaptation compared to conventional FS.

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APPENDICES

Appendix 1 - Sequences and efficiencies of primer pairs.

Enzyme/ Plasmid	Gene	Primer/ Plasmid	Sequence	Amplicon size	Reference
APA9	APA9	APA9 forward	CGAACCTGGACTGTTATGATG	3054	Thonar et al., 2011
		APA9 reverse	AATAAACAATCCCCTGTATTTCAC		
Alkaline metalloprotease	*apr*	*apr* forward	TAYGGBTTCAAYTCCAAYAC	194	Bach et al., 2001
		apr reverse	VGCGATSGAMACRTTRCC		
Neutral metalloprotease	*npr*	*npr* forward	GTDGAYGCHCAYTAYTAYGC	233	Bach et al., 2001
		npr reverse	CMGCATGBGTYADYTCATG		
16S ribosmal RNA	16S	16S forward	CCT ACG GGA GGC AGC AG	466	Muyzer et al. 1993 Nadkarni et al. 2002
		16S reverse	GGA CTA CCA GGG TAT CTA ATC CTG TT		

Appendix 2 - Quantitative real-time PCR cycling profiles.

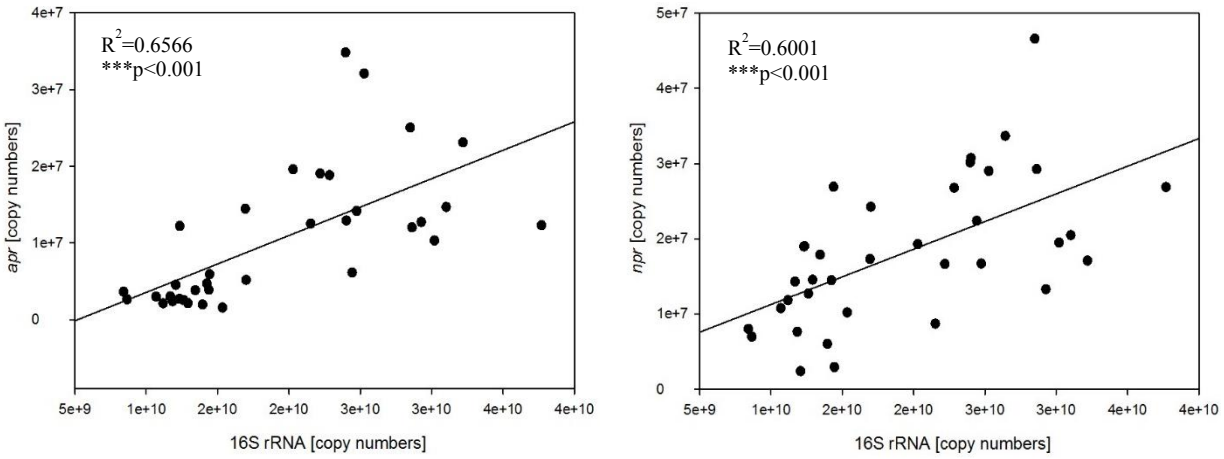
APA 9			*Apr & npr*			16S rDNA		
Number of cycles	Temperature	Duration	Number of cycles	Temperature	Duration	Number of cycles	Temperature	Duration
1	95°C	3:00 min	1	95°C	3:00 min	1	95°C	3:00 min
34	95°C	0:10 min	39	95°C	0:15 min	35	95°C	0:10 min
	52°C	0:15 min		55°C	0:15 min		61,5°C	0:20 min
	72°C	0:20 min		72°C	0:20 min		72°C	0:20 min
1	55, 95°C	0:05 min	1	55, 95°C	0:05 min	1	55, 95°C	0:05 min

Appendix 3 - Soil parameters & gene abundances before incubation.

Soil biogeochemical parameters before the start of the experiment. Differences between soils from an organic farming system (FS) and a conventional FS were determined with a Student's t-test or a Wilcoxon signed-rank test for data with in-homogenously distributed variances. Significance levels: * $p<0.05$, ** $p<0.01$ and *** $p<0.001$, n.s.=not significant, (n=12).

		Organic FS		Conventional FS		
Parameter	Unit	mean	SE	mean	SE	T-test/ Wilcoxon signed-rank test
N_{min}	mg N/kg soil	47.76	0.86	39.01	0.52	***
NO_2, NO_3	mg N/kg soil	42.91	0.82	35.75	0.52	***
NH_4	mg N/kg soil	5.10	0.07	3.53	0.07	**
C_{mic}	mg C/kg soil	408	8	337	29	n.s.
N_{mic}	mg N/kg soil	47.33	4.60	41.17	4.71	n.s.
C_{mic}:N_{mic}	ratio	7.83	0.06	7.67	0.64	n.s.
DON	mg N/kg soil	1.9E-02	1.7E-03	1.3E-02	1.4E-03	*
$N_{available}$	mg N/kg soil	95.12	4.32	80.19	4.95	*
C_{total}	mg C/ kg soil	18297	46	18015	158	n.s.
N_{total}	mg N/ kg soil	1843	4	1819	16	n.s.
pH		6.08	0.02	6.03	0.01	*
apr	copy numbers	4.52E+06	1.30E+06	3.37E+06	1.03E+06	n.s.
npr	copy numbers	1.85E+06	1.66E+05	1.13E+06	1.08E+05	**
16S rRNA	copy numbers	1.19E+10	1.25E+09	9.48E+09	1.58E+09	n.s.

Appendix 4 - Relationships between the 16S rRNA and *apr*/*npr*



Relationships between functional microbial genes for proteolysis and the 16S rRNA subunit. Correlations between alkaline metalloprotease (*apr*) and the neutral metalloprotease (*npr*) transcript abundances, respectively, with the abundance of the 16S rRNA, were investigated with a Pearson's correlation. Significance levels: * p<0.05, ** p<0.01 and *** p<0.001, n.s.=not significant, (n=36).

STATUTORY DECLARATION

I declare that I have authored this thesis independently, that I have not used other than the declared sources / resources, and that I have explicitly marked all material which has been quoted either literally or by content from the used sources.

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