Soluble phosphorus in forest soil: Effects of drying and rewetting

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MAI-VAN DINH

born on January 17th , 1985

in Hanam (Vietnam)

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Acting dean: Prof. Dr. Stefan Peiffer

Doctoral Committee:

Prof. Dr. Egbert Matzner (1st reviewer)

J Prof. Dr. Johanna Pausch (2nd reviewer)

Prof. Dr. Gerhard Gebauer (Chairman)

Prof. Dr. Andrea Carminati

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ANOVA	analysis of variance
ATP	adenosine triphosphate
BayCEER	Bayreuth Center of Ecology and Environmental Research
CFE	chloroform fumigation-extraction
D/W	drying-rewetting
DIP	dissolved inorganic phosphorus
DNA	deoxyribonucleic acid
DOP	dissolved organic phosphorus
DSMZ	Deutsche Sammlung von Mikroorganismen und Zellkulturenund (German Collection of Microorganism and Cell Cultures)
GWC	gravimetric water content
HPLC	high-performance liquid chromatography
HSD	honest significant difference
ICP-OES	inductively coupled plasma optical emission spectroscopy
MBC	microbial biomass carbon
MGMPB	malt extract glucose meat extract peptone liquid medium
MPa	megapascal
n.d.	not detected
pF	logarithm of the absolute of soil matric potential
Pi	inorganic phosphorus
Pmic	microbial biomass phosphorus
Po	organic phosphorus
RNA	ribonucleic acid
SE	standard error
SIR	substrate induced respiration
TDP	total dissolved phosphorus
TN	total nitrogen
TOC	total organic carbon
TP	total phosphorus
WHC	water holding capacity

Summary

Drying and rewetting (D/W) of soils might have strong effects on the release of phosphorus (P) into soil solution and on the turnover of P in terrestrial ecosystems. Former studies on the release of dissolved P after D/W only have focused on mineral soils from arable or grassland systems. Many open questions with regard to the effect of D/W on the release of P in forest soils and the role of organic layers still await answers.

This study aimed to determine the effects of repeated D/W of forest floors on soluble P. The response of specific groups of microorganism to D/W was investigated as well as the dynamic of soluble P and the microbial biomass P (Pmic) following D/W of forest floors.

Forest floor samples were analysed from European beech and Norway spruce sites in Germany for study I and III. In study II, an artificial soil amended with growth medium was inoculated separately with the bacteria species *Pseudomonas fluorescens* (gram-negative), and *Micrococcus luteus* (gram-positive), or with the fungus *Penicillium chrysogenum*. All experiments were conducted at 20°C. Samples were adjusted to 50% water holding capacity and pre-incubated from one to three weeks before starting D/W experiments. D/W samples were desiccated up to pF 6 (–100 MPa), while the controls were kept permanently at 50% water holding capacity (WHC). In study I, three D/W cycles were applied, while in study III the samples were maintained at 50% WHC after only one D/W cycle. In study II, samples of the artificial soil with the defined microbial cultures was collected at different degrees of desiccation to extract water soluble P. Water soluble P (total dissolved phosphorus (TDP), dissolved inorganic phosphorus (DIP), dissolved organic phosphorus (DOP)) and microbial biomass was measured.

The largest increase in TDP concentration after D/W was observed in Oe layers (average concentrations 120-130 mg P kg^{-1}). In all samples, the net release of TDP after D/W cycles was mostly in the form of DIP except for the A horizons. The net release of DIP after D/W was largest from the Oe horizons (average net release 30-60 mg P kg^{-1}) of both beech and spruce forest soils. In the A horizons, net DIP release was similar in beech and spruce soils with 0.4 mg P kg^{-1}. The release of DIP and DOP was positively correlated to the initial microbial biomass in Oe and Oa layers but not in Oi layers. Repeated cycles did not increase the release of DIP and DOP.

The TDP concentrations decreased after rewetting strongly with time in forest floor Oi layers (within 1 day in beech and 4 days in spruce), while the TDP concentrations kept rather stable in Oe layers for 14 days. The net release of TDP still amounted to 30 mg P kg^{-1} in beech and 40 mg P kg^{-1} in spruce Oe at day 14 after rewetting. After rewetting, Pmic in spruce Oi and Oe was reduced by the D/W treatment. Pmic recovered in spruce Oi already at day 1, while the reduction of Pmic in spruce Oe persisted until day 14. For beech, there was only a tendency Pmic reduction after desiccation. In the spruce samples, the release of TDP and its dynamic was linked to the decrease of the microbial P pool after desiccation and its recovery after rewetting.

The average ratio of TDP net release/Pmic in soils incubated with *P. fluorescens* was from 0.4 to 1.2 mg mg^{-1}. The net release of TDP/Pmic from the soils incubated with *P. fluorescens* started at pF 3.9 and increased with the degree of desiccation prior to rewetting. The ratio of net release of TDP/Pmic in the *P. chrysogenum* incubation was similar to the ratio in the *M. luteus* incubation with 0.25 mg mg^{-1} after desiccation to pF 6.

As a conclusion, D/W cycles of forest floors contribute significantly to water soluble and plant available P and to the overall P cycling as related to the annual P flux with litterfall and the net P mineralization in forest ecosystems. The effect of D/W on P release from microbial biomass depends largely on the microbial community composition, with fungi and gram-positive bacteria being less susceptible to D/W than gram-negative bacteria. The decline and recovery of the microbial biomass P affect the temporal dynamics of soluble P after D/W cycles.

Zusammenfassung

Austrocknung und Wiederbefeuchtung (A/W) von Böden kann einen großen Einfluss auf die Menge an gelöstem Phosphor (P) haben und damit eine Bedeutung für den gesamten P Kreislauf in terrestrischen Ökosystemen erlangen. Bisherige Arbeiten zur Freisetzung von gelöstem P nach A/W haben sich auf Mineralböden unter Grünland oder Ackernutzung beschränkt. Unklar war hingegen die Bedeutung von A/W für den P Umsatz in organischen Auflagen in Waldböden.

Das Ziel der Arbeit war es daher die Effekte von A/W auf gelösten P in organischen Auflagen zu untersuchen und die zugrundeliegenden Mechanismen zu verstehen. Daher wurden Experimente mit wiederholten A/W Zyklen und an Kulturen definierten Mikroorganismen durchgeführt. Ferner wurde die Dynamik des an gelösten P nach Wiederbefeuchtung verfolgt und die Relation zur Dynamik der mikrobiellen Biomasse untersucht.

In den Studien I und III wurden Proben aus verschiedenen Horizonten der Auflage und aus dem A Horizont in einem Buchenbestand und einem Fichtenbestand in Deutschland entnommen. In Studie II wurde ein künstlicher Boden jeweils mit einem definierten Mikroorganismus inokuliert (*Pseudomonas fluorescens* (gram-negatives Bakterium), *Micrococcus luteus* (gram-positives Bakterium), *Penicilium chryosporum* (Pilz)). Alle Experimente wurden bei 20^{o}C durchgeführt. Der Wassergehalt der Proben wurde zunächst auf 50% der Wasserhaltekapazität eingestellt und die Proben wurden 1 bis 3 Wochen vorinkubiert bevor die A/W Experimente begannen. In Studie I wurden die Proben 3 mal hintereinander bis auf pF 6 (-100 MPa) ausgetrocknet und anschließend wiederbefeuchtet. Die Kontrollen verblieben bei 50% Wasserhaltekapazität. In Studie III wurden die Proben nur einmal bis auf pF 6 ausgetrocknet und dann bei einem Wassergehalt von 50% der Wasserhaltekapazität weiter inkubiert. In Studie II wurde der künstliche Boden mit den definierten Kulturen nach Erreichen definierter Austrocknungsstufen beprobt und wiederbefeuchtet. In den wässrigen Extrakten wurde der gelöste P gesamt (TDP) sowie als inorganischer P (DIP) und organischer (DOP) bestimmt. Hinzu kamen Messungen der mikrobiellen Biomasse und des darin gebundenen P (Pmic).

Der höchste Anstieg der TDP Konzentrationen nach A/W wurde für die Of Horizonte gefunden (im Mittel bis auf 120-130 mg P kg^{-1}). In allen Proben erfolgte die Freisetzung

von P vornehmlich in Form von DIP, mit Ausnahme der A Horizonte. Die Netto-Freisetzung von DIP war ebenfalls am größten in den Of Horizonten (im Mittel 30-60 mg P kg^{-1}). Für die A Horizonte lag die Netto-Freisetzung bei 0.4 mg P kg^{-1}. Die Freisetzung von DIP und DOP war positiv mit der initialen mikrobiellen Biomasse in den Of und Oh Horizonten, Korreliert aber nicht in den L Horizonten. Wiederholte A/W Zyklen führten nicht zu einer Verstärkung der P Freisetzung.

In den L Horizonten nahmen die Konzentrationen an TDP nach Wiederbefeuchtung rasch ab (in 1 bis 4 Tagen). Hingegen blieben die Konzentrationen in den Of Horizonten auch nach 14 Tagen noch hoch mit 30-40 mg P kg^{-1}. Der Pool von P in der mikrobiellen Biomasse nahm nach Austrocknung in den L und Of Horizonten der Fichtenauflagen ab und erholte sich in den L Horizonten bereits nach einem Tag. Hingegen war Pmic auch nach 14 Tagen in den Of Horizonten reduziert. Für die Proben aus dem Buchenstandort war nur eine Tendenz zur Verringerung der Pmic Gehalte nach Austrocknung zu finden. Für die Fichtenproben konnte die Dynamik des TDP nach A/W mit der Dynamik der Veränderungen der Pmic Gehalte erklärt werden.

Die Freisetzung von TDP aus den Kulturen von *P. fluorescens* im künstlichen Boden war am höchsten (0.4-1.2 mg P mg^{-1} Pmic), stieg mit der Austrocknungsintensität an und begann bereits bei pF 3.9. Die TDP Freisetzung aus Kulturen von *P. chysogenum* und *M. luteus* war ähnlich aber erst nach starker Austrocknung zu beobachten mit Werten von 0.25 mg P mg^{-1} Pmic bei pF 6.

Zyklen von A/W führen zu einer deutlichen Erhöhung des Gehaltes an gelöstem P in Auflagen von Waldböden. Die freigesetzten Mengen sind auch vor dem Hintergrund der P Umsätze im Ökosystem durch Mineralisation oder Streufall signifikant. Der Effekt von A/W wird stark von der vorherrschenden mikrobiellen Gemeinschaft abhängen mit geringeren Effekten bei Dominanz von gram-positiven Bakterien und Pilzen. Die Schädigung der mikrobiellen Biomasse und ihre Erholung nach Wiederbefeuchtung beeinflusst die Dynamic des gelösten P.

1. Synthesis: Soluble phosphorus in forest soils: Effects of drying and rewetting

1.1 Introduction

1.1.1 Phosphorus in forest soils

Phosphorus (P) is an essential element for all living organisms as P is active in the energy metabolism, the formation of phospholipids in cell membranes and P is essential for synthesis of nucleic acids (DNA and RNA) (Marschner, 1996; Raghothama and Karthikeyan, 2005). In soils, P is present as inorganic and organic phosphorus.

Inorganic phosphorus (Pi) usually accounts for 35% to 75% of total P in soil (Harrison, 1987). At least 170 different mineral forms of P in inorganic fraction occur naturally and the most common primary mineral form of P is apatite (Holford, 1997). Apatite weathers slowly, releasing Pi as orthophosphate ($H_2PO_4^-$ and HPO_4^{2-}). Secondary P minerals are calcicum (Ca) and less often iron (Fe) and aluminium (Al) phosphates. The dissolution rates of P containing minerals mainly depend on the size of the mineral particles and on the soil pH (Oelkers and Valsami-Jones, 2008; Pierzynski and McDowell, 2005). With decreasing soil pH, the solubility of Fe and Al phosphates increases, but the solubility of Ca phosphates decreases except for pH values more than 8.0 (Hinsinger, 2001).

Organic phosphorus (Po) comprises 30% to 65% of the total P in soil, although soil with high organic matter contents can contain up to 90% Po (Harrison, 1987). Soil Po originates from plant residues, animal wastes and the soil microbial biomass (Nash and Halliwell, 1999). The majority of Po is present as phosphate esters including inositol phosphates, nucleic acids and phospholipids, and phosphonates (Condron et al., 2005; Turner et al., 2002). Po occurs in soil primarily as phytates ($C_6H_6(OHPO_3)_6$) or related forms and as nucleic acids and their derivatives (Plante, 2007). Phytin, a polymeric inositol hexaphosphate, is synthesized by plants and accounts for roughly 40% of the Po in soils (Plante, 2007). Cellular membranes include phospholipids which can comprise up to 30% of the P in microbial biomass (Magid et al., 1996). The size of the Po pool in the soil is in the order inositol phosphate > polymer organic phosphate > nucleic acid P > phospholipid P.

A large proportion of Po in soils is bound in the microbial biomass. Microbial biomass accounts from 2 to 5% of total organic phosphorus in cultivated soil, but up to 20% in grassland soils (Plante, 2007) and up to 50% in forest floors (Achat et al., 2010).

The concentration of soluble P in soils is very low from 0.1 to 1 μg g^{-1}, representing less than 1% of the total P (Plante, 2007). The availability of soluble inorganic P is greatest between pH 6 and 7 (White, 2006) with $H_2PO_4^-$ as the dominant species at pH < 7.2, and HPO_4^{2-} dominating at pH > 7.2 (Plante, 2007).

In soil solutions, a significant proportion of P is represented by dissolved organic P (DOP) (Guggenberger and Kaiser, 2003; Pant et al., 1994; Shand et al., 1994). The concentrations of DOP in soils varies strongly with land use and soil type in a range of < 0.02 to 1 mg L^{-1} (Turner, 2005). DOP is a complex mixture of different compound classes and comprises mono-esters of inositol, diesters in DNA, esters with sugars, phospho-lipids, phosphonates, and a significant proportion of undefined esters with humic substances. DOP was found mostly in the hydrophilic fraction (Guggenberger and Kaiser, 2003; Weng et al., 2012). Several methods have been used to characterize DOP in surface waters and soil solutions, like ion chromatography, ^{31}P-NMR, molecular size separation and enzymatic methods (McKelvie, 2005; Monbet et al., 2007). Dynamics of DOP in agricultural soils have been widely studied, mainly with focus on the effects of organic fertilizer application (Fuentes et al., 2012). On the contrary, studies on DOP in forest soils are scare. In forest soils, the transport of DOP is a major vertical P transfer from the forest floor into the mineral soil and may also contribute to the establishment of the organic P pool in the soil profile. DOP was shown to contribute over 95% of the P leached from deeper layers of a calcareous forest soil (Guggenberger and Kaiser, 2003) and represented about 66% of the solute P fluxes in a tropical montane forest (Goller et al., 2006). In Hawaiian forest soils, fluxes of DOP in soils were positively related to the soil P availability (Neff et al., 2000). Furthermore, DOP is influencing the P nutrition of plants, since fractions of DOP might be also taken up directly by roots (Richardson et al., 2005).

1.1.2 Phosphorus cycle in ecosystems

Phosphorus cycling in soils is strongly influenced by the nature of the inorganic and organic solid phase of soils, by plant and microbial activity, chemistry of the soil solution, and many environmental factors (Fig 1.1) (Pierzynski and McDowell, 2005). The soil solution is the primary source of P for plants and microorganism and most P is taken up as phosphate

(HPO_4^{2-}, $H_2PO_4^-$). The processes involved in soil P cycle are dissolution-precipitation, sorption-desorption, mineralisation and immobilization. Sorption, precipitation, plant uptake and microbial immobilization decrease P content in soil solutions while dissolution, desorption and mineralisation increase P content in soil solutions.

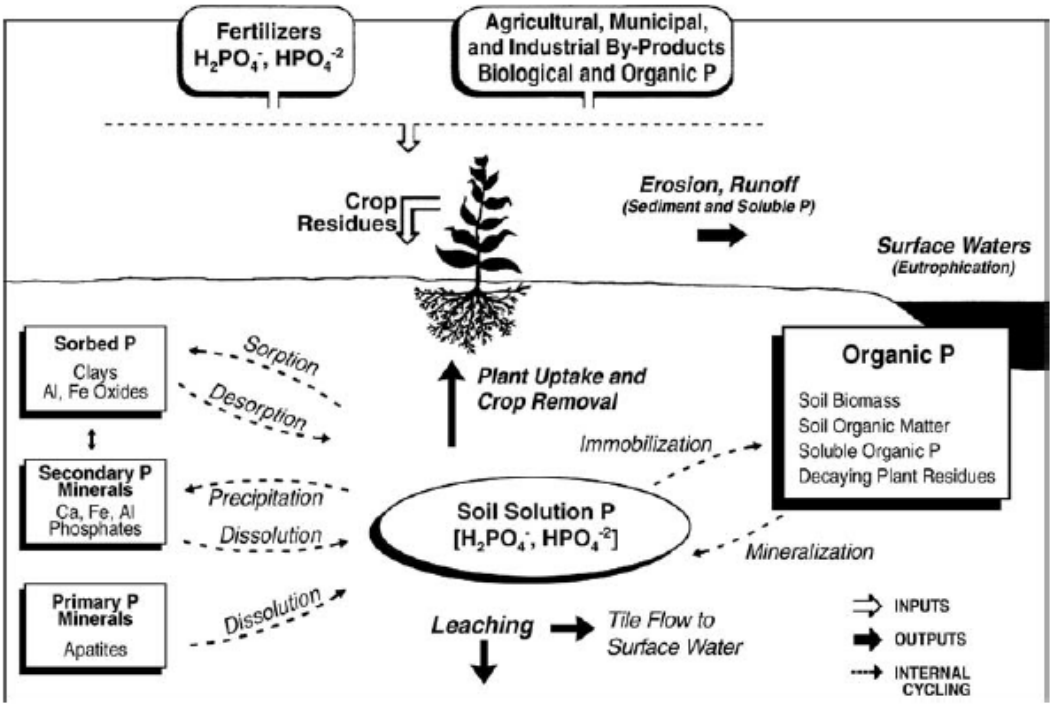

Figure 1. 1. The soil P cycle in terrestrial ecosystems (from Pierzynski et al., 2000)

Sorption and desorption

Sorption removes soluble phosphorus from solution by a physico-chemical reaction with the solid phase of the soil. Orthophosphates are mostly adsorbed to the surfaces and edges of Fe and Al hydroxides as well as clay minerals and the sorption increases with decreasing pH (Hinsinger, 2001).

The adsorption of P compounds on minerals was often described using the Langmuir equation. The Freundlich equation is used instead at lower phosphorus concentration (Barrow, 1983; Colombo et al., 1994).

Phosphate is bound strongly in inner-sphere complexes to soil minerals by a covalent bond between the metal and the electron-donating oxygen ions (Brusseau and Chorover, 2006). Inner-sphere complex formation is termed chemisorption, whereas formation of outer-sphere complexes is often referred to as a physical adsorption (Brusseau and Chorover, 2006).

Outer-sphere surface complexes are dominantly the result of electrostatic interaction (Bard, 2010).

Desorption releases P from the solid phase into the soil solution. Desorption occurs when runoff or leaching deplete soluble P concentrations to very low levels (Pierzynski and McDowell, 2005). Phosphorus adsorbed by the solid phase cannot easily be desorbed and adsorption isotherms do not coincide with desorption isotherms, resulting in a hysteresis (Sander et al., 2005). The formation of bidentate and binucleate bonds is seen as the main cause of hysteresis (Bohn et al., 1985). Residence time, P loading, generation of strong and stable bonds affect on the mechanisms of hysteresis (Okajima et al., 1983). Soils differ in their capacity to adsorb and desorb P. Soils with larger clay and Al/Fe oxide contents will adsorb more P. Adsorption also varies with soil depth and is affected by cultural operations that alter soil P levels, soil pH and organic matter content.

Organic phosphates are bound in mineral soils to the same sites as orthophosphate, forming binuclear inner-sphere complexes (Anderson et al., 1974; Goldberg and Sposito, 2008). The extent and the rate of sorption of organic phosphorus in soils depends on the structure of the organic phosphorus compound. Monoesters such as inositol phosphate are sorbed strongly by mineral components in the soil (Anderson et al., 1974). The sorption of inositol phosphates to clay minerals is greater than the one of nucleic acids, phospholipids and sugar phosphates (Anderson et al., 1974; Leytem et al., 2002). In addition, soil type influences the sorption capacity. In acids soils, the sorption of inositol phosphates was dependent on the content of amorphous aluminium and iron oxides (Anderson et al., 1974), while it was affected by clays and organic matter in neutral and alkaline soils (McKercher and Anderson, 1989). Organic matter does not necessarily sorb phosphate directly, but it can have a strong influence on the sorption or desorption of phosphate by minerals (Addiscott and Thomas, 2000).

Dissolution-precipitation of phosphates

Precipitation can be defined as the reaction of P with metal ions in solution to form salts. The most common forms of precipitates include the products of P with Ca, Al, and Fe. In calcareous soils, soluble Ca is the dominant cation and the addition of soluble P initially results in the formation of dicalcium-phosphate dehydrate (Pierzynski and McDowell, 2005). In acidic soils, Fe- and Al-phosphates are the dominant precipitates (Pierzynski and McDowell, 2005). As mentioned above, the dissolution of the different P containing salts is highly pH dependent.

Mineralisation and immobilization

Mineralisation is the process whereby phosphate is released from organic phosphorus by biological processes (McGill and Cole, 1981). Pi is released from organic matter during oxidation of carbon by soil organisms (McGill and Cole, 1981). Therefore, P mineralization is not only controlled by the need of microorganism for phosphate but often connected to the carbon mineralization (Gressel et al., 1996; McGill and Cole, 1981). Pi is released from organic compounds via the activity of phosphatase enzymes (Condron and Tiessen, 2005). The most important enzymes in P mineralization are phosphomonoesterases, phosphodiesterases, pyrophosphatease and polyphosphatase which hydrolyze P containing anhydrides (Nannipieri et al., 2011). Acid and alkaline phosphomonoesterases dominate in acid and alkaline soils, respectively (Juma and Tabatabai, 1978). In general, the pH optimum for phosphomonoesterase activity in forest soil is 7.0 (Pang and Kolenko, 1986). Seasonal effects also play an important role in soil enzyme activities (Schneider et al., 2001). Activities of phosphomonoesterases are higher than phosphodiesterases activities (Criquet et al., 2007). The mineralization process significantly contributes to the plant P requirements (Frossard et al., 2000; Magid et al., 1996). Typical gross organic P mineralization rates fluctuated from 0.1 to 2.5 mg P kg^{-1} day^{-1}, but rates up to 12.6 mg P kg^{-1} day^{-1} were reported in grassland and forest soil (Bünemann, 2015). Phosphorus mineralization in forest soils occurs mostly in the litter layer (Attiwill and Adams, 1993; Yanai, 1992). Soil with higher soil organic matter concentration and higher soil microbial biomass and activity had larger rates of mineralization (Oberson and Joner, 2005).

Plant root uptake

The most dominant form of P take up by roots is orthophosphate ($H_2PO_4^-$ and HPO_4^{2-}) (White, 2006). Orthophophate P can be taken up by the transport systems of plant roots (Schachtman et al., 1998).

The available P concentration required for adequate plant growth depend on the plant species (Reuter and Robinson, 1997). In intensive agriculture, a maize crop yield of 6-9 t ha^{-1} needs up of 30-50 kg P ha^{-1} (Johnston et al., 2005; Vance et al., 2003) with two-thirds of that turned into the harvested portion of the crop. Small grains yielding 3 t ha^{-1} take up 15-22 kg P ha^{-1}, again with a 70% removal rate (Johnston et al., 2005). In forests, the annual P uptake is in the range of 4-7 kg ha^{-1} a^{-1} with the largest proportion being returned as litterfall (Compton and Cole, 1998; Ilg et al., 2009; Yanai, 1992).

Because of low P concentrations in soil solutions, many plants have enhanced their uptake of P by establishing mycorrhizal symbiosis (White, 2006). Mycorrhizal fungi develop specialized areas, called symbiotic interfaces, to interact with the host plant (Bonfante, 2001; Parniske, 2008). The two major mycorrhizal symbiosis groups are referred to as ectomycorrhizas and endomycorrhizas, based on whether the fungus colonizes the root intercellular spaces or develops inside the cells (Smith and Read, 2009). Under low nutrient conditions and under conditions where the movement of nutrients in soil is reduced, mycorrhizal hyphae can enhance the nutrient uptake of the roots by expanding the zone of nutrient uptake and/or increasing the nutrient uptake and transport efficiency (Powell and Klironomos, 2007).

Limitation of phosphorus in forest ecosystems

Generally, only a small proportion of total stocks of P is in the soil solution, which is the source that is directly used by plant roots (Holford, 1997). The release of P from soils is always slow and often not sufficient to supply the requirements of plants (Schachtman et al., 1998). At 50% of German national forest soils, low P concentrations were found in leaves and needles in Scots pine and Norway spruce (< 1.5 mg P g^{-1} dry weight), in Oak forest (< 1.6 mg P g^{-1}) and in European beech (< 1.3 mg P g^{-1} dry weight) compared to large P pools of P in the humus layer and rooting zone. The median P pool for German forests was 44 kg P ha^{-1} in the humus layer and 2250 kg P ha^{-1} in the rooting zone (Ilg et al., 2009). Talkner et al., (2015) showed that foliar P concentrations declined by 13% from 1.31 to 1.14 mg P g^{-1} during 20 years from 1991 to 2010. They also showed the clear decreasing trend in P concentrations in European forests indicating that the P nutrition of forest sites is detrimentally influenced, the causes being not totally identified. The nutrient imbalances and P limitations in forest stand may be the result of the high N inputs (Braun et al., 2010; Gradowski and Thomas, 2008; Jonard et al., 2015). The P supply of trees by mycorrhiza may be deteriorated by inputs of H^+ and N (Hutchinson et al., 1999).

1.1.3 Drying-rewetting cycles in soils modifying nutrient availability

General

Drying and rewetting (D/W) of soils is a natural phenomenon that mainly affects top soil horizons in temperate climate. In many regions of the world, e.g, semi-arid, or seasonally arid regions, D/W cycles will also influence the deeper mineral soil. The first report on the effects of D/W on the release of mineral nutrients from soil was by Birch, (1958). When rewetting a dried soil, a pulse of organic matter mineralization was observed that released nutrients into the soil solution (Birch, 1958; Bünemann et al., 2013; Butterly et al., 2011; Fierer and Schimel, 2002). The mechanisms behind the increased nutrient availability after D/W include physical and biological processes as outlined for P in Fig 1.2.

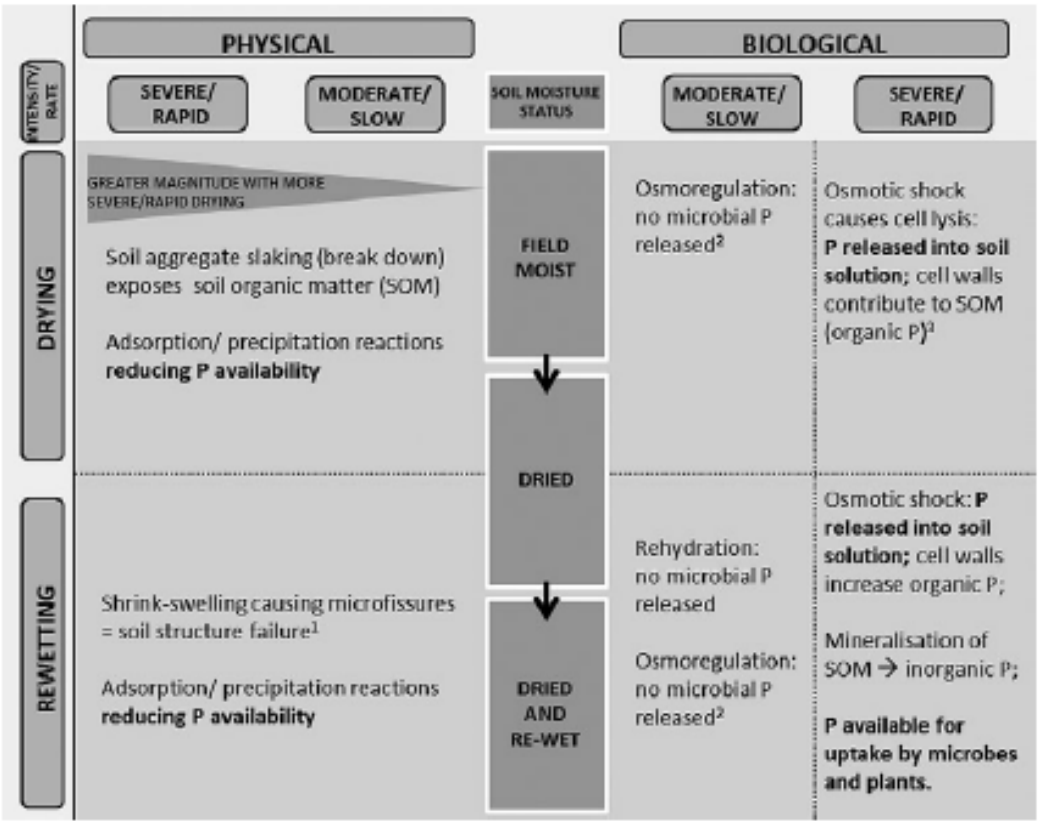

Figure 1. 2. Effect of soil drying and rewetting on P availability (from Dodd et al., 2015)

Physical processes

Changes in the water content of soil may affect physical processes due to various mechanisms, resulting in different fragment sizes of aggregates. Aggregates may break down by compression of entrapped air during wetting (slaking). In addition, micro-aggregates may

break down by differential swelling of clays (Bissonnais, 1996). The disruption of soil aggregates after D/W releases organic matter (Denef et al., 2001) and nutrients, which are potentially available to plants (Bünemann et al., 2013). However, D/W can also increase P sorption capacity of soils by exposing additional binding sites from disrupted aggregates (Haynes and Swift, 1985).

Biological processes

Biological processes include the death of soil microbes due to desiccation and lysis caused by osmotic shock upon rapid rewetting, releasing nutrients into the soil solution. In moist conditions, microbes are fully hydrated and the cellular water potential equilibrates with the surrounding water. D/W cycles affect soil microorganism by "double stresses" (drought and waterlogging) (Ouyang and Li, 2013). As soil water potential declines cells may be damaged and nutrients are releases into soil solution and the cell walls become part of the organic matter. Some of the nutrients released during the drying period may be taken up by other soil microbes or might maintain in solution when the soil is rewetted. Microorganisms may survive the desiccation by production of cell osmotytes. Many microorganism use simple organics with a good balance of high solubility and limited direct physiological effects (Csonka, 1989). Bacteria use potassium ions, glutamate, proline, glycine betaine and proline betain (Csonka, 1989; Miller and Wood, 1996), while fungi use polyols such as glycerol, erythritol and mannitol (Witteveen and Visser, 1995). After rewetting the cellular water potential equilibrates with the surrounding water causing rapid uptake of water into the cell and finally causing it to disrupt unless it the cell rupture is prevented by strong cell walls (Kieft et al., 1987). Because of thick cell walls with crooslinked polymers, preventing water losses, fungi are less sensitive and better adapted to D/W than bacteria (Bapiri et al., 2010; Yuste et al., 2011). In case of procaryotes, gram-positive bacteria are often more resistant to D/W than gram-negative bacteria. Gram-positive bacteria have a strong, thick cell wall with interlinked peptidoglycans to reduce water losses, while the cell wall of gram-negative bacteria consist of a single layer and an outer membrane (Madigan, 2012).

The soil microbial biomass accounts for about 1-5% of soil organic matter but it is the main agent in most biogeochemical processes in terrestrial ecosystems (Paul and Voroney, 1980) and significantly contributes to the plant available nutrients (Inubushi and Watanabe, 1987; Lynch, 1983). After D/W, the nutrients stored in the cells of the soil microbial biomass are partly mineralized and become available for plant uptake (Inubushi and Watanabe, 1987; Lynch, 1983). Microbial biomass P (Pmic) is a main component of the soil P pool. Pmic

accounts for about 2 to 10% of total P in mineral soils, but in in litter layers it represents be up to 50% of the total P (Achat et al., 2010; Oberson and Joner, 2005). After D/W, Pmic was shown to decrease resulting in the release of P into the soil solution (Blackwell et al., 2009; Bünemann et al., 2013; Butterly et al., 2011; Thanh Nguyen and Marschner, 2005; Turner and Haygarth, 2001). The increase of available P after D/W was related to the size of the microbial biomass (Sparling et al., 1985; Turner et al., 2003).

The nutrient release after D/W is not only affected by the size of microbial biomass but also the properties of the microbial community (Bapiri et al., 2010; Fierer et al., 2003; Hamer et al., 2007; Yuste et al., 2011). Soil microbial biomass comprises bacteria, fungi, actinomycetes, archaea and protozoa with fungi and bacteria being most dominant (Anderson and Domsch, 1973). Fungi and bacteria have different tolerances to desiccation with bacteria often being more sensitive and less adapted than fungi (Bapiri et al., 2010; Yuste et al., 2011). Fungi tend to be drought tolerant (Harris, 1981) and drought conditions tend to benefit gram-positive bacteria (Nazih et al., 2001; Uhlířová et al., 2005). Rewetting of dried soil may hence change the microbial community (Butterly, 2008; Ouyang and Li, 2013).

1.1.4 Effect of drying-rewetting cycles on nutrients in soil solution

The effects of D/W on C and N mineralization have been documented in numerous laboratory studies during recent decades (Borken and Matzner, 2009). Rewetting of dry soils usually results in a pulse of C and N mineralization (Fierer and Schimel, 2002; Miller et al., 2005) and this effect is attributed to the mineralization of previously unavailable, easily decomposable organic substrates (Borken and Matzner, 2009). Theoretically, the cumulative mineralization of C and N decrease with increasing duration and intensity of desiccation and the size of mineralization pulse is expected to increase with the amount of the applied water (Borken and Matzner, 2009).

Some studies also indicated that rewetting of dry soil led to an increase in soluble P (Achat et al., 2012; Bünemann et al., 2013; Butterly et al., 2009, 2011; Turner and Haygarth, 2001). Dissolved inorganic phosphorus (DIP) increased up to 2.1 mg P kg^{-1}soil after rewetting and DOP increased up to 3 mg P kg^{-1} (Butterly et al., 2011). Butterly et al., (2009) reported an increase in resin extractable P of about 7 mg P kg^{-1} after D/W in an arable soil, representing a 40% increase compared to the moist control. In grassland soils, the largest concentration of DIP was 0.14 mg P kg^{-1} and the net release of DIP was about 0.1 mg P kg^{-1} after 2h rewetting (Blackwell et al., 2009). In forest floors, the net DIP release increased up to 48-76 mg P kg^{-1}

after the soil was dried at 60°C (Achat et al., 2012). Soil drying can kill up to 58% of the total microbial biomass (Blackwell et al., 2009; Van Gestel et al., 1993; Wu and Brookes, 2005) and several authors concluded that the biomass could be an important source of P in soil solution after D/W (Bünemann, 2003; Turner and Haygarth, 2001). The degree of drying also played an important role in releasing P after D/W. Bünemann et al., (2013) observed that the P release after rewetting of soils dried to 2-5% of gravimetric water content was much larger than from soils desiccated to 10% of gravimetric water content.

The recovery of the microbial biomass may immobilize the previously released P (Butterly et al., 2009; Chen et al., 2016; Mondini et al., 2002). Chen et al., (2016) reported the recovery of microbial biomass to the level before D/W treatment after 7 days, while Mondini et al., (2002) found that the biomass did not recover to pre-drying conditions 12 days after rewetting.

Former studies on the release of dissolved P after D/W have concentrated on mineral soils from arable or grassland systems. Hence, there is a lack of knowledge on the release of dissolved P in forest soils after D/W.

1.2 Aims and hypotheses

There are still many open questions with regard to the effect of D/W on the release of P in forest soils and the role of organic layers. The aim of this thesis was to address some of these questions. The influence of repeated D/W of forest floors on soluble P was determined in the first study (study I). The response of specific groups of microorganism to D/W and the related release of P was determined in the second study (study II). The effect of D/W on the dynamic of soluble P and the microbial biomass P was tested in the third study (study III).

In detail, the following hypotheses were tested:

1. The release of soluble P from forest soils is enhanced by D/W cycles. Repeated cycles will increase the effects.
2. The release of P from the microbial biomass increases with drought stress prior to rewetting.
3. The amount of P released after D/W depends on the microbial community with bacteria releasing more P than fungi.
4. The recovery of the microbial biomass after D/W influences the fate of P released by D/W.

1.3 Materials and Methods

1.3.1 Sampling and experimental design

A diagram of the experimental design of the three studies is provided in Fig 1.3.

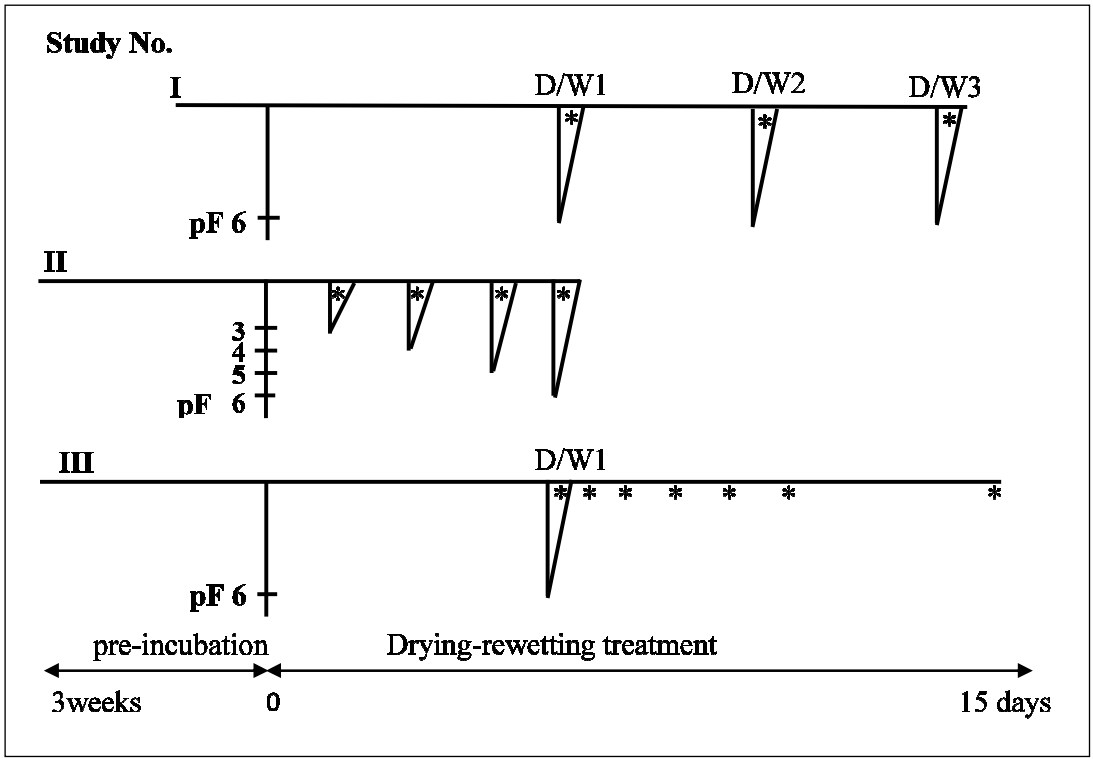

Figure 1. 3. Scheme of the experimental design and samplings (*) for the drying and rewetting treatments (D/W). Study I: samples were taken at drying-rewetting cycles 1, 2, 3. Study II: samples were taken at different desiccation intensities prior to rewetting (at app. pF 3, 4, 5 and 6). Study III: samples were taken at time 0, 3h, 8h, 1 day, 3 days, 7 days and 14 days after rewetting.

All experiments were conducted in a climate chamber at 20°C. All D/W treatments and controls were set up with 4 replicates. In the control treatment, the boxes were kept close during the whole experiment in order to prevent desiccation. In the D/W treatment, the boxes were kept open after the pre-incubation in order to allow the soil to dry.

Study I

Sampling

Soil samples were collected from three mature European beech stands at Bad Brückenau (N 50°21.26', E 6°59.07'), Bayreuth (N 49°58.37', E 11°35.13') and Lüss (N 52°50.68', E 6°53.91') and from three mature Norway spruce sites at Wülfersreuth (N 50°3.28', E 11°45.59'), Waldstein (N 50°8.62', E 11°51.98') and Oberwarmensteinach (N 49°59.45', E 11°49.93') in Germany. All soils have a loamy texture except for the soil at Lüss with a sandy texture. The parent material at Lüss is a sandy Pleistocene sediment, an alkaline igneous rock at Bad Brückenau, upper Triassic sandstone at Bayreuth, while the parent bedrock at Wülfersreuth and Oberwarmensteinach is Phyllite and at Waldstein is Granite.

In the beech forest, soils were collected from Oi, Oe layers and A horizon in the summer season. For the spruce forest, soils were sampled for Oi, Oe, Oa layers and A horizon in the spring season. Samples were homogenized by hand, roots and twigs were removed, and the Oi samples were cut into pieces of 1-2 cm; the A horizon samples were sieved (< 2 mm).

Experimental design

Moist (30 g) soil was arranged as a shallow layer in small plastic boxes (4 cm x 14 cm x 2.5 cm) in eight replicates per soil sample. Four replicates were subjected to D/W and four served as a control. All samples were brought to a water content equivalent to 50% of the maximum water holding capacity (WHC) and were kept at 5°C for 1 week until further processing in order to allow the microbial activity to reach basal rates after sample preparation. Soil water potentials were measured daily by a dew point potentiometer (WP4C, Decagon Devices Inc. Pullman WA, USA). After 3-4 days of desiccation, a water potential of about –100 MPa (pF 6) was reached. At this point of desiccation, subsamples were collected to measure dissolved P. The remaining soil was rewetted to 50% WHC by spraying with deionized water and subjected to the next drying-rewetting cycle. In total, three D/W cycles were applied to the samples, and the total experiment lasted for 12 days.

Study II

Sampling

An artificial soil was used defined by a 3:1 mixture of quartz sand (Dorsilit Nr. 9, particle size: 0.1-0.5 mm, 97% SiO_2, Dorfner GmbH & Co. KG, Hirschau, Germany) and quartz silt (Sikron SF300, particle size: 2-64 mm, 98% SiO_2, Quarzwerke GmbH, Frechen, Germany), which was cleaned by rinsing with deionized water. The bulk density of the mixture was 1.06 g cm^{-3}. The P content of the artificial soil measured in Bray-1 extracts (0.025 M HCl + 0.03 M NH_4F) was less than the detection limit of ICP-OES (< 2 mg P kg^{-1} soil).

Malt extract glucose meat extract peptone liquid medium (MGMPB: 0.3% malt extract, 0.3% meat extract, 0.5% peptone and 1% glucose, w/v) was used as growth medium. The addition of nutrients with the MGMPB solution to the artificial soil amounted to (in mg kg $soil^{-1}$): 3.1 dissolved inorganic P (DIP), 5.3 dissolved organic P (DOP), 1.070 organic carbon, and 111 total N (with 108 organic N)

Experimental design

The experimental unit of this experiment was a petri dish with artificial soil amended with growth medium, steam-sterilized, and inoculated with one out of three different soil microorganisms. The artificial soil (360 g) was arranged in a 1 cm layer in petri-dishes with 200 mm diameter. The soil was inoculated separately with the bacteria (*Pseudomonas fluorescens* MIGULA (*P. fluorescens;* gram-negative, DSMZ-No: 4358) or *Micrococcus luteus* (Schroeter) Cohn (*M. luteus*; gram-positive, DSMZ-No: 20030)) or the fungus *Penicillium chrysogenum* Thom (*P. chrysogenum*). 1 ml of a liquid pre-culture was mixed with 43 ml of a MGMPB. The amount of medium was chosen to reach 50% of the maximum WHC of the soil. In total, 40 petri dishes were established for each bacterium, and 64 for the fungus. After inoculation, petri-dishes were closed and incubated for 7 days (bacteria) or for 25 days (fungus) at 20°C to allow growth. At the end of the pre-incubation period, the desiccation experiment was started. Soil water potentials were measured daily by a dew point potentiometer (WP4C). The experiment lasted until a water potential of about -100 MPa (pF 6) was reached. At each day, 4 petri dishes and 4 controls for each of the three microorganisms were destructively harvested to measure dissolved P.

Study III

Sampling

Soil samples were collected from a mature European beech stand near Bayreuth (N 49°58.37', E 11°35.13') and from a mature Norway spruce site at Waldstein (N 50°8.62', E 11°51.98') in Germany (the same position as study I). Soils were sampled for Oi, Oe layers in autumn for both beech and spruce forest. Samples were prepared like in study I.

Experimental design

Moist soil samples were arranged in a 1 cm layer in petri-dishes with 200 mm diameter. All samples were adjusted to a water content equivalent of 50% of the maximum WHC and pre incubated for 3 weeks in a climate chamber at 20°C in order to allow the microbial activity to adjust. At the end of the incubation period, the D/W experiment was started. Soil water potentials were measured daily by a dew point potentiometer (WP4C) until a water potential of about -100 MPa (pF 6) was reached. At this point of desiccation, the D/W samples were rewetted immediately to 50% WHC by spraying with deionized water. Afterwards, D/W samples were maintained at 50% WHC throughout. The D/W treatment and controls were sampled at 0 h (directly after rewetting), 3 h, 8 h, 1 d, 3 d, 7 d and 14 d after rewetting, totalling 56 petri-dishes for each layer and species. At each time point, 4 D/W and 4 control petri dishes were destructively harvested to measure dissolved P and microbial biomass P.

1.3.2 Analytical methods

Total P, total C and total N

For chemical analysis, a subsample of each soil was dried at 60°C, mineral soil was dried at 105°C. Total P was determined after digestion with HNO_3 using an ICP-OES (Jobin-Yvon Horiba Group, JY2000, Varian Inc, Palo Alto, California, USA). Total C and N were determined by an elemental analyzer (Vario MAX, Elementar, Hanau, Germany).

Total dissolved phosphorus (TDP), dissolved inorganic phosphorus (DIP), dissolved organic phosphorus (DOP)

Soil samples were extracted in deionized water in a soil: water ratio of 1:10 by shaking the soil for 140 min on a horizontal shaker in order to determine dissolved phosphorus. For TDP and DIP determination, samples were filtered through a cellulose membrane acetate filter (0.45 µm, Sartorius AG, Göttingen, Germany). DIP was measured spectrophotometrically by using the colorimetric molybdate- ascorbic acid method (Murphy and Riley, 1962). TDP was

determined by ICP-OES. The difference between TDP and DIP was considered as dissolved organic P (DOP). The net release of P (DIP, DOP) was determined as the difference between the P concentrations in the samples subjected to D/W and in the controls. The concentration of P in soil (TDP, DIP, DOP, Pmic) was calculated based on the soil dry weight (mg P kg^{-1} soil).

Microbial biomass

Substrate induced respiration (SIR) method

In study I, the microbial biomass carbon (MBC) was measured in all samples after 1 week of pre-incubation by the SIR method at 22°C. Soil samples were placed in a 120-mL glass jars to measure CO_2 in the headspace (bottle R 100-D, CS-Chromatography service GmbH, Germany). Each sample was amended with two different glucose concentrations in 3 replicates. Oi layers were supplemented with 20 mg g^{-1} soil and 60 mg g^{-1} soil of glucose, and 25 mg g^{-1} soil and 75 mg g^{-1} soil of glucose were used for Oe and Oa layers. A horizons were added with 8 mg g^{-1} soil and 24 mg g^{-1} soil of glucose. The CO_2 production was determined hourly for 6 hours at 22°C by a gas chromatograph (SRI 8610C, SRI Instruments, Torrance, USA). The microbial biomass C was calculated according to the maximum initial respiration rate following the equation (Anderson and Domsch, 1978):

$MBC = 40.04x + 0.37$,

where x is the maximum initial rate of CO_2 respiration, expressed

in mL CO_2 (g soil)$^{-1}$ h^{-1}; MBC: mg microbial C g $soil^{-1}$.

Chloroform fumigation-extraction method (CFE)

In study II and study III, Pmic was measured by the chloroform fumigation-extraction (Brookes et al., 1982; Vance et al., 1987). After fumigation, soils were extracted in Bray-1 solution (0.025 M HCl + 0.03 M NH_4F) with a soil: solution ratio of 1:10 (Aponte et al., 2010; Bray and Kurtz, 1945; Heuck et al., 2015). Total P in the Bray-1 extracts was measured by ICP-OES (Jobin-Yvon Horiba Group, JY2000, Varian Inc, Palo Alto, California, U.S.A.). Pmic was calculated as the difference of inorganic P in the fumigated and non-fumigated soil extracts, using a conversion factor of 2.5 (Brookes et al., 1982; Jenkinson, 2004).

Ergosterol as an indicator of fungal biomass

In study II, soils (5 g moist soil) were extracted with 25 ml ethanol for 30 min at 5°C by shaking (250 rpm) following the method of Djajakirana et al., (1996). Then, samples were centrifuged for 30 min at 4100 rpm. The ethanol extracts were evaporated in a vacuum rotary evaporator at 44°C in the dark. The residues were collected in 2 ml methanol, filtered (cellulose membrane acetate, 0.45 µm) and stored in brown glass HPLC-vials at 2°C until analysis. Ergosterol was quantified using high performance liquid chromatography (HPLC, System Gold 125 Solvent Module, Beckman Coulter, Brea, U.S.A. column: MZ Spherisorb ODS-2 C18, 150 x 3mm, MZ Analysetechnik, Germany) and detected using an UV-detector at a wavelength of 282 nm (System Gold 166 UV-Detector, Beckman Coulter, Brea, U.S.A.). Ergosterol was determined at the Department of Soil Biology, University Hohenheim, Germany.

1.3.3 Statistical analyses

All statistical analyses were conducted in R environment for statistical computing (R Core Team, 2014).

Normal distribution and homogeneity of variance was confirmed using Shapiro–Wilk test and Levene test, respectively. In order to test differences in the release of P, linear mixed effect models were calculated using the R package lme. Fixed effects were P release (DIP, DOP), treatment (control, D/W), and experiment (the D/W cycles), and random effects accounted for different sites. In case the data were not normally distributed or variances were not homogeneously distributed among groups, data were ranked. Post-hoc tests were done using the linear hypothesis test as implemented in R package multcomp. The relationship between P release and microbial biomass was analysed by linear and nonlinear regressions for both beech forest and spruce forest (study I).

The differences between species and pF values with respect to P release, analysis of variance (ANOVA) followed by Tukey-HSD test as post-hoc test, were used (study II). The differences between control and D/W were tested by a t-test (study III).

1.4 Main results

1.4.1 Study I

In the controls, the concentration of total dissolved P (TDP) increased with time of incubation in all samples (Fig 1.4). The largest TDP concentration in controls was found in beech Oi with 133 mg P kg^{-1} and the smallest concentration was 4 mg P kg^{-1} in the spruce A horizon. After D/W, the TDP concentration was significant larger than in the controls in all layers, except for beech Oi and A horizon. In beech Oi, the increase of TDP in D/W compared to control only occurred after the first D/W cycle and was not significant. The biggest increase in TDP concentration in D/W samples was observed in the Oe layers of both tree species with average concentrations of about 130 mg P kg^{-1}.

The TDP concentrations in the D/W samples of the Oe layers were 10 times higher than those in A horizons. In spruce Oi, the TDP concentration in D/W sample ranged from 60 to 73 mg P kg^{-1} after D/W cycles. The response of TDP to D/W in the spruce Oa was remarkable with the concentration from 44 to 59 mg P kg^{-1}. The second and the third D/W cycles did not significantly increase the release of TDP.

The net release of TDP following D/W were lowest in the A horizons (< 8 mg P kg^{-1}) for both beech and spruce soils. After the first D/W cycle, the net release of TDP from the beech soils were 8.3, 68.7, 3.3 mg P kg^{-1} in Oi, Oe, and A horizon, respectively. In spruce soils, these rates were 33.7, 63.3, 33.8 and 6.2 mg P kg^{-1} in Oi, Oe, Oa and A horizon, respectively. Generally, the net release of TDP in the Oi layer was less than in the Oe layers.

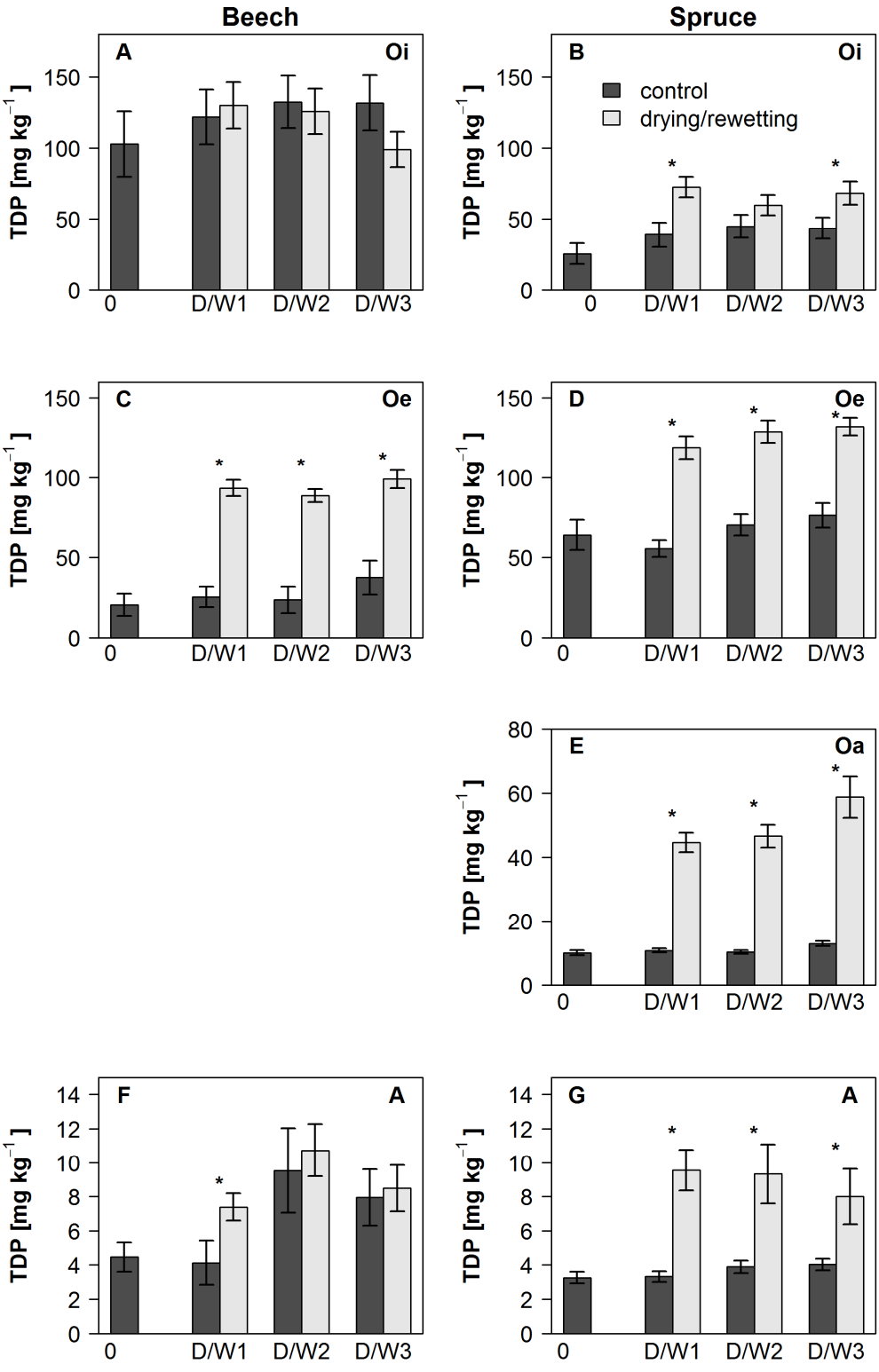

Figure 1. 4. Total dissolved phosphorus (TDP) in Oi, Oe, Oa layers and in A horizons of beech (A, C, F) and spruce forest soils (B, D, E, G); 3 drying-rewetting (D/W1-D/W3); 0: initial DIP measured after 1 week pre-incubation before starting the first D/W cycle. (*: $p < 0.05$).

1.4.2 Study II

The net release of TDP per unit Pmic was much larger in soils incubated with *P. fluoresces* than in soils incubated with *M. luteus* and *P. chrysogenum* (Fig 1.5). The net release from the soils incubated with *P. fluorescens* started already after desiccation to pF 3.9 and further increased strongly with the degree of desiccation prior to rewetting. In soils incubated with *P. fluorescens*, the average TDP net release ranged from 0.4 to 1.2 mg mg^{-1}. The net release of TDP in the *P. chrysogenum* incubation started at pF 5.0 to reach a maximum of 0.25 mg mg^{-1} after desiccation to pF 6.2 and was similar to the ratio in the *M. luteus* incubation at pF 6.2. The net release of TDP from the soils incubated with *M. luteus* had no clear pattern. An increase at pF 3.9 was followed by a decrease at pF 5.0-6.0 and an increase at pF 6.2.

1.4.3 Study III

Net release of TDP per unit Pmic (in control at time 0) was always largest at time 0 immediately after rewetting and was smallest at day 14 after rewetting for both Oi and Oe layers in beech and spruce forest (Fig 1.6). The maximum net release of TDP/Pmic after rewetting at time 0 was observed in spruce Oi reaching up to 0.13 mg mg^{-1}, while a minimum of 0.02 mg mg^{-1} was observed in beech Oi. In beech Oe and spruce Oe, a similar net release of TDP at time 0 with 0.09 mg mg^{-1} was observed. The net release of TDP per unit Pmic decreased with time in all samples after rewetting, but differences between the forest floor layers emerged. In beech and spruce Oi, the net release of TDP/Pmic decreased substantially with time from 0.02 mg mg^{-1} to near zero following day 1 (beech) and from 0.11 to 0.015 mg mg^{-1} in spruce, with a sharp decline at day 4 (Fig 1.6). In contrast, the net release of TDP per Pmic from Oe layers decreased much less. For beech Oe, the net release of TDP/Pmic decreased continuously from 0.09 mg mg^{-1} at time 0 until day 14 with 0.05 mg mg^{-1}. For spruce Oe, the net TDP release/Pmic also decreased from 0.09 mg mg^{-1} to about 0.08 mg mg^{-1} during day 1.

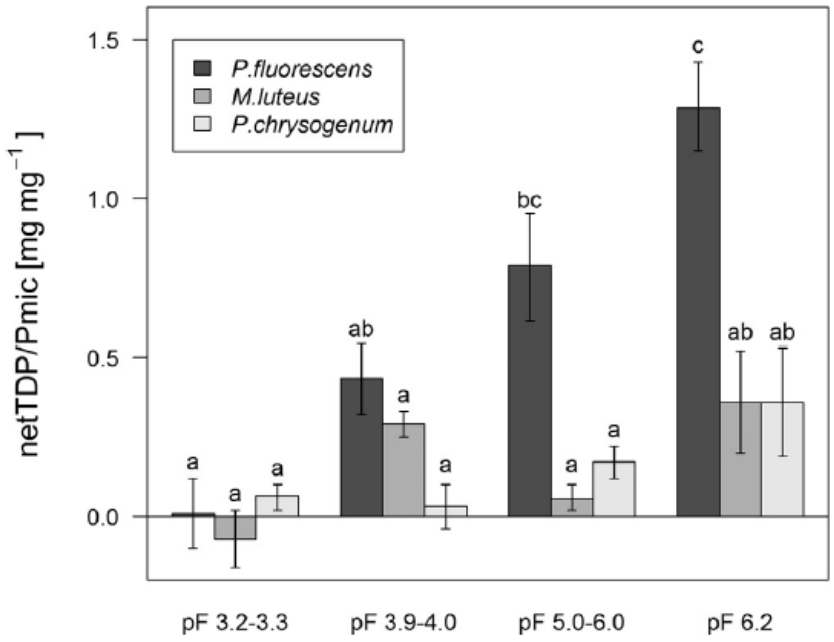

Figure 1. 5. Net release (D/W – controls) of total dissolved P (TDP) after D/W in relation to initial microbial biomass P (Pmic) for inoculations with *Pseudomonas fluorescens*, *Micrococcus luteus* and *Penicillium chrysogenum* (Mean ± SEM; n = 4). Different letters indicate significant differences between groups ($p < 0.05$).

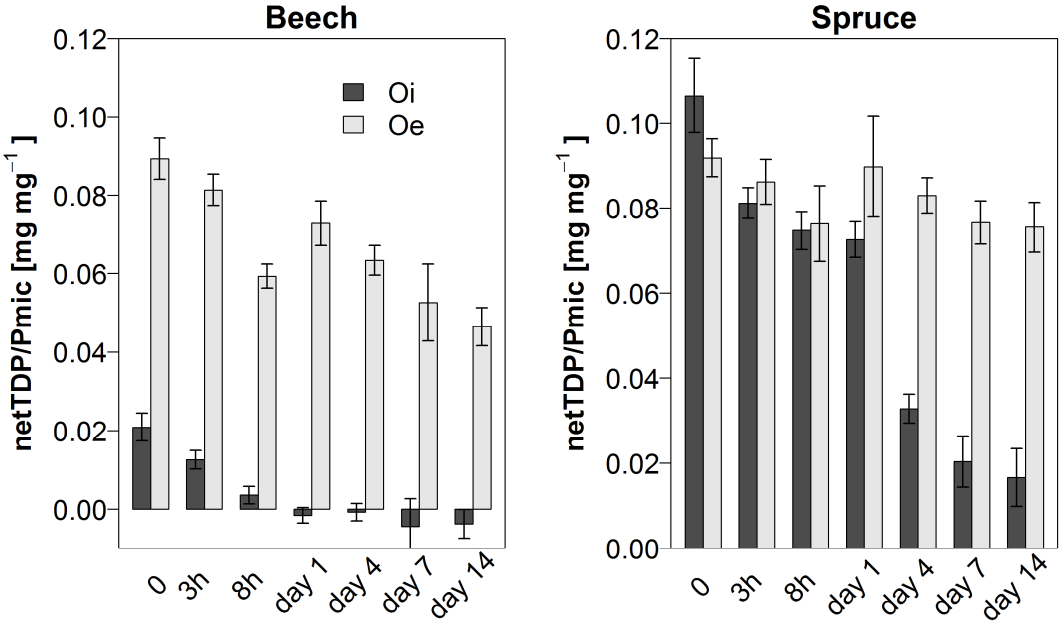

Figure 1. 6. Temporal development of the TDP net release (D/W – controls) after rewetting in relation to initial microbial biomass P (Pmic) (in control at time 0) for Oi and Oe layers of beech and spruce forest floor (Mean ± SEM; n = 4).

1.5 Discussion

1.5.1 Release of soluble P in response to D/W

The results from these experiments indicate that D/W cycles are important for enhancing P cycling in forest soils. The release of P started immediately after the first D/W cycle and the effect of the D/W cycles on the release of soluble P in organic layers was stronger than in the mineral soil (study I). The amount of P released from O layers confirmed the important role of the organic layer for the effects of D/W cycles in the forest soils. Previous studies investigated the effect of D/W on P release from mineral soils (Blackwell et al., 2009; Bünemann et al., 2013; Butterly et al., 2009, 2011). Only one study of Achat et al., (2012) conducted experiments on forest floors under rather extreme conditions (drying soil at 60°C). The amount of P release in their study accounted for 30% of the total P compared to 1-5% of the total soil P in our studies. The net release of TDP from forest soil A horizons was similar to the release of TDP reported by Blackwell et al., (2009) for arable mineral soils.

The TDP net release after rewetting of dried soil decreased strongly with time in forest floor Oi layers. In contrast, TDP concentration kept rather stable in Oe layers where a substantial part of the release was still observed at day 14 (study III). The net release of TDP was already very low after day 1 of rewetting in beech Oi and after day 4 in spruce Oi (study III). In contrast, in Oe layers, the net release of TDP was still 30 mg P kg^{-1} in beech and 40 mg P kg^{-1} in spruce at day 14 after rewetting (study III). The dynamic of TDP after rewetting seem to be related to the recovery of the microbial biomass as discussed under 1.5.4.

The release of DIP after D/W from the organic layers in most cases exceeded the release of DOP (study I, study III). The lysis of microbial cells by D/W, as the main source of soluble P, should initiate a flush of DOP rather than of DIP, as P in cells is mostly organically bound (Butterly et al., 2009; Turner et al., 2003; Turner and Haygarth, 2001). The dominance of DIP over DOP can be explained by fast mineralization of released DOP to DIP (Bünemann et al., 2013; Macklon et al., 1997).

Repeated D/W cycles did not change the amount of net released P (study I). The hypothesis that repeated D/W cycle will increase the effects was not confirmed. Our results are supported by Butterly et al., (2009) who indicated that Pmic and MBC strongly decreased after the first D/W cycle but did not change much after the second and the third D/W cycle. This might be due to two factors: First, the adaption of microbial community to D/W cycles, such that more resilient species dominate in subsequent D/W. Second, the microbial biomass

reacting to D/W did not recover until the following D/W cycle (Parr et al., 1981; Turner et al., 2003).

Extrapolating the net release of dissolved P observed from our laboratory D/W study to the field scale, the release of TDP from O layers and A horizon following a D/W cycle was about 2 kg P ha^{-1} (beech) to 3 kg P ha^{-1} (spruce) (study I). With a similar calculation, the TDP net release from Oi + Oe layers accounted to 1.4 kg P ha^{-1} (beech) and to 2.0 kg P ha^{-1} (spruce) directly after rewetting and maintained at 0.7 kg P ha^{-1} (beech) and 1 kg P ha^{-1} (spruce) 14 days after rewetting (study III). The release represents a substantial pool of available P compared to the total annual P uptake of trees in the range 4-7 kg P ha^{-1} a^{-1} (Ilg et al., 2009) and also confirms the role of D/W cycles for P cycling in forest soils.

1.5.2 Effect of desiccation intensity prior to rewetting on the release of P

The P net release upon rewetting occurred already after desiccation to pF 4 for the gram-negative bacterium (*P. fluorescens*), while a substantial P net release was only found at the most severe desiccation (pF 6.2) for the gram-positive bacterium (*M. luteus*) and the fungus (*P. chrysogenum*) (study II). Kakumanu et al., (2013) found that the soluble C concentrations after D/W only increased after desiccation to pF 5.6, but no data on P were given in their study. To our knowledge, there is only one study that has investigated the release of P after D/W from soils in response to different desiccation degree (Bünemann et al., 2013). In mineral soils, an increase of P release was reported after desiccation to a volumetric water content of less than 10%, and the P release peaked at 2-5% prior to rewetting (Bünemann et al., 2013). However, pF values were not given in the study of Bünemann et al., (2013). Our finding of pF 4 (gravimetric water content of about 2-4%) as a critical desiccation degree for P release from gram-negative bacteria seems in accordance with their results.

1.5.3 Effect of microbial community on the release of P after D/W

The initial microbial biomass C in beech forest floors was larger than in spruce forest floors with averages of 35 mg g^{-1} (beech) and 17 mg g^{-1} (spruce). However, the release of P after D/W from beech forest floors was similar to that in spruce forest floors. In addition, there was a relation between the net release of DIP after D/W to the microbial biomass carbon in the Oe and in the Oa layers, but not in Oi layers, although the microbial biomass carbon in the Oi layers and in Oe layers was similar (study I). Moreover, in Oi layer, the net DIP release from spruce forest were 3 times higher than that from beech although the microbial biomass P in beech samples was similar to that in spruce samples (study III). In addition, the net DIP

release was about 5 times larger in the Oe layer compared to the Oi layer of the beech forest, at similar microbial biomass P concentrations (study III). An explanation for these findings may be seen in different microbial communities inhabiting the Oi and the Oe layers of the deciduous and coniferous forest. Bacteria are more sensitive to D/W than fungi (Blackwell et al., 2010; Schimel et al., 1999), and the reason for the difference between Oi and Oe layers might be the higher ratio of fungi to bacteria in the Oi layers (Baldrian et al., 2012; Fierer et al., 2003; Schmitt et al., 2010).

As abiotic sources of P after D/W were excluded in study II, the differences in TDP release after D/W between the species are attributed to the release of P from Pmic. The different cell wall architecture seems to modify the stress tolerance and the net release of P from Pmic due to cell lysis and osmotic regulation. Fungi are better adapted than bacteria at low soil water potential because of their thick cell wall with crosslinked polymers, preventing water loss (Gordon et al., 2008; Holland and Coleman, 1987). Fungal cell walls can be further stabilized by thickening and crosslinking of polymers (Kollar et al., 1997; Sietsma and Wessels, 1981). During drought hydrophobic substances are released from the fungal hyphae to prevent desiccation (Spohn and Rillig, 2012). Further, filamentous fungi can extent their hyphal networks over long distances and cross air filled soil pores to access nutrient and water and counteract water stress (Guhr et al., 2015). In case of procaryotes, gram-positive bacteria have a strong, thick cell wall with interlinked peptidoglycans to reduce water losses. Gram-negative bacteria seem to be less resistant to D/W than gram-positive bacteria because of their specific cell wall properties (Madigan, 2012).

The largest net release of TDP/Pmic from soils incubated with *P. fluorescens* (1.2 mg mg^{-1}) supported that gram-negative bacteria are more sensitive to D/W than gram-positive bacteria and fungi. After D/W, the release of P was in the order gram-negative bacterium (*P. fluorescens*) >> gram-positive bacterium (*M. luteus*) = fungus (*P. chrysogenum*) (study II). As the gram-positive bacterium behaves similar to the fungi, this result did not completely support the hypothesis that the amount of P release after D/W depends on the microbial community with bacteria generally releasing more P than fungi.

Gram-negative bacteria have the highest abundancy in the upper soil and their proportion decreases in relation to gram-positive bacteria with depth (Fierer et al., 2003; Potthoff et al., 2006). Gram-negative bacteria in soils seem to be more dependent on the availability of labile organic matter like fresh litter or root exudates (Fierer et al., 2003; Griffiths et al., 1998; Paterson et al., 2007; Potthoff et al., 2006). In addition, gram-negative bacteria are often

associated with the rhizosphere (including *Pseudomonas* species like *P. flurescence*, (Chan and Katznelson, 1961; Gottel et al., 2011). Since root density decrease with depth, this also contributes to the distribution patterns of gram-positive bacteria in soils. Hence, the strongest effect of D/W on P release is to be expected in soil horizons or compartments with high abundance of gram-negative bacteria. Less effects of D/W are expected in soil compartments with microbial communities dominated by fungi and gram-positive bacteria.

1.5.4 Effect of recovery of microbial biomass after D/W on the fate of P released

Our hypothesis was that the dynamics of the microbial biomass and its P pool (Pmic) after D/W influenced the release and fate of soluble P in forest floors. The concentration of Pmic ranged from 350 to 700 mg P kg^{-1} soil in the controls (study III). After rewetting of the dried soils (time 0), Pmic decreased by 6% to 28% in spruce Oi, Oe, respectively. Pmic in spruce Oi recovered already at day 1 after rewetting, while the reduction of Pmic in spuce Oe remained until day 14. In contrast to spruce, there was no significant difference in Pmic between D/W and controls in beech Oi and Oe. In beech Oe, Pmic had a tendency for decline at time 0, but this tendency was no longer obvious at day 1. Generally, the reduction of Pmic after D/W in our study in Oi layer was quite low. In Oe layers the reduction of Pmic was larger and comparable to D/W effects in grassland or arable mineral soils. Chen et al., (2016) showed a decline of Pmic by 21% after drying, and a full recovery 7 days after rewetting while Thanh Nguyen and Marschner, (2005) indicated that P mic decreased by 25% after drying and rapidly increased 1 day after rewetting. De Nobili et al., (2006) also indicated that soil ATP had rapidly recovered from 60% to 80% of the controls in 2 days after rewetting of dried soil.

Although the amount of TDP released after D/W did not exceed 10% of the Pmic pool, the decrease of Pmic after rewetting in dried Oe layer (by 90 mg kg^{-1}) and Oi layer (by 30 mg kg^{-1}) of spruce supported the conclusion that Pmic is a major source of the TDP release. Microbial biomass in the beech Oi layer had no clear change after rewetting which coincided to the small net TDP release.

The immobilization of P by the recovering microbial biomass after rewetting might be an important fate of the soluble P released by D/W. In our study, the rather stable TDP net release after rewetting of Oe layers indicted that the immobilization of P in the microbial biomass was minor, at least in the fourteen days after rewetting. During this time the Pmic concentration in the D/W samples were still lower than in the controls. Overall, the decrease

and recovery of microbial biomass seems responsible for the release of soluble P and its temporal dynamics after rewetting.

1.5.5 Critical assessment of the experimental conditions

Soil samples were sieved and homogenized (A horizons) and cut (beech Oi) in all experiments (study I, study II). These treatments destroyed soil aggregates and likely increased microbial activity and the availability of substrates (Degens and Sparling, 1995; Denef et al., 2001). Thus, the release of P after D/W from undisturbed soils is hard to estimate based on the release of P after D/W in laboratory condition.

We conducted the desiccation (study I, study II, study III) from pF 2 (50% WHC) to pF 6 in some days (from 3 to 5 days) at 20°C which is considered as a moderate stress. Drought can impact on microorganism by inducing surface hydrophobicity in soils (Doerr et al., 2006; Goebel et al., 2007). The hydrophobicity increases with the intensity of drought (Franco et al., 2000; Verheijen and Cammeraat, 2007). Hydrophobicity prevents microbial degradation of soil organic matter, reduces the availability of soil moisture and nutrients (Doerr et al., 2006; Goebel et al., 2007). In mineral soils, hydrophobicity plays a minor role because of low organic content, while hydrophobicity in the organic layer of forest soils occuring during dry season may sustain for several weeks or months (Mataix-Solera et al., 2007). During rainfall, the increase in water potential, is then inhibited. This depends on the degree of hydophobicity, the initial water potential, and the intensities and durations of rainfall (Borken and Matzner, 2009). In our experiments (study I, study II, study III), the desiccation regime was moderate, while the rewetting was very intense. The abrupt change in soil water potential poses a strong stress to soil microorganisms (Schimel et al., 2007), which under natural soil and climatic conditions is only seldom reached. Because of the hydrophobicity of surfaces and the formation of preferential flow paths (Bogner et al., 2008), the rewetting of soil often proceeds more slowly under natural conditions and the P net release after rewetting of a forest floor under the field conditions might be less than in the laboratory experiments.

Study II was limited to a single species per group (fungi, gram-positive bacteria, gram-negative bacteria). More species belonging to these groups should be studied to verify the results for the different groups.

1.6 Conclusions and outlook

This study highlighted the importance of D/W cycles for the pool of water soluble and plant available P in forest soils. The amounts of TDP released made a significant contribution to the overall P cycling in forest ecosystems as related to the annual P flux with litterfall and the net P mineralization in forest ecosystems. The release of available P after D/W from litter layers was larger than from mineral soils and had a positive relation to the microbial biomass in Oe and Oa layer. Immediately repeated D/W cycles did not increase the release of P. Microbial biomass seems the main source of P release after D/W. The differences between forest floor layers in response to D/W are likely due to differences in the total microbial biomass and microbial community composition with different proportions of fungi and gram-negative and gram-positive bacteria.

Available P was released already after desiccation to pF 4 (gravimetric water content (GWC): 2-4%) in case of the gram-negative bacterium, while only a tendency for P release from the fungus and the gram-positive bacterium was measured after desiccation to pF 6 (GWC: <1%). Hence, gram-negative bacteria seem to play major role in microbial source of the P pulse in soils after D/W and can be an important source for the release of soluble P in soils.

The decrease and recovery of the P pool in the microbial biomass influences the temporal dynamics in the TDP released after D/W cycle. The different response of Oi and Oe layers of beech and spruce forest soil suggested that the degree of decline and recovery of the Pmic pool after D/W is specific for the soil microbial community inhabiting the different layers of the forest floor.

Future experiments on D/W effects on soils should better mimic field conditions either by using undisturbed soil columns or by direct field studies to overcome some of the shortcoming of laboratory incubations with disturbed soils. In addition, the fate of TDP released by D/W should be investigated in more detail. Here, the leaching from forest floors to the mineral soil and the uptake by plant roots needs more attention. Furthermore, it is also important that we better understand the effects of a prolonged drought on the release of P in soil upon rewetting. Finally, the results on the sensibility of different soil microbial species to D/W require further confirmation in more complex microbial communities.

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Manuscripts

2. Study I: Drying–rewetting cycles release phosphorus from forest soils

Mai-Van Dinh, Thilo Schramm, Marie Spohn and Egbert Matzner

Soil Ecology, University of Bayreuth, Bayreuth Center of Ecology and Environmental Research (BayCEER), Dr.-Hans-Frisch-Str. 1-3, 95448 Bayreuth, Germany

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2.1 Abstract

Drying-rewetting cycles (D/W) occur frequently in topsoils and may mobilize phosphorus (P). We investigated the effect of repeated D/W on the release of dissolved inorganic (DIP) and organic P (DOP) from forest floors and A horizons. Samples were taken from 3 European beech sites and from 3 Norway spruce sites. Soils were desiccated up to pF 6 (-100 MPa) in three D/W cycles in the laboratory, while the controls were kept permanently at 50% water holding capacity. After each drying, P was extracted from the soils in water. D/W caused the release of DIP and DOP especially from O layers. There was no general difference in response to D/W between samples from beech and spruce. The net release of DIP after D/W was largest from the Oe horizons (average 50-60 mg P kg^{-1}) for both beech and spruce forest soils. The net release of DIP from Oi layers was on average 7.8 mg P kg^{-1} and from spruce Oa layers 21.1 mg P kg^{-1}. In the A horizons, net DIP release was similar in beech and spruce soils with 0.4 mg P kg^{-1}. The release of DOP was less than the release of DIP except for the A horizons. Repeated cycles did not increase the release of DIP and DOP. The release of DIP and DOP was positively correlated to the microbial biomass in Oe and Oa layers but not in Oi layers. Our results suggest that D/W may significantly influence the short term availability of dissolved P in both beech and spruce forest soils.

2.2 Introduction

Drying and rewetting (D/W) is a common phenomenon in soils and cycles of D/W are considered as the most common abiotic stressors in many soils (*Blackwell* et al., 2013), which affects mainly the topsoil. D/W leads to lysis of microorganisms *(Schimel* et al., 1999*; Kaiser* et al., 2015) and soil aggregate disturbance (*Blackwell* et al., 2013). The effect of D/W on soil processes have been studied for a long time with focus on C and N in arable and grassland soils (*Degens* and *Sparling,* 1995*; Magid* et al., 1999*; Mikha* et al., 2005*; Borken* and *Matzner,* 2009*; Butterly* et al., 2009). The rewetting of dried soil in laboratory incubations often resulted in a burst of CO_2 ("Birch effects", *Birch*, 1964) and an increase in net organic N mineralization (*Borken* and *Matzner*, 2009). In forest soils, however, such effects were rarely observed in field and laboratory experiments (*Hentschel* et al., 2007; *Muhr* et al., 2010; *Chen* et al., 2011). A short term peak of CO_2 emissions that exceeded the permanently wet controls was only found in incubation experiments with organic layers after extreme desiccation (soil water potentials < –400 MPa; *Muhr* et al., 2010).

Several authors have investigated the effects of D/W on P release, focusing on arable and grassland soils. In general, a release of DIP and DOP after D/W was observed. DIP increased up to 2.1 mg P kg^{-1} [0.5% total phosphorus (TP)] (*Butterly* et al., 2011) after rewetting and DOP increased up to about 3 mg P kg^{-1} (0.7% TP) (*Butterly* et al., 2009). Specific factors such as the degree of drying (*Kieft* et al., 1987; *West* et al., 1992; *Lado-Monserrat* et al., 2014), the duration of drying (*De Nobili* et al., 2006) and the frequency of D/W cycles *(Van Gestel* et al., 1992; *Mikha* et al., 2005; *Butterly* et al., 2009; *Yu* et al., 2014) have been investigated. The reasons for the release of P after D/W are seen in the lysis of microbial cells after rewetting, leading to the release of P rich cell components and the disruption of aggregates, exposing occluded organic and inorganic P *(Turner* and *Haygarth,* 2001*; Turner* et al., 2003; *Butterly* et al*.,* 2009). *Bünemann* et al. (2013) showed that microbial biomass in grassland soils was the original source of C and P release after D/W, while the physicochemical processes, such as the disruption of soil aggregates also played an important role for the release of P. Yet, little is known about P release due to D/W in forest soils. *Achat* et al. (2012) studied P release caused by D/W in forest soils. However, they dried forest soils at 60°C, which is an unrealistic temperature even for Mediterranean soils.

The organic layer is a hotspot of microbial activity in forest soils and comprises the largest microbial biomass per dry weight in the pedon. As the microbial biomass is rich in P, it may represent up to 50% of the total soil P content and the turnover rate of this P-pool seems to be

only a few days (*Achat* et al., 2010). This suggests a high susceptibility of the forest floor to D/W effects on the P availability. Yet, the different layers of the forest floor and the mineral soil may react differently to D/W. The forest floor Oi layer is composed of fresh litter, the Oe layer has a larger degree of decomposition and fragmentation while the Oa comprises humified and decomposed soil organic matter (SOM). In A horizons, humified SOM is to a large extent associated with soil minerals. Soil minerals might adsorb inorganic and organic phosphorous once it is mobilized by D/W. Furthermore, the different horizons of the forest floor and mineral soil are inhabited by specific microbial communities (*Matejek* et al., 2010), that differ in their susceptibility to drought. The ratio of fungi/bacteria decreases with soil depths in forest soils (*Scheu* and *Parkinson*, 1994; *Fritze* et al., 2000; *Schmitt* et al., 2010). Bacteria are known to be more sensitive to soil desiccation than fungi (*De Vries* et al., 2012; *Barnard* et al., 2013) and fungi seem more competitive in the top of the organic layer, which is more often subjected to desiccation.

The quality of litter, its decomposition rate, the morphology of the forest floor and the inhabiting microbial community differ between coniferous and deciduous tree species (*Reich* et al., 2005; *Hobbie* et al., 2006). These differences likely influence the effect of D/W, but to our knowledge, no comparative study on this subject is available so far.

In summary, past studies on the release of dissolved P after D/W have concentrated on mineral soils from arable or grassland systems. Information on the response of dissolved P to D/W in forest soils is still lacking. In this study, we aimed at investigating the effect of repeated D/W cycles on the release of soluble organic and inorganic P in forest soils. We hypothesized that (1) the release of organic and inorganic P caused by drying-rewetting cycles increases with the soil microbial biomass concentration, that (2) the release of organic and inorganic P due to drying-rewetting is higher in soils of beech than in soils of spruce forests, and that (3) the repeated D/W cycles have a larger effect than a single D/W event on the net release of P. To test these hypotheses, we conducted a laboratory experiment using soils from different European beech and Norway spruce sites.

2.3 Materials and Methods

2.3.1 Study sites and sample preparation

Soil samples were collected from three mature European beech stands at Bad Brueckenau (N 50°21.26′, E 6°59.07′), Bayreuth (N 49°58.37′, E 11°35.13′) and Luess (N 52°50.68′, E 6°53.91′) and from three mature Norway spruce sites at Wuelfersreuth (N 50°3.28′, E 11°45.59′), Waldstein (N 50°8.62′, E 11°51.98′) and Oberwarmensteinach (N 49°59.45′, E 11°49.93′) in Germany. All soils have a loamy texture, except for the soil at Lüss, which has a sandy texture. The parent material is a sandy Pleistocene sediment at Lüss, an alkaline igneous rock at Bad Brückenau, upper Triassic sandstone at Bayreuth, while parent material at Wülfersreuth and Oberwarmensteinach is Phyllite and at Waldstein is Granite. In the beech forests samples were collected from Oi, Oe layers and A horizon in summer (Table 2.1). For the spruce forests, soils were sampled for Oi, Oe, Oa layers and A horizon in spring (Table 1). Samples were homogenized by hand, roots and twigs were removed, and the Oi samples were cut into pieces of 1-2 cm; the A horizon samples were sieved (< 2 mm).

2.3.2 Experimental design

Moist soil (30 g) was arranged as a shallow layer in small plastic boxes (4 cm x 14 cm x 2.5 cm) in eight replicates per soil sample. Four replicates were subjected to D/W and four served as a control. All samples were brought to a water content equivalent to 50% of the maximum water holding capacity and were kept at 5°C for 1 week until further processing in order to allow the microbial activity to reach basal rates after sample preparation. The soil field capacity was determined previously by saturating the soil for three hours in an immersed funnel equipped with a cellulose-nitrate filter, and subsequently drying it at 105°C for A horizons and at 60°C for Oi, Oe, Oa layers after determination of the water saturated weight. The pre-incubation and the experiment were conducted in a climate chamber at 20°C. In the control treatment, the boxes were kept close during the whole experiment in order to prevent desiccation. In the D/W treatment, the boxes were kept open after the pre-incubation in order to allow the soil to dry. Three D/W cycles were applied to the samples. Soil water potentials were measured daily by a dew point potentiometer (WP4C, Decagon Devices, USA). After 3-4 days of desiccation, a water potential of about -100 MPa (pF 6) was reached. At this point of desiccation, subsamples of 1.5 g were extracted in deionized water in a soil:water ratio of 1:10 by shaking for 140 min on a horizontal shaker. The remaining soil was rewetted to 50% water holding capacity by spraying with deionized water and subjected to the next drying-

rewetting cycle. In total, three D/W cycles were applied to the samples, and the total experiment lasted for 12 days.

2.3.3 Analytical methods

For chemical analysis, a subsample of each soil was dried at 60°C. Total P was determined after digestion with HNO_3 using a ICP-OES (Jobin-Yvon Horiba Group, JY2000, Varian Inc, Palo Alto, California, USA). Total C and N were determined by an elemental analyzer (Vario MAX, Elementar, Hanau, Germany).

For TDP and DIP, samples were filtered through a cellulose membrane acetate filter (0.45 µm). DIP was measured spectrophotometrically by using the colorimetric molybdate-ascorbic acid method (*Murphy* and *Riley*, 1962). Total water-soluble P was determined by ICP-OES. The difference between total water soluble P and DIP is considered as DOP. The net release of P (DIP, DOP) was determined as the difference between the P concentrations in the samples subjected to D/W and in the controls.

Microbial biomass carbon (MBC) was measured in all samples after 1 week of pre-incubation by the substrate induced respiration method at 22°C. Soil samples were placed in a 120-mL glass jars to measure CO_2 in the headspace (bottle R 100-D, CS-Chromatography service GmBH, Germany). Each sample was amended with two different glucose concentrations in 3 replicates. Oi layers were supplemented with 20 mg g^{-1} soil and 60 mg g^{-1} soil of glucose, and 25 mg g^{-1} soil and 75 mg g^{-1} soil of glucose were used for Oe and Oa layers. A horizons were added with 8 mg g^{-1} soil and 24 mg g^{-1} soil of glucose. The CO_2 production was determined hourly for 6 hours at 22°C by a gas chromatograph (SRI 8610C, SRI Instruments, Torrance, USA). The lowest glucose concentration that gave maximum initial respiratory response was determined. The microbial biomass C was calculated according to the maximum initial respiration rate following the equation (*Anderson* and *Domsch*, 1978):

$$MBC = 40.04x + 0.37 \quad (1)$$

where x is the maximum initial rate of CO_2 respiration, expressed in mL CO_2 (g soil)$^{-1}$ h^{-1}

MBC: mg microbial C g $soil^{-1}$

2.3.4 Statistical analyses

Normal distribution and homogeneity of variance was confirmed using Shapiro-Wilk test and Levene test, respectively. In order to test differences in the release of P, linear mixed effect models were calculated using the R package lme. In this models, fixed effects were P release

(DIP, DOP), treatment (control, D/W), and experiment (the D/W cycles), and random effects were accounted for different sites. In case the data were not normally distributed or variances were not homogeneously distributed among groups, data were ranked. Post-hoc tests were done using the linear hypothesis test as implemented in R package multcomp. The relationship between P release and microbial biomass was analyzed by linear and nonlinear regressions for both beech forest and spruce forest. All statistical analyses were conducted in R environment for statistical computing *(R Core Team,* 2014*).*

2.4 Results

2.4.1 Soil properties

Total P (TP) concentrations in the forest floor and in the mineral soil differed among the three beech sites (Table 2.1). Lowest TP concentrations were observed in the Oi and Oe layers at Bayreuth and in the A horizon at Lüss, the latter coincided with the lowest C and N concentration of all A horizons. By far the largest TP concentrations were observed in samples from Bad Brückenau. TP concentrations of the soil layers were similar in the three spruce sites. MBC was generally lowest in the A-horizons of both tree species with values of < 7 mg g^{-1}. At the beech sites, the largest MBC was observed in the Oi and Oe layers at Bayreuth (31.8 and 38.9 mg g^{-1}, respectively). MBC in the Oi and Oe layers of the spruce sites was 25-50% less than in the corresponding beech layers, but there was no significant difference between the spruce sites. MBC of the Oa layers under spruce was similar to those of the A horizons under spruce although the C content was much higher in Oa layers.

2.4.2 Release of dissolved phosphorus by D/W

In the controls, DIP concentrations in the Oi (beech and spruce) and Oe (spruce) layers slightly increased compared to the initial DIP in soils over the duration of the experiment (Fig. 2.1). After D/W, the DIP concentration was significantly larger than the DIP concentration in the controls in all layers (Fig. 2.1, Table 2.2). The largest increase in DIP occurred in the Oe layers in both beech and spruce forest soils, with average concentrations reaching about 80 mg P kg^{-1} under beech and 110 mg P kg^{-1} under spruce. The net release of DIP from Oe layers amounted to about 50-60 mg kg^{-1} (5% of the TP) and this was similar for both beech and spruce forest soils (Table 2.2). The net release of DIP following D/W were lowest in the A horizons (< 1 mg kg^{-1}) of both beech and spruce forest soils (Table 2.2). Following the first D/W cycle, the DIP concentrations in the Oi layer amounted to 35 mg kg^{-1} in the beech and 15 mg kg^{-1} in the spruce forest soils. The net release of DIP in the Oi layers

was less than in the Oe layers. The response of DIP to D/W in the Oa layer of the spruce sites was also remarkable with average concentrations reaching up to 40 mg P kg^{-1} and a net release of about 21 mg kg^{-1}. Repeating the D/W cycles had no effect on the amount of released DIP (Fig. 2.1).

The DOP concentrations in the controls of all the forest floor layers were in the same order of magnitude as the DIP concentrations (Fig. 2.2). However, the DOP concentrations in the controls of the A horizons (on average between 2.5 and 7.0 mg kg^{-1}) were 5-times higher than the DIP concentrations. With the exception of beech Oi layers, the D/W resulted in larger DOP concentrations than in controls. In case of the spruce Oi horizons the increase was largest (Table 2.2). For the A horizons, the net release of DOP (2.5-6 mg kg^{-1}) after D/W was larger than for DIP (Table 2.2). The second and third D/W cycles did not increase the release of DOP. On the contrary, in some samples (Oi spruce, Oi beech, A spruce) there was a tendency to decreasing DOP concentrations after repeated D/W.

The net release of DIP after the first D/W cycle was not related to the total P content of the samples (data not shown), but was positively correlated to the initial MBC in an asymptotic way for Oe and Oa layers (Fig. 2.3A). No such correlation was observed for Oi layers and A horizons.

The net release of DOP after the first D/W cycle in Oe and Oa layers was also positively related to the initial MBC concentration although not as strongly as the net release of Pi. For Oi layer and A horizon, this relationship was not found (Fig. 2.3B).

2.5 Discussion

Here we found that D/W led to a net release of DIP in the organic layers and in the A horizon of beech and spruce forest soils. The effect of the D/W cycles on the release of DIP differed between organic layers and mineral soil. More specifically, the largest net release of DIP was observed in the Oe layers and the increase in DIP was lowest in the A horizons for both beech and spruce forest soils. So far, the effects of D/W on P release were investigated mostly in mineral soils. *Butterly* et al., (2009) reported an increase in resin extractable P of about 7 mg kg^{-1} after D/W in an arable soil, representing a 40% increase compared to the moist control. In grassland soils, the maximum DIP concentration after 2 h rewetting was 0.14 mg kg^{-1} and the net release of DIP was about 0.1 mg kg^{-1} (*Blackwell* et al., 2009). To our knowledge there is only one study that investigated D/W effects on forest floor samples. In an extreme treatment, forest floor samples (sum of Oi, Oe and Oa layers) were dried at 60°C and after

rewetting the net DIP release increased up to 48-76 mg kg^{-1}, which represented almost 30% of the total P (*Achat* et al., 2012). The release rate observed by Achat et al. (2012) is comparable to our findings for Oe layers, however in our study the net release of DIP represented only 1-5% of the total soil P. The smaller relative release in the present study is most likely due to the lower incubation temperature and a longer desiccation period causing less stress during desiccation in our experiment. The study by *Achat et al.* (2012) also showed that the effect of D/W on DIP release increased with organic matter content of the soil samples, emphasizing the critical role of the organic layer for the effects of D/W cycles on forest soils.

Against our expectation, the response of beech and spruce forest floors to D/W cycles in terms of net DIP release did not differ. The quality of litter and forest floor layers differs in several aspects between deciduous and coniferous species (*Reich* et al., 2005), and the rate of P turnover and the rate of litter decomposition were lower in coniferous forest floors (*Oostra* et al., 2006; *Vesterdal* et al., 2008). In our study, the MBC was larger in beech than in spruce forest floors. The MBC estimated here was in the same range as reported for other forest floors by *Joergensen* and *Scheu* (1999). The microbial biomass is likely the main source of P release after D/W (*Grierson* et al., 1998; *Turner* and *Haygarth*, 2001). Assuming a molar C/P ratio of 74 of the microbial biomass in the forest soils (*Cleveland* and *Liptzin*, 2007), the microbial biomass P pool in Oi and Oe horizons is 289 to 308 mg kg^{-1}. The net release of DIP after D/W can be explained by the lysis of microbial cells as the net release was 3 to 16 % of the microbial P pool. However, despite the larger MBC in beech samples, the release of P after D/W was similar. This might be caused by different microbial communities in the organic layers of beech and spruce forests, but also between the layers of one forest. Yet, it has to be considered that the microbial biomass might be overestimated in this study due to the use of the SIR method, which tends to overestimate the biomass of microbiota in active state (*Martens*, 1995; *Joergensen* and *Scheu*, 1999).

The net release of DIP was related to the MBC in the Oe and in the Oa layers, but not in the Oi layers, although the MBC in the Oi layers was similar to those in the Oe layers. The reason for this discrepancy might be seen in differences between microbial communities inhabiting the Oi and the Oe layer. Under field conditions, the Oi layer is more often subjected to D/W cycles than the Oe layer. This likely affects the structure of the soil microbial communities due to the different levels of tolerance of soil microorganisms to drought stress (*Van Gestel* et al., 1992; *Chen* et al., 2003; *Su* et al., 2004; *Ouyang* and *Li*,

2013). Fungi are more resistant to D/W than bacteria (*Schimel* et al., 1999; *Blackwell* et al., 2010), and it has been reported that the ratio of fungi to bacteria was larger in Oi than in the Oe and Oa layer (*Scheu* and *Parkinson*, 1994; *Fierer* et al., 2003; *Šnajdr* et al., 2008; *Schmitt* et al., 2010; *Baldrian* et al., 2012). Hence, the reason for the difference between Oi and Oe layers might be the higher ratio of fungi-to-bacteria in the Oi layers.

The lower release of DIP after D/W in the Oa than in the Oe layers of the spruce forest soils corresponded to the lower MBC in the Oa layer and further supports the role of MBC as a source of P. The smallest effect of D/W on net DIP release was observed in the A horizons of both the beech and the spruce forest soils. Low rates of P release after D/W in the A horizons are in accordance with *Achat* et al. (2012) and coincide with the low MBC of the A horizons studied here.

In contrast to the A horizons, the release of DIP after D/W from the organic layers exceeded the release of DOP. The lysis of microbial cells by D/W as the main source of P should initiate a flush of DOP rather than of DIP, as P in cells is mostly organically bound (*Turner* and *Haygarth*, 2001; *Turner* et al., 2003; *Butterly* et al., 2009). In fact, we observed a positive relation between DOP net release and MBC, similar to findings of *Turner* and *Haygarth* (2001) for grassland soils. The dominance of DIP over DOP can be explained by the mineralization of released DOP during the 2.5 h extraction procedure, as the mineralization of organic P in soil is very fast and can be substantial at time scales of less than 2 h (*Macklon* et al., 1997; *Fransson* and *Jones*, 2007; *Bünemann* et al., 2013). Thus, it seems likely that the net release of DIP exceeded the net release of DOP due to a high phosphatase activity that converted initially released DOP into DIP.

Repeated D/W cycles did not change the amount of net released P. This might be due to two factors: First, to the adaption of the microbial community to D/W or second, to the fact that the microbial biomass which reacts to D/W by lysis, did not recover until the following D/W cycle. Our results are supported by *Butterly* et al. (2009) who found that microbial biomass P and C strongly decreased after the first D/W cycle but they changed not much after the second and the third D/W cycle. These results indicate that the microbial community changed likely towards a dominance of species more resistant to D/W such as fungi (*Parr* et al., 1981; *Turner* et al., 2003).

Extrapolating the net release of dissolved P observed from our laboratory D/W study to the field scale has to be done with caution. While the degree of drying in the laboratory (up to pF

6) might be reached in forest floors during dry summer periods, the rewetting in the laboratory was fast and complete and thereby differed from field conditions. Such a drastic rewetting posed a maximum osmotic stress on microbial cells with subsequent lysis and P release. Under field conditions, the rewetting of a dry forest floor is not homogeneous because of its hydrophobicity. The resulting preferential flow paths cause only partly rewetting (*Bogner* et al., 2007). Hence, under field conditions, rewetting will likely be less intense and the effect of D/W will be less than in the laboratory. The simple extrapolation of the P net release per kg soil to the field scale, by assuming a typical forest floor stock for beech (*Wunderlich* et al., 2012) and spruce (*Hentschel* et al., 2007, 2009), yields maximum values. Doing so, the release of total P from O and A horizons following a D/W cycle would equal about 2 kg P ha^{-1} (beech) to 3 kg P ha^{-1} (spruce). Most of the release would be due to the O layer. These rates represent a substantial short term change in the P availabile for plants and microorganisms as they equal the annual P flux with litterfall (*Meier* et al., 2005; *Huang* and *Spohn*, 2015) and the annual net P mineralization in forest ecosystems (*Yanai*, 1992; *Bridgham* et al., 1998).

2.6 Conclusions

This study demonstrates that P dynamics in forest floors are sensitive to D/W cycles. The findings emphasize the role of D/W cycles for the short term availability of P in forest soils. The microbial biomass seems to be the main source of P release after D/W. The differences between soil layers are likely due to differences in the total microbial biomass and in the fungi-to-bacteria ratios. There was no general difference in the response to D/W between samples from beech or spruce sites. Future research on effects of D/W cycles should be more field oriented such that realistic release rates can be deduced. Also the recovery times of the reactive source pools following a first D/W cycle should be addressed. Furthermore, the effect of D/W on different microbial communities differing in their fungi-to-bacteria ratio should be studied.

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Table 2. 1. Properties of the samples of the three beech and three spruce forest sites

Site	Layer	Depth	TOC	TN	TP	TOC : TN	TOC : TP	MBC	MBC : TOC	pH_{H2O}	Water content	Humus layer
		[cm]	[g kg^{-1}]					[mg g^{-1}]			[g g^{-1}]	
Beech												
Lüss	Oi	8-5	431	15	1.15	29.3	376	23.0	0.05	6.0	3.2	Mor - Moder
	Oe	5-1.5	432	17	0.86	25.0	501	23.6	0.05	4.4	2.9	
	A	0-5	17	1	0.11	32.4	159	1.6	0.09	4.0	0.2	
Bad Brückenau	Oi	5-3	460	14	1.23	32.8	374	22.8	0.05	5.2	2.6	Mull - Moder
	Oe	3-1	438	21	1.59	20.6	276	28.2	0.06	4.4	2.4	
	A	0-5	144	10	3.45	14.4	42	5.9	0.04	4.4	1.2	
Bayreuth	Oi	5-3.5	480	12	0.56	40.4	856	31.8	0.07	5.5	3.0	Moder
	Oe	3.5-1	502	16	0.70	32.3	717	38.9	0.08	4.9	2.8	
	A	0-5	131	4	0.30	33.2	437	2.8	0.02	3.5	0.4	
Spruce												
Wülferseuth	Oi	4-2.5	498	14	0.88	36.8	569	15.3	0.03	4.6	0.9	Mor
	Oe	2.5-1	425	15	1.12	27.7	380	18.3	0.04	5.8	2.9	
	Oa	1-0	405	17	0.93	23.0	434	8.6	0.02	4.3	2.4	
	A	0-5	74	4	0.57	20.8	131	4.4	0.06	3.4	0.6	
Waldstein	Oi	3-2	486	14	1.04	33.8	469	16.9	0.03	4.8	1.7	Moder
	Oe	2-0.5	515	21	1.20	25.0	429	17.6	0.03	4.6	3.1	
	Oa	0.5-0	391	18	1.03	22.2	382	4.7	0.01	3.7	1.9	
	A	0-5	74	3	0.61	21.8	121	5.5	0.07	3.5	0.6	
Oberwarmensteinach	Oi	4-2.5	499	14	0.83	34.8	603	18.5	0.04	4.0	1.7	Mor
	Oe	2.5-1	519	21	1.08	24.6	479	10.2	0.02	4.1	3.4	
	Oa	1-0	407	17	0.98	23.4	417	7.4	0.02	3.0	2.2	
	A	0-5	133	7	0.64	18.3	208	6.7	0.05	3.4	1.0	

TOC = Total Organic Carbon, TN = Total Nitrogen, TP = Total Phosphorus, MBC = Microbial Biomass Carbon

Table 2. 2. Net release of DIP and DOP after the first D/W cycle[a]

Site	Layer	Net release of DIP [mg kg^{-1}]	Net release of DOP [mg kg^{-1}]
Beech			
Lüss	Oi	10.1*	8.6 ns
	Oe	68.2***	15.0***
	A	-0.1 ns	2.5***
Bad Brückenau	Oi	7.5**	-25.8**
	Oe	7.9*	17.9***
	A	0.2**	-0.2 ns
Bayreuth	Oi	5.8*	13.2**
	Oe	72.6***	22.6***
	A	1.1***	4.8***
Average Beech			
	Oi	7.8***	-1.3 ns
	Oe	49.6***	18.5***
	A	0.4**	2.4**
Spruce			
Wülferseuth	Oi	3.9***	15.3***
	Oe	59.2***	17.3***
	Oa	27.0***	12.3***
	A	1.0***	9.0***
Waldstein	Oi	10.6***	17.6***
	Oe	44.5***	9.7***
	Oa	8.4***	10.0***
	A	0.1**	2.8***
Oberwarmensteinach	Oi	8.8***	29.0***
	Oe	48.3***	10.9***
	Oa	27.9***	16.0***
	A	0.04*	5.4***
Average Spruce			
	Oi	7.8***	20.7***
	Oe	50.7***	12.6***
	Oa	21.1***	12.7***
	A	0.4***	5.7***

[a]no significant difference between control and D/W sample: ns; significant difference between control and D/W at significance level of *$p<0.05$; **$p<0.01$; ***$p<0.001$

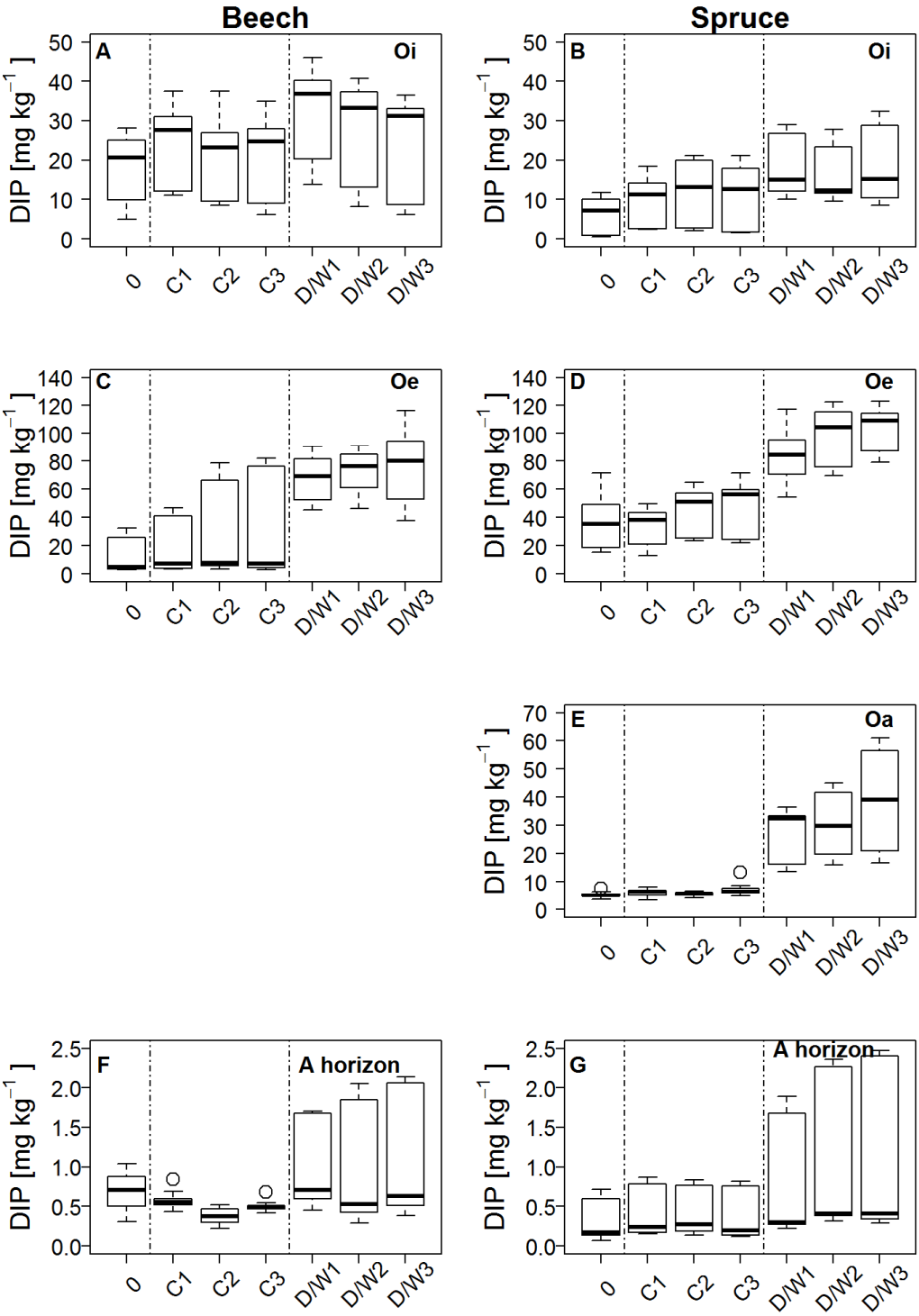

Figure 2. 1. Dissolved inorganic phosphorus (DIP) in Oi, Oe, Oa layers and in A horizons of beech (A, C, F) and spruce forest soils (B, D, E, G): controls (C1-C3) and 3 drying-rewetting cycles (D/W1-D/W3); 0: initial DIP measured after 1 week pre-incubation before starting the first D/W cycle.

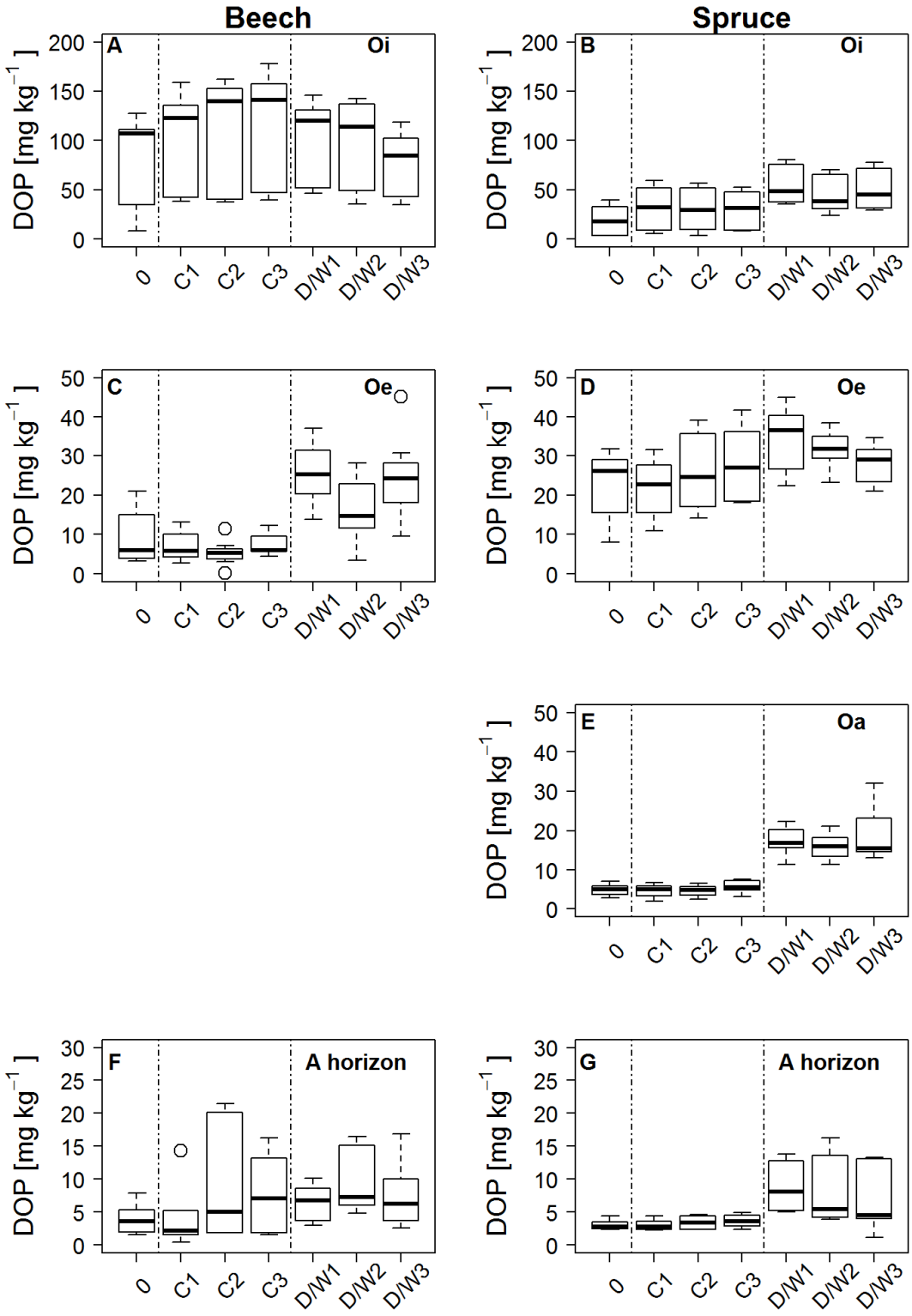

Figure 2. 2. Dissolved organic phosphorus (DOP) in Oi, Oe, Oa layers and in A horizons of beech and spruce forest soils: controls (C1-C3) and 3 drying-rewetting cycles (D/W1-D/W3); 0: initial DOP measured after 1 week pre-incubation before starting the first D/W cycle .

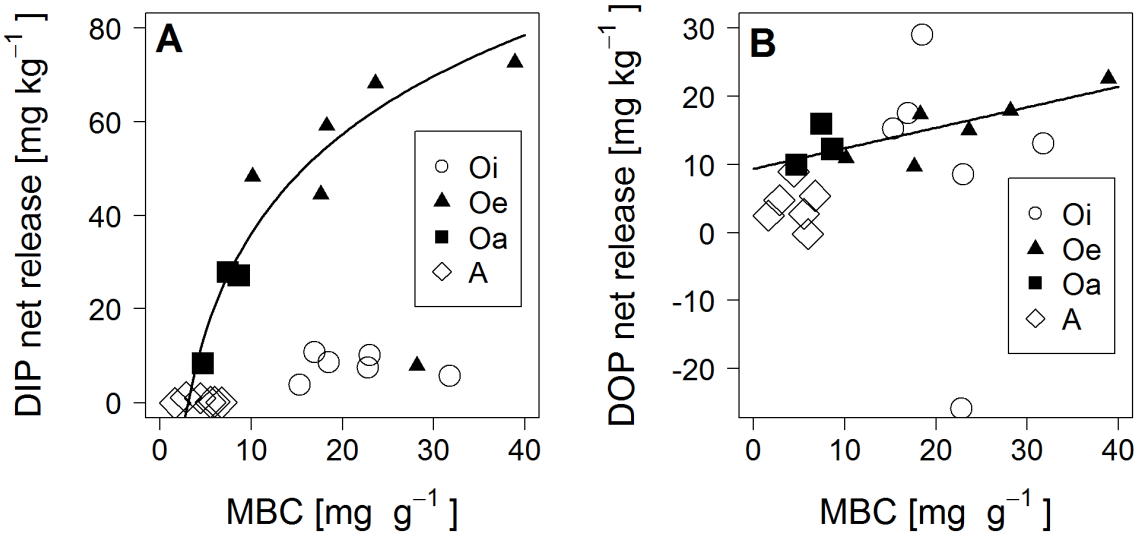

Figure 2. 3. Relation between net release of DIP (A) and DOP (B) after the first cycle to initial microbial biomass carbon (MBC). Each point represents the average net release for one site (n = 3 for Oa and n = 6 for each layer left). DIP net release in Oe and Oa layers ($y = 30.5*\ln(x) - 34$, $r^2 = 0.90$, $p = 0.003$); DOP net release in Oi, Oe and Oa layers ($y = 0.3*x + 9.4$, $r^2 = 0.56$, $p = 0.01$). Oe layer of Bad Brückenau was not included in the regression for DIP in the Figure 2. 3A.

3. Study II: Release of phosphorus from soil bacterial and fungal biomass following drying/rewetting

Mai-Van Dinh[a], Alexander Guhr[a, *], Marie Spohn[b] and Egbert Matzner[a]

[a]Soil Ecology, University of Bayreuth, Bayreuth Center of Ecology and Environmental Research (BayCEER), Dr. Hans-Frisch-Str. 1-3, 95448 Bayreuth, Germany

[b]Soil Biogeochemistry, University of Bayreuth, Bayreuth Center of Ecology and Environmental Research (BayCEER), Dr. Hans-Frisch-Str. 1-3, 95448 Bayreuth, Germany

Keywords: drying–rewetting; dissolved phosphorus; soil microbial biomass; saprotrophic fungi; Gram-positive bacteria; Gram-negative bacteria

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3.1 Abstract

Previous work has shown that the drying/rewetting (D/W) of soils mobilizes phosphorus (P), and that the effect of D/W on P release likely depends on the soil microbial community composition. We tested the hypotheses that (i) P release after D/W from fungi is lower than from bacteria and that (ii) gram-positive bacteria are less susceptible to D/W than gram-negative bacteria. We investigated the release of dissolved organic (DOP) and inorganic phosphorus (DIP) from bacterial and fungal biomass after rewetting of an artificial soil that was desiccated to different degrees. For this purpose, sterilized soil amended with growth medium was inoculated separately with one of two bacterial strains (*Pseudomonas fluorescens,* gram-negative and *Micrococcus luteus,* gram-positive) or with one fungal strain (*Penicillium chrysogenum*). The bacterial strains were grown for 7 days, the fungus for 25 days at 50% soil water holding capacity. After the pre-incubation period, microbial biomass P (Pmic) was determined by chloroform fumigation extraction, and soils were desiccated at 20°C for 5-8 days until pF 6 (-100 MPa) was reached, while the controls were kept permanently at 50% water holding capacity. At different degrees of desiccation, samples were destructively harvested and soils were extracted with water to measure the release of DIP and DOP. The net release of total dissolved P per unit Pmic following D/W was in the order *P. fluorescens >> M. luteus = P. chrysogenum.* In case of *P. fluorescens*, net release started already after desiccation to pF 4 (-1.0 MPa) and increased with further desiccation. For *M. luteus* and *P. chrysogenum*, a tendency for net release was only observed after severe desiccation up to pF 6. Our results suggest that the effect of D/W on P release from microbial biomass depends largely on the microbial community composition, with fungi and gram-positive bacteria being less susceptible to D/W than gram-negative bacteria.

3.2 Introduction

Previous studies have shown that the drying-and-rewetting of soils (in the following called D/W) leads to the lyses of microbial cells, resulting in the release of C, N and P (Blackwell et al., 2010; Fierer et al., 2003; Halverson et al., 2000; Schimel et al., 1999; Turner et al., 2003). However, it is still not well understood how different groups of soil microorganisms respond to D/W of soil.

The change in soil water potential during drying-and-rewetting (D/W) of soils exerts physiological stress and energetic challenges to microbial communities (Kakumanu et al., 2013; Schimel et al., 2007). More than 50% of the microbial biomass may become necromass after soil D/W (Van Gestel et al., 1993; Wu and Brookes, 2005). Desiccation of soil exposes soil microorganisms to low soil water potentials, since water can cross microbial cell membranes. Thus, the cell internal osmotic potential of unicellular organisms without effective protective structures has to be equilibrated with the external soil water potential to maintain cell integrity. The sudden change in the water potential after rewetting of a dry soil requires the release of organic solutes (Halverson et al., 2000; Kieft et al., 1987) or those solutes are released after the lysis of cells (Blackwell et al., 2010; Fierer et al., 2003).

As a consequence, rewetting of dry soil often increased C and N mineralisation (Gordon et al., 2008; Schimel et al., 1999) and led to an increase in soluble P in the soil solution (Achat et al., 2012; Bünemann et al., 2013; Dinh et al., 2016; Turner et al., 2003; Turner and Haygarth, 2001). The impact of D/W on the release of C, N and P in soils increased with decreasing soil water content prior to rewetting (Bünemann et al., 2013; Kakumanu et al., 2013). Several authors proposed that the release of P from microbial biomass is the main cause for the pulse of P after D/W of soils (Turner et al., 2003; Turner and Haygarth, 2001). However, Butterly et al., (2009) suggested a major contribution of abiotic sources.

The effect of D/W on the release of elements in soils seems to be influenced by the soil microbial community composition with fungi often being less sensitive and better adapted to D/W than bacteria (Bapiri et al., 2010; Yuste et al., 2011). One reason for the better adaptation of fungi compared to bacteria at low soil water potentials is seen in their thick cell walls with crosslinked polymers, preventing water losses (Gordon et al., 2008; Holland and Coleman, 1987). Under drought stress, fungal cell walls can be further stabilized by thickening and crosslinking of polymers (Kollár et al., 1997; Sietsma and Wessels, 1981). Fungi also release hydrophobic substances that prevent them from desiccation during drought

(Spohn and Rillig, 2012). Further, filamentous fungi can extent their hyphal networks over long distances and across soil pores to access nutrient and water and counteract water stress (Guhr et al., 2015). With regard to procaryotes, gram-positive bacteria seem to be more resistant to D/W than gram-negative bacteria because of their specific cell wall properties. Gram-positive bacteria have a strong, thick cell wall with interlinked peptidoglycans to reduce water losses, while the cell wall of gram-negative bacteria consist of a single layer and an outer membrane (Madigan, 2012).

Experimental evidence for the better adaptation of soil fungi than of bacteria to D/W is not fully consistent. Barnard et al. (2013) and Cosentino et al. (2006) showed that fungal communities were less affected by D/W than bacterial communities. Kakumanu et al. (2013) also found that the fungi-to-bacteria ratio increased with drying of the soil. Gordon et al. (2008) showed that D/W led to a more intensive leaching of nutrients from grassland soils with a low fungal abundance. However, some studies observed that the ratio of fungi-to-bacteria remained unchanged after D/W stress (Hamer et al., 2007; Schmitt et al., 2010). Bapiri et al. (2010) investigated the effect of repeated D/W on bacterial and fungal growth based on leucine and ergosterol incorporation and found that D/W decreased bacterial growth while fungal growth remained unaffected. While this method very elegantly allows to distinguish bacterial and fungal growth is does not allow to determine differences between gram-negative and gram-positive bacteria.

In a previous study, we showed that the release of P following D/W differed between the layers of the forest floor, which might be due to different microbial communities inhabiting the different layers (Dinh et al., 2016). Hence, here we investigated the release of P after D/W from a fungus, a gram-positive and a gram-negative bacterium. We hypothesized that following D/W (1) the release of phosphorus from bacterial biomass is larger than from fungal biomass, (2) that the release of phosphorus from gram-positive bacteria is lower than from gram-negative bacteria, and (3) that the release of P from the microbial biomass increases with drought stress prior to rewetting. To test these hypotheses, we conducted a laboratory experiment using an artificial soil inoculated separately with different species.

3.3 Materials and Methods

3.3.1 Experimental setup

The experimental unit of this experiment was a petri dish with artificial soil amended with growth medium, both steam-sterilized, and inoculated with one out of three different soil microorganisms. The artificial soil used in this experiment was a 3:1 mixture of sand (Dorsilit Nr. 9, particle size: 0.1-0.5 mm, 97% SiO_2, Dorfner GmbH & Co. KG, Hirschau, Germany) and silt (Sikron SF300, particle size: 2-64 µm, 98% SiO_2, Quarzwerke GmbH, Frechen, Germany) from pure quartz which was cleaned by rinsing with deionized water. The bulk density of the mixture was 1.06 g cm^{-3}. 360 g of the artificial soil was arranged in a 1 cm layer in petri-dishes with 200 mm diameter. The P content of the artificial soil measured in Bray-1 extracts (0.025 M HCl + 0.03 M NH_4F) was less than the detection limit of ICP-OES (< 2 mg P kg^{-1} soil). The soil was inoculated separately with the bacteria (*Pseudomonas fluorescens* MIGULA *(*gram-negative*,* DSMZ-No.: 4358) or *Micrococcus luteus* (Schroeter) COHN (gram-positive*,* DSMZ-No.: 20030)) or the fungus (*Penicillium chrysogenum* Thom*)*. The species were chosen since they are well studied and commonly found in soils. Further, they are fast growing and can be considered as r-strategists. Hence, they likely have a high RNA and consequently cellular P content since both are strongly linked to the growth rate and the growth strategy (Keiblinger et al., 2010; Sterner and Elser, 2002). In addition, *P. fluorescens* can be considered as a phosphate-accumulating organism under conditions of excessive available P (Sidat, M et al., 1999). Bacterial and fungal cultures were kindly provided by the Department of Ecological Microbiology and the Department of Mycology, respectively, University of Bayreuth. 1 ml of a liquid pre-culture was mixed with 43 ml of a malt extract glucose meat extract peptone liquid medium (MGMPB: 0.3% malt extract, 0.3% meat extract, 0.5% peptone and 1% glucose, w/v). The amount of medium was chosen to reach 50% of the maximum water holding capacity of the soil. The addition of nutrients with the MGMPB solution to the artificial soil amounted to (in mg kg $soil^{-1}$): 3.1 dissolved inorganic P (DIP), 5.3 dissolved organic P (DOP), 1.070 organic carbon, and 111 total N (with 108 organic N). The pre-incubation and the experiment were conducted in a climate chamber at 20°C. All treatments and controls were set up with 4 replicates. In total, 40 petri dishes were established for each bacterium, and 64 for the fungus.

After, inoculation petri-dishes were closed and incubated for 7 days (bacteria) and for 25 days (fungus) at 20°C to allow growth. At the end of the pre-incubation period, the

desiccation experiment was started by opening the petri-dishes of the D/W treatment to allow the soil to dry. The control petri-dishes were kept close during the whole experiment. Soil water potentials were measured daily by a dew point potentiometer (WP4C, Decagon Devices Inc., Pullman WA, U.S.A.). The experiment lasted until a water potential of about -100 MPa (pF 6) was reached. At each day, 4 petri dishes and 4 controls for each of the three microorganisms were destructively harvested. A subsample of 6 g per petri dish was extracted in deionized water (rewetting event) in a soil: water ratio of 1:10 by shaking the soil for 140 minutes on a horizontal shaker in order to determine DOP and DIP.

3.3.2 Determination of microbial biomass P and ergosterol

At day 1 of the desiccation, microbial biomass P (Pmic) was measured by chloroform fumigation-extraction (Brookes et al., 1982; Vance et al., 1987). After fumigation, soils were extracted in Bray-1 solution (0.025 M HCl + 0.03 M NH_4F) with a soil: solution ratio of 1:10 (Aponte et al., 2010; Bray and Kurtz, 1945; Heuck et al., 2015). Total P in the Bray-1 extracts was measured by ICP-OES (Jobin-Yvon Horiba Group, JY2000, Varian Inc, Palo Alto, California, U.S.A.). Pmic was calculated as the difference of inorganic P in the fumigated and non-fumigated soil extracts, using a conversion factor of 2.5 (Brookes et al., 1982; Jenkinson, 2004).

Ergosterol was measured as an indicator of fungal biomass as well as a quality control in petri dishes with bacterial inoculation at day 1 of the desiccation. For this purpose, soils from 4 petri-dishes per species were extracted in ethanol following the method of (Djajakirana et al., 1996). Ergosterol was quantified using high performance liquid chromatography (HPLC, System Gold 125 Solvent Module, Beckman Coulter, Brea, U.S.A., column: MZ Spherisorb ODS-2 C18, 150x3mm, MZ Analysetechnik, Germany) and detected using an UV-detector at a wavelength of 282 nm (System Gold 166 UV-Detector, Beckman Coulter, Brea, U.S.A.).

3.3.3 Analytical methods

For total dissolved P (TDP) and DIP analysis, samples were filtered through a cellulose membrane acetate filter (0.45µm, Sartorius AG, Göttingen, Germany). The molybdate-ascorbic acid method (Murphy and Riley, 1962) was used to measure DIP. TDP was determined by ICP-OES (Jobin-Yvon Horiba Group, JY2000, Varian Inc, Palo Alto, California, U.S.A.). Dissolved organic phosphorus (DOP) was calculated by the difference between TDP and DIP.

3.3.4 Calculation and statistic

The net release of DIP, DOP and TDP was calculated as the difference of DIP, DOP and TDP between the samples subjected to D/W and the controls.

In order to test differences between species and pF values with respect to P release, analysis of variance (ANOVA) followed by Tukey-HSD test as post-hoc test, were used. All statistical analyses were conducted in R environment for statistical computing (R Core Team, 2014).

3.4 Results

The ergosterol content at day 1 of desiccation was close to zero in the artificial soil inoculated with the bacterial strains (Table 3.1), and was substantially higher in the soil inoculated with the fungus *P. chrysogenum* than in the soil inoculated with the two bacterial strains, indicating that the bacterial cultures were not contaminated with fungi The Pmic content was lowest in the soil inoculated with *P. fluorescens* (1.5 mg kg^{-1} soil) and about twice as high in the soil with *M. luteus* and *P. chrysogenum* (Table 3.1).

DIP in the water extracts of the controls of both bacterial incubations did not exceed 0.2 mg kg^{-1} throughout the desiccation (Fig 3.1), which was much less than the amount of DIP applied with the nutrient solution. The release of DIP following the D/W treatment was largest for *P. fluorescens* (Fig 3.1), it exceeded the controls already at pF 3.9 and continued to increase at pF 5.5 and pF 6.2. In contrast, DIP release after D/W was in no case observed for *M. luteus*, even after the most severe desiccation to pF 6.2. There was no effect of the D/W treatment on the release of DIP from *P. chrysogenum* throughout the desiccation, either. The amount of DIP extracted from the *P. chrysogenum* incubations was generally much larger (4-5 mg kg^{-1}) than from the two bacterial incubations and decreased with time of incubation in controls and treatments.

The amount of DOP extracted was less than the amount of DOP applied with the nutrient solution in all controls (Fig 3.2). D/W lead to the release of DOP in both bacterial incubations at pF > 3.9, while for the *P. chrysogenum* incubation a D/W effect on DOP was only observed at pF 6.1 with low statistical significance ($p < 0.1$). While there was no temporal trend in extractable DOP in controls of the bacterial incubations, DOP increased over time in controls and treatments of *P. chrysogenum*.

Net release of total dissolved P (TDP) per unit Pmic was much larger in soil incubated with *P. fluoresces* than in soil incubated with *M. luteus* (Fig 3.3). Net release of TDP per unit Pmic

from the soils incubated with *P. fluorescens* started at pF 3.9 and increased with the degree of desiccation prior to rewetting. In soils incubated with *P. fluorescens*, the average ratio of TDP net release/Pmic ranged from 0.4 to 1.2 mg mg^{-1}. The ratio of net release of TDP/Pmic in the *P. chrysogenum* incubation was about 0.25 mg mg^{-1} after desiccation to pF 6.2 and was similar to the ratio in the *M. luteus* incubation.

3.5 Discussion

3.5.1 Experimental conditions

We used an artificial and sterilized soil with low P content and low P sorption capacity to study P release from soil microbial biomass after D/W. Other abiotic sources of P after D/W, as proposed by Butterly et al. (2009), can thus be mostly neglected in our study.

The amount of Pmic formed during the pre-incubation in the artificial soil (1.5 to 5.0 mg kg^{-1}, Table 3.1) was rather low as compared to natural soils, which has a Pmic content of 12-217 mg kg^{-1} (Xu et al., 2013).

The intensity of drying influences the effect of D/W on the release of P (Bünemann et al., 2013). We desiccated the artificial soil from pF 3 to pF 6 in 6 days at 20°C which is considered as a moderate stress. Higher soil temperatures and faster desiccation in natural soils might pose a stronger stress on soil microorganisms. In other laboratory studies, the desiccation regime was more harsh and samples were dried at 60ºC in 2 days (Achat et al., 2012) or at 110ºC in 3 days (Butterly et al., 2011).

While the desiccation regime in our experiment was moderate, the rewetting was very intense. The abrupt change in soil water potential poses a strong stress to soil microorganisms (Schimel et al., 2007), which under natural soil and climatic conditions is only seldom reached. Under natural conditions, the rewetting of soil often proceeds more slowly due to hydrophobicity of surfaces and the formation of preferential flow paths (Borken and Matzner, 2009). Thus, the observed amplitude of effects in the laboratory cannot easily be transferred to natural soils.

3.5.2 Organismic response to D/W

Our results support the hypothesis that gram-negative bacteria in soils are more sensitive to D/W than gram-positive bacteria and fungi. The amount of P released after D/W was in the order gram-negative bacterium >> gram-positive bacterium = fungus. In case of the gram-negative bacterium, P net release occurred already after desiccation to pF 4, while only a

tendency for a net release was found after the most severe desiccation (pF 6) for the gram-positive bacterium and the fungus. Our finding of a gram-negative bacterium being more sensitive to D/W is in accordance with other studies on soil bacteria. Gram-positive bacteria released less of their cellular solutes after a change in the water potential than gram-negative bacteria (Halverson et al., 2000). Orwin et al. (2016) showed that the ratio of gram-positive-bacteria-to-gram-negative bacteria was negatively correlated to the water soluble P content after D/W.

In the *P. fluorescens* incubation the ratio of net release TDP/Pmic ranged from 0.4 to 1.2. Ratios of > 1.0 are questionable as abiotic sources of P caused by D/W should be negligible in the artificial soil. Ratios > 1.0 might be due to the relatively large variations and due to the calculation of net release and Pmic as differences between controls and treatments resulting in uncertainties. In addition, we used the conversion factor of 2.5 for quantifying Pmic by the chloroform fumigation method. This factor is most often used for soils (Brookes et al., 1982; Jenkinson, 2004), but the validity for the specific organisms and conditions in our experiment is open (Yevdokimov et al., 2016).

Most of the P was released in the form of DIP in case of *P. fluorescens*, while DOP was the dominant form of P released after D/W in soil incubated with *P. chrysogenum* and *M. luteus*. The lysis of microbial cells should initiate a flush of DOP rather than of DIP, as organically bound P is the dominant form of P in microbial cells (Bünemann et al., 2011). Released DOP might be mineralized to DIP by phosphatases during extraction and sample storage as the mineralization of DOP can be substantial at time scales of less than 2 h (Bünemann et al., 2013). In addition, *Pseudomonas* species have been reported to accumulate inorganic polyphosphates under stress conditions and excessive P availability (Nikel et al., 2013; Sidat et al., 1999). Taking into account the potential enzymatic hydrolysis of polyphosphates (Dick and Tabatabai, 1986) during extraction, this might partly explain the higher DIP release in case of *P. fluorescens*.

3.5.3 Effect of desiccation intensity prior to rewetting

DIP release from *P. fluorescens* became significant after desiccation to pF 4 (gravimetric water content (GWC) of the artificial soil: 2-4%) and further increased to pF 6 (GWC: < 1 %). In contrast, P release from *M. luteus* and *P. chrysogenum* was only detectable at pF 6. To our knowledge, there is only one study that investigated the release of P after D/W from soils as affected by different degrees of desiccation. Bünemann et al. (2013) showed an increase of

P release after desiccation to a GWC of 10% or lower in experiments with natural soils, and the P release was maximum at GWC of 2-5% prior to rewetting. While pF values were not given in the study of Bünemann et al., (2013), our finding of pF 4 as a critical degree of desiccation for P release from a gram-negative bacterium seems to be in accordance with their results. Regarding the release of C after D/W, Kakumanu et al. (2013) reported an increase of soluble C compounds after D/W of natural soils only after desiccation to pF 5.6, but no data on P were given in their study.

3.5.4 Relevance of the findings for D/W effects in soils

The stock of Pmic in our artificial soil was rather low in relation to natural soil and the release of TDP after D/W in natural soils might be generally larger than in our experiment (Dinh et al., 2016; Turner and Haygarth, 2001).

D/W effects on P release were most pronounced for the gram-negative bacterium. Hence, the strongest effect of D/W on P release is to be expected in soil compartments with high abundance of gram-negative bacteria. Smaller effects of D/W are expected in soil compartments with microbial communities dominated by fungi and gram-positive bacteria. Gram-negative bacteria tend to have the highest abundance in organic layers and upper mineral soils representing 15-39 % of the total bacterial community (Fierer et al., 2003; Hamer et al., 2007; Potthoff et al., 2006). Those soil layers are frequently subjected to D/W cycles. Gram-negative bacteria in soils seem to be more dependent on the availability of labile organic matter like fresh litter or root exudates, and their proportion often decreases with depth in relation to gram-positive bacteria (Fierer et al., 2003; Potthoff et al., 2006).

While this study was limited to a single species per group, it is likely that the results hold true for the majority of species belonging to these groups since they have similar cell morphology. In addition, the studied species are very common in soils and the corresponding genera often belong to the dominant taxa in soils (e.g., Aneja et al., 2006; Janssen, 2006).

3.6 Conclusions

This study showed that different groups of soil microorganisms vary in their reaction to D/W and P release, with a gram-negative bacterium being most sensitive. Furthermore, the water potential prior to rewetting strongly modifies the P release. While for the gram-negative bacterium P release was already observed after desiccation to pF 4, only a tendency for P release from the fungus and the gram-positive bacterium was observed after desiccation to pF 6. Hence, gram-negative bacteria seem to represent the main microbial source of the P pulse in soils after D/W and can be an important source for the release of soluble P in soils. The effect of D/W on the release of soluble P from microorganisms in soils will be most pronounced in soil horizons or compartments dominated by gram-negative bacteria.

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Table 3. 1. Ergosterol content and microbial biomass phosphorus (Pmic) in the artificial soil at the beginning of desiccation (Mean ± SEM; n=4)

Inoculation		**Ergosterol [mg kg^{-1}]**	**Pmic [mg kg^{-1}]**
Pseudomonas fluorescens (gram-negative)	D/W	n.d.	1.56 ± 0.12
	Control	n.d.	1.45 ± 0.20
Micrococcus luteus (gram-positive)	D/W	0.16 ± 0.23	3.19 ± 0.50
	Control	0.10 ± 0.12	3.56 ± 0.37
Penicillium chrysogenum	D/W	15.5 ± 2.1	5.03 ± 1.46
	Control	16.2 ± 1.4	2.95 ± 0.26

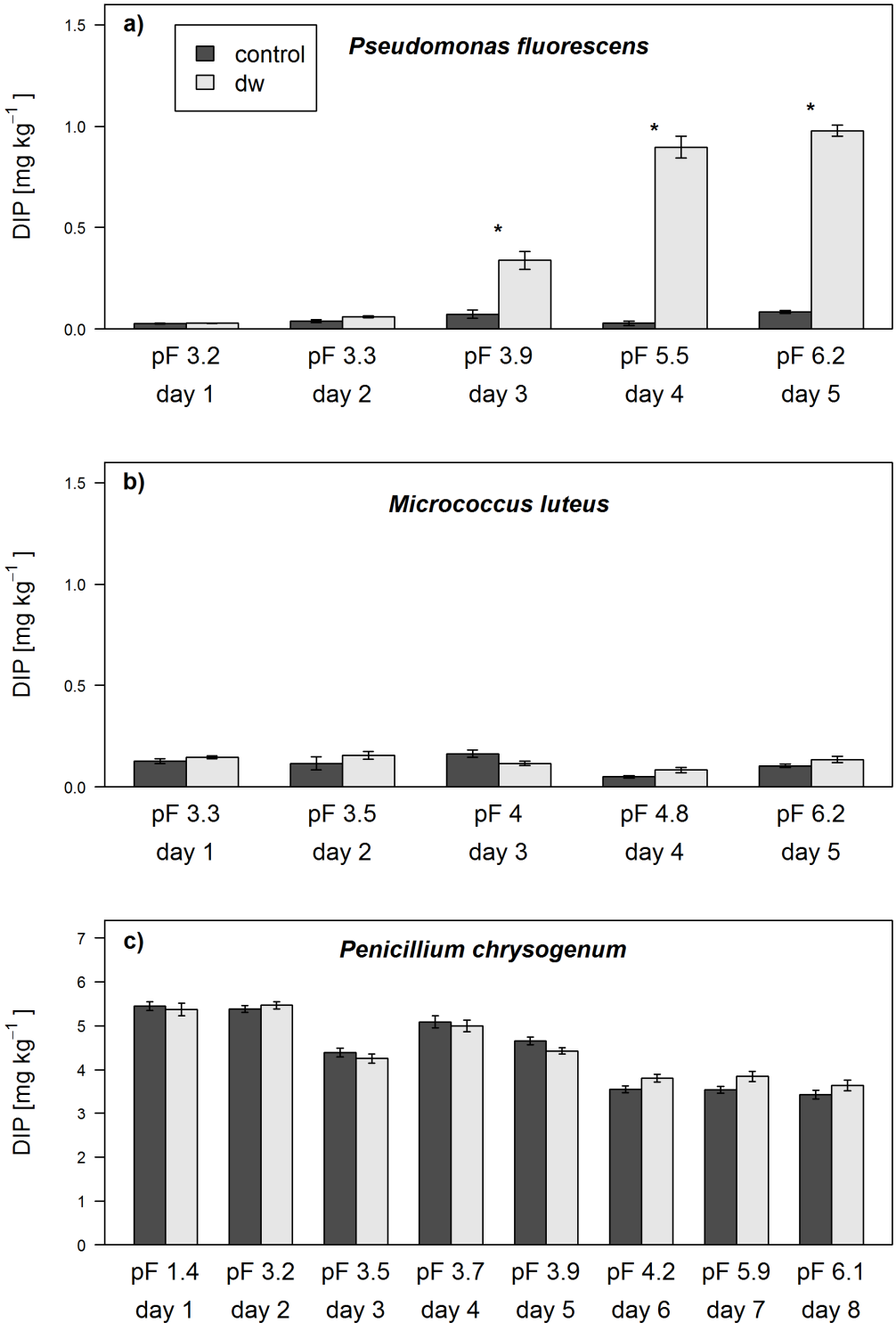

Figure 3. 1. Dissolved inorganic phosphorus (DIP) in water extracts following D/W of the artificial soil inoculated with *Pseudomonas fluorescens* (a), *Micrococcus luteus* (b) and *Penicillium chrysogenum* (c) (Mean ±SEM; n= 4) (*: $p < 0.05$).

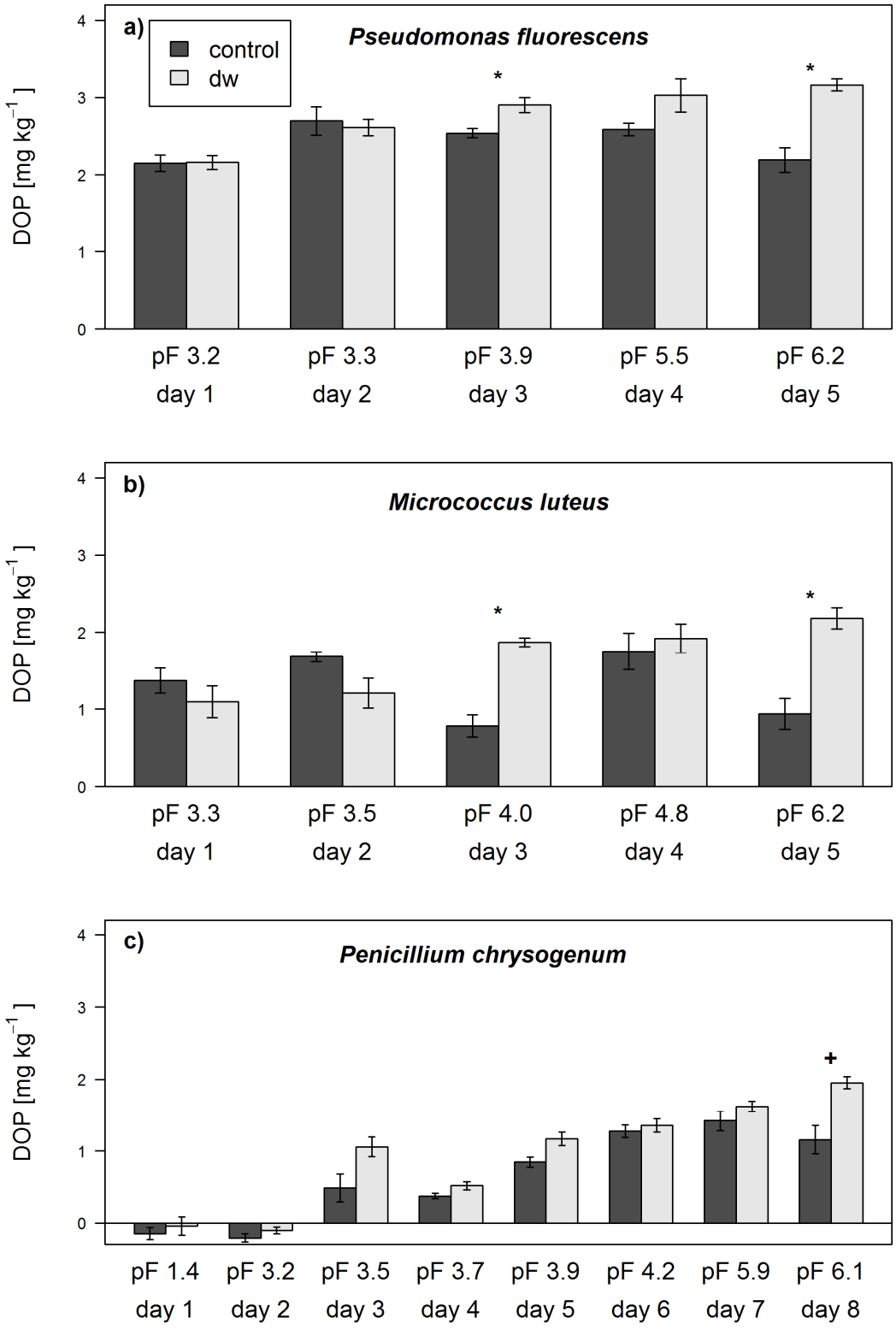

Figure 3. 2. Dissolved organic phosphorus (DOP) in water extracts following D/W of the artificial soil inoculated with *Pseudomonas fluorescens* (a), *Micrococcus luteus* (b) and *Penicillium chrysogenum* (c) (Mean ±SEM; n= 4) (*: $p < 0.05$; +: $p < 0.1$).

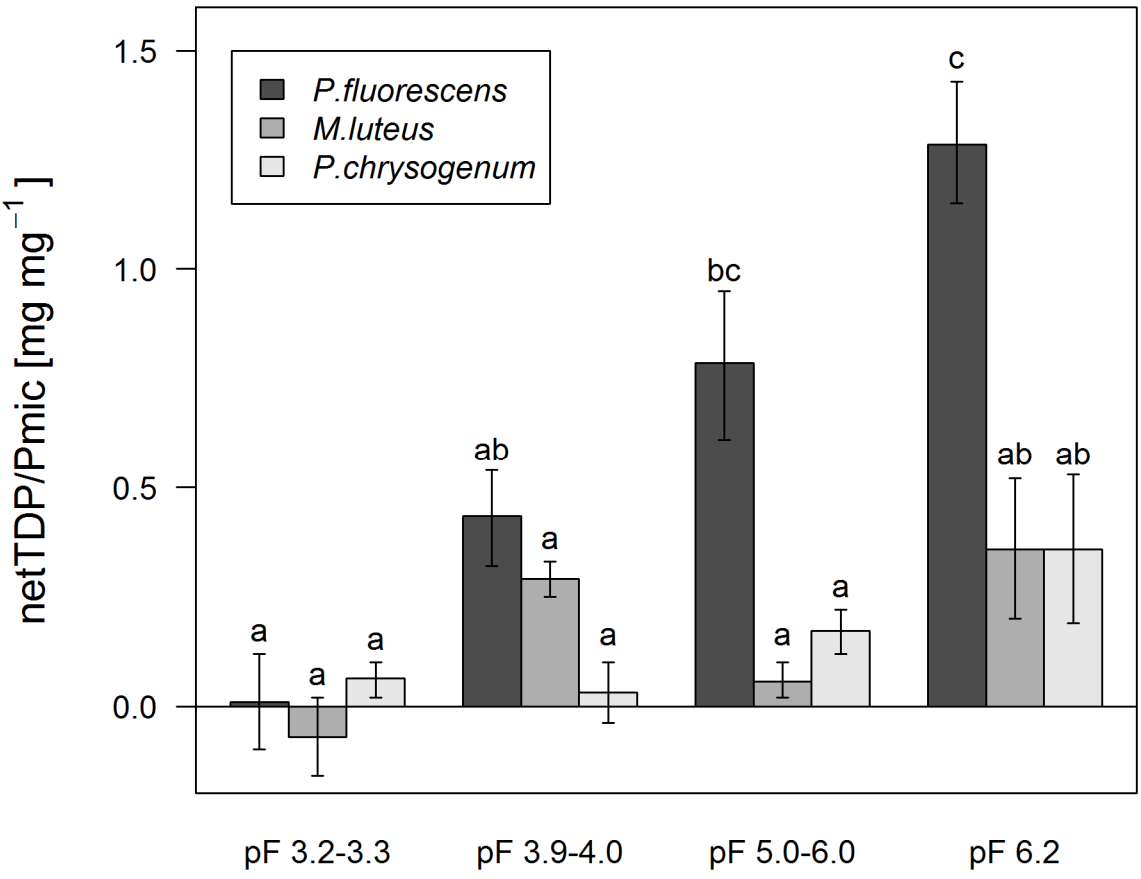

Figure 3. 3. Net release (D/W – controls) of total dissolved P (TDP) after D/W in relation to microbial biomass P (Pmic) for inoculations with *Pseudomonas fluorescens*, *Micrococcus luteus* and *Penicillium chrysogenum* (Mean ±SEM; n= 4). Different letters indicate differences between groups ($p < 0.05$).

4. Study III: Drying and rewetting of forest floors: Dynamics of soluble phosphorus, microbial biomass phosphorus

Mai-Van Dinh, Alexander Guhr and Egbert Matzner

Soil Ecology, University of Bayreuth, Bayreuth Center of Ecology and Environmental Research (BayCEER), Dr. Hans-Frisch-Str. 1-3, 95448 Bayreuth, Germany

Keywords: drying–rewetting / total dissolved phosphorus / inorganic dissolved phosphorus/ soil microbial biomass/ soil microbial community

In preparation

4.1 Abstract

Previous work has shown that drying and rewetting (D/W) of soils leads to an increase of water soluble P. The effect of D/W on the P availability for root uptake depends on the duration of the P release. Hence, our goal was to investigate the kinetics of water soluble P and the microbial P pool (Pmic) after rewetting of desiccated forest floor layers. Samples were taken from Oi and Oe layers of a European beech site and a Norway spruce site. After the pre-incubation period, soils were desiccated up to pF 6 (-100 MPa) at 20°C in the laboratory, while the controls were kept permanently at 50% of water holding capacity. The D/W samples were then rewetted and maintained at 50% WHC throughout. Water soluble P and Pmic was measured at different times after rewetting up to 14 days.

After rewetting, the largest net release of total dissolved P (TDP) was from beech and spruce Oe layers and from spruce Oi, amounting to 40-50 mg P kg^{-1}. The lowest net release of TDP was from beech Oi layers (12 mg P kg^{-1}). Dissolved inorganic P was the dominant fraction of TDP. The TDP concentrations decreased after rewetting strongly in the Oi layers of both tree species within 1 (beech) to 4 (spruce) days, while the TDP concentrations kept rather stable in both Oe layers for 14 days. The release of TDP and its dynamic was linked to the decrease after desiccation and recovery after rewetting of Pmic. The decline of Pmic after D/W and its recovery differed between Oi and Oe layers and tree species, suggesting the influence of different soil microbial communities.

Our results suggest that D/W of forest floors significantly increases the plant available P thereby influencing the ecosystem P cycle.

4.2 Introduction

Drying and rewetting (D/W) of soils represents a stress to soil microorganisms (Blackwell et al., 2010; Fierer and Schimel, 2002). Often the rewetting of desiccated soil causes a pulse of soluble N and P and an increase in soil respiration (Borken and Matzner, 2009; Bünemann et al., 2013; Butterly et al., 2009; Dinh et al., 2016; Gordon et al., 2008; Turner and Haygarth, 2001). The release of soluble N and P after rewetting is often attributed to the lysis of soil microorganisms, their biomass representing a significant pool of organic N and P in soils (Achat et al., 2012; Bünemann et al., 2013; Dinh et al., 2016; Turner and Haygarth, 2001). The microbial biomass pools of C, N and P may be substantially reduced by D/W up to 50% (Chen et al., 2016; Gordon et al., 2008; Mondini et al., 2002; Wu and Brookes, 2005; Yevdokimov et al., 2016). The release of soluble N and P in turn of D/W can be substantial and may be essential for plants and surviving microorganisms especially at nutrient limited sites (Appel, 1998; Dinh et al., 2016).

The effect of D/W cycles and the related release of solute nutrients on other processes, like plant uptake or leaching, will depend on the development of their concentrations following the rewetting. Investigations on the dynamics of soluble N and P released after D/W are sparse and absent for forest floors being hotspots of microbial activity in forest soils. In experiments with mineral soils, highest concentrations of dissolved P were observed 2 hours after rewetting and concentrations decreased by about 50% after 50 hours (Blackwell et al., 2009). In contrast, Butterly et al., (2011) showed that the dissolved P after rewetting of a mineral soil remained constant for 50 hours.

The immobilization of N and P into the recovering microbial biomass after rewetting might be an important sink for the soluble nutrients released by D/W. Intensive microbial immobilization of dissolved P into the microbial biomass (Pmic) within 12 h after rewetting was reported in a ^{33}P labelling experiment with mineral soils (Yevdokimov et al., 2016). Several studies reported a recovery of the microbial biomass within 1 to 9 days after rewetting of desiccated mineral soils (Chen et al., 2016; Gordon et al., 2008; Hamer et al., 2007; Wu and Brookes, 2005). In contrast, Yevdokimov et al., (2016) found no recovery of the Pmic to control levels within 12 h after rewetting.

The effect of D/W on the release of P differed between the layers of the forest floor, with larger release from Oe layers than from Oi layers despite similar microbial biomass in both layers (Dinh et al., 2016). As the different forest floor layers are inhabited by different

microbial communities (Voříšková et al., 2014), this raises the question if the soil microbial community composition influences the effects of D/W cycles. Soil fungal biomass is considered to be more resistant to desiccation than bacterial biomass (Bapiri et al., 2010; Sheik et al., 2011; Yuste et al., 2011). Different groups of soil microorganisms reacted differently to D/W and P release, with fungi and gram-positive bacteria being less sensitive than gram-positive bacteria (Bapiri et al., 2010; Dinh et al., 2017). Vice versa, D/W might also change the microbial community as the ratio of bacteria to fungi was reduced (Kakumanu et al., 2013) and bacterial communities changed (Fierer and Schimel, 2002).

Here we investigated the dynamics of soluble P, the microbial biomass P and the changes in microbial community composition following the drying and rewetting of forest floors. We hypothesized that after rewetting (1) the soluble P will decrease with time due to the recovering soil microbial biomass, (2) the decrease and the recovery of the soil microbial biomass after D/W is different between Oi and Oe layers.

To test these hypotheses, we conducted a laboratory experiment using forest floor samples from a European beech and a Norway spruce forest.

4.3 Materials and Methods

4.3.1 Study sites and sample methods

Samples were collected from a European beech stand near Bayreuth (N 49°58.23', E 11°35.8') and from a Norway spruce site at Waldstein (N 50°8.33', E 11°32.20') in Germany. Samples were collected from Oi (intact needles and leaves) and Oe layers (moderately decomposed needles and leaves) in late autumn. Samples were homogenized by hand, roots and twigs were removed, and the Oi samples were cut into pieces of 1-2 cm. The initial litter properties of beech and spruce forest are presented in table 4. 1.

4.3.2 Experimental design

Moist samples were arranged as a 1 cm layer in petri-dishes with 200 mm diameter. All samples were adjusted to a water content equivalent to 50% of the maximum water holding capacity (WHC) and pre-incubated for 3 weeks in a climate chamber at 20°C in order to allow the microbial activity to adjust. At the end of the incubation period, the D/W experiment was started by opening the petri-dishes to allow the soil to dry. The control petri-dishes were kept closed. Soil water potentials were measured daily by a dew point

potentiometer (WP4C, Decagon Devices Inc. Pullman WA, USA) until a water potential of about -100 MPa (pF 6) was reached. At this point of desiccation, the D/W samples were rewetted immediately to 50% WHC by spraying with deionized water. Following rewetting, the D/W samples were maintained at 50% WHC throughout. The D/W treatment and controls were sampled at 0 h (directly after rewetting), 3 h, 8 h, 1 d, 3 d, 7 d and 14 d after rewetting. At each time point, 4 D/W and 4 control petri dishes were destructively harvested and subsamples of 8 g were extracted in deionized water in a soil: water ratio 1:10 by shaking for 140 minutes on a horizontal shaker.

Microbial biomass phosphorus (Pmic) was measured immediately after rewetting (time 0) and at days 1, 3, 7, and 14 by the chloroform fumigation-extraction method (Brookes et al., 1982; Vance et al., 1987). After fumigation the soils were extracted with Bray-1 solution (0.025 M HCl + 0.03 M NH_4F) with a soil: solution ratio of 1:10 (Aponte et al., 2010; Bray and Kurtz, 1945; Heuck et al., 2015). Pmic was calculated as difference of inorganic P in the fumigated and non-fumigated soil extracts using a conversion factor of 2.5 (Brookes et al., 1982; Jenkinson, 2004). Inorganic P in the solutions was measured spectrophotometrically by using the colorimetric molybdate-ascorbic acid method (Murphy and Riley, 1962).

4.3.3 Analytical methods

Samples from each forest were dried at 60°C to measure total C, total N, total P. Total P was determined after digestion with HNO_3 using an ICP-OES (Jobin-Yvon Horiba Group, JY2000, Varian Inc, Palo Alto, California, USA). Total C and total N were determined by an elemental analyser (Vario MAX, Elementar, Hanau, Germany).

Water extracts were filtered through a cellulose membrane acetate filter (0.45µm, Sartorius AG, Göttingen, Germany) to measure total dissolved P (TDP) and dissolved inorganic P (DIP). DIP was measured spectrophometrically by using the colorimetric molybdate-ascorbic acid method (Murphy and Riley, 1962). TDP was determined by ICP-OES (Jobin-Yvon Horiba Group, JY2000, Varian Inc, Palo Alto, California, USA). Dissolved organic phosphorus (DOP) was calculated as the difference between TDP and DIP.

The net release P (TDP, DIP, DOP) was determined as the difference between the P concentrations in the samples subjected to D/W in the controls.

4.3.4 Statistical analyses

All statistical analyses were conducted in R environment for statistical computing (R Core Team, 2014). The differences between control and D/W were tested by a t-test.

4.4 Results

The concentrations of TDP in the controls were generally lowest in the beech Oi samples and largest in spruce Oe samples (Fig 4.1). The rewetting of the dried soil caused a significant increase of TDP in all samples ($p < 0.05$). The increase of TDP was always largest at time 0 immediately after rewetting. The maximum TDP concentrations after rewetting at time 0 were observed in spruce Oe reaching up to 118 mg TDP kg^{-1}, while minimum concentrations of 20 mg TDP kg^{-1} were observed in beech Oi.

The net release of TDP decreased with time in all samples, but differences between the forest floor layers emerged: In beech and spruce Oi, the net release decreased substantially with time from 12 mg TDP kg^{-1} to near zero following day 1 (beech) and from 55 to 10 mg TDP kg^{-1} in spruce, with a sharp decline at day 4 (Fig 4.2). In contrast, the net release of TDP from Oe layers decreased much less. For beech Oe, the net release of TDP decreased continuously from 50 at time 0 to 30 mg TDP kg^{-1} at day 14. For spruce Oe, the net release of TDP decreased from 47 to about 40 mg TDP kg^{-1}, but the decrease was already observed after 8 h.

In all samples the net release of TDP was mostly in the form of DIP (Fig 4.2). DOP amounted to about 50% of DIP in spruce Oi and Oe and in beech Oi, but only to about 15% of DIP in beech Oe. The time trends of the net release of DIP and DOP were both similar to those observed for TDP. The maximum net release of both compounds was at time 0. Like with TDP, the net release DIP and DOP decreased stronger in Oi than in Oe samples.

In the controls, the amount of P in the microbial biomass (Pmic) was in the range of 400 to 700 mg kg^{-1} in beech Oi, Oe and in spruce Oi, but Pmic was less (around 350 mg kg^{-1}) in spruce Oe (Fig 4.3). After rewetting (time 0), the D/W treatment caused a reduction in Pmic in spruce Oi and Oe. In spruce Oi, Pmic decreased by about 32 mg kg^{-1}, the difference representing 6 % of the controls. In spruce Oe the decrease of Pmic at time 0 was 94 mg kg^{-1}, 28% less than the controls. While Pmic recovered in spruce Oi already at day 1, the reduction of Pmic by D/W in spruce Oe persisted until day 14.

In contrast to spruce, the effect of D/W on Pmic for beech Oi and Oe was not statistically significant. However, for beech Oe there was a tendency for a decrease of Pmic at time 0 by

about 92 mg kg^{-1} the difference representing 16 % of the controls. This tendency was no longer observed at day 1.

4.5 Discussion

Drying and rewetting caused a release of TDP from the forest floors of both tree species. The largest net release of TDP and DIP was from the Oe layer with 50 mg P kg^{-1} and 40 mg P kg^{-1} for beech and spruce, respectively, similar to the effects observed by Dinh et al., (2016) on the same samples. The net TDP release in the present study was more than 30 times higher than the release of TDP after D/W of mineral soils (Butterly et al., 2011). In contrast to Dinh et al., (2016), the net release of DOP from beech forest floors was only 30% of the rates observed in the previous study and DOP generally represented only a smaller part of TDP. These differences in the release dynamics may be attributed to seasonal effects, the sampling previously in late summer and now in late autumn and to the related differences in the beech Oi quality. The C:N ratio of the beech Oi was 40 in the previous study and 54 in this study.

In agreement with Butterly et al., (2009) and Blackwell et al., (2013), DOP release decreased with ongoing incubation after rewetting and DOP concentrations were smaller compared to DIP pools. DOP may be rapidly mineralised during the 2h of rewetting (Bünemann et al., 2013; Macklon et al., 1997) to convert initially released DOP to DIP.

The TDP from forest floors strongly decreased with incubation time after rewetting in the Oi layers of both tree species and was at very low levels after day 1 in beech. For spruce Oi, the strongest decrease was observed after day 4. In contrast, TDP released after rewetting was rather stable in Oe layers and a substantial part of the release was still observed at day 14. In mineral soils, Blackwell et al., (2009) reported a sharp decline of the TDP release already 1 day after rewetting. The continuing TDP net release in the Oe layers for more than 14 days indicate that the D/W might have a significant relevance for the P transport from the forest floor into the mineral soil by leaching and for the uptake of P by plant roots. Extrapolating the TDP net release to the mass of a beech and spruce forest floor under field conditions (data on forest floor stocks from Gerstberger et al., 2004), the TDP net release from Oi + Oe layers amounts from 1.4 kg P ha^{-1} (beech) to 2.0 kg P ha^{-1} (spruce) at time 0 after rewetting and from 0.7 kg P ha^{-1} (beech) to 1 kg P ha^{-1} (spruce) 14 days after rewetting. These release rates may provide a substantial pool of available P, as the total annual P uptake of trees in temperate forest (litterfall + increment) is in the range of 4-7 kg P ha^{-1} a^{-1} (Ilg et al., 2009). Bünemann et al., (2013) reported that plant roots took up about 30% of the P released by

D/W in a grassland soil, emphasizing the relevance of D/W cycles for the P cycling in the soil-plant system. However, as the rewetting in our laboratory experiment was fast and massive, the P net release after rewetting of a forest floor under field conditions might be less due to only partial rewetting and hydrophobicity of surfaces (Bogner et al., 2008). Hence, the P release in the laboratory experiment likely represents the upper limit of D/W effects.

The dynamic of the P pool in the microbial biomass (Pmic) is seen as the driver of the TDP net release after D/W and the subsequent immobilization of TDP by the growing, previously declined microbial biomass. Relating the dynamics of Pmic to those of the TDP net release in forest floors is hampered as the amount of TDP released after D/W was only less than 10% of the Pmic pool. However, the observed decrease of Pmic after drying in spruce Oe (by 90 mg kg^{-1}) and spruce Oi (by 30 mg kg^{-1}) supports the conclusion that Pmic is a major source of the TDP release. No clear change in microbial biomass was seen in the beech Oi layer following D/W which coincided with low TDP net release.

The TDP net release was most stable after rewetting in Oe layers indicating only minor immobilization of P in the microbial biomass. In case of spruce, the Pmic in the D/W samples did not reach the control level after 14 days which coincides to the rather stable TDP concentrations. In case of beech, there was only a tendency of continuing less Pmic in D/W than in controls, but differences were not statistically significant. Overall, the dynamics of Pmic supports the postulated role of microbial biomass for the TDP net release and its temporal development after rewetting.

In our study, the reduction of Pmic by D/W in Oi layers was quite low and more pronounced in Oe layers. The relative reduction of Pmic in Oe layers was similar to studies with grassland or arable mineral soils. Chen et al., (2016) found a decrease of Pmic by 21% after drying, and a full recovery 7 days after rewetting. Nguyen and Marschner, (2005) observed that Pmic had a decrease by 25% after drying followed by a rapid increase after 1 day rewetting. In contrast, in the spruce Oe layer, the differences in Pmic after D/W maintained until day 14.

The proportion of microbial biomass being sensitive to D/W in forest floor Oi layers seems to be less than in Oe layers and in grassland or arable mineral soils. In a previous study, we showed that the net release of TDP after D/W was in the order gram-negative bacteria >> gram-positive bacteria = fungi (Dinh et al., 2017). This suggests that the effect of D/W on Pmic largely depends on the microbial community composition, with fungi and gram-positive bacteria being less susceptible to D/W than gram-negative bacteria. Schmitt and Glaser,

(2011) observed that the ratio of fungi to bacteria was much larger in spruce Oi layer than in Oe + Oa layer. This conclusion is supported by Yevdokimov et al., (2016) who observed a strong reduction of Pmic after D/W in pH neutral Chernozem and Phaeozem soils, but not in an acidic Podzol with microbial biomass likely dominated by fungi. Moreover, a slower recovery of declined Pmic is then to be expected in fungal dominated than in bacterial dominated soils.

4.6 Conclusions

This study demonstrated that D/W increases the pool of water soluble and plant available P in forest floors, as the release of P lasted for at least 14 days. The release of soluble P and its temporal dynamics seem to be matched by the decline and recovery of the P pool in the microbial biomass. Effects of D/W were found specific for Oi and Oe layers and for beech and spruce. This suggests that the degree of decline and recovery of the Pmic pool after D/W is specific for the soil microbial community inhabiting the different layers of the forest floor.

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Table 4. 1. Properties of the forest floor layers

Site	Layer	TOC	TN	TP	TOC : TN	TOC : TP	pH_{H2O}
		[g kg^{-1}]					
Beech							
Bayreuth	Oi	491	9	0.6	54.5	877	7.0
	Oe	493	20	0.7	24.7	704	5.3
Spruce							
Waldstein	Oi	503	17	1.0	29.6	484	5.5
	Oe	493	20	1.2	24.7	411	4.7

TOC = Total Organic Carbon, TN = Total Nitrogen, TP = Total Phosphorus

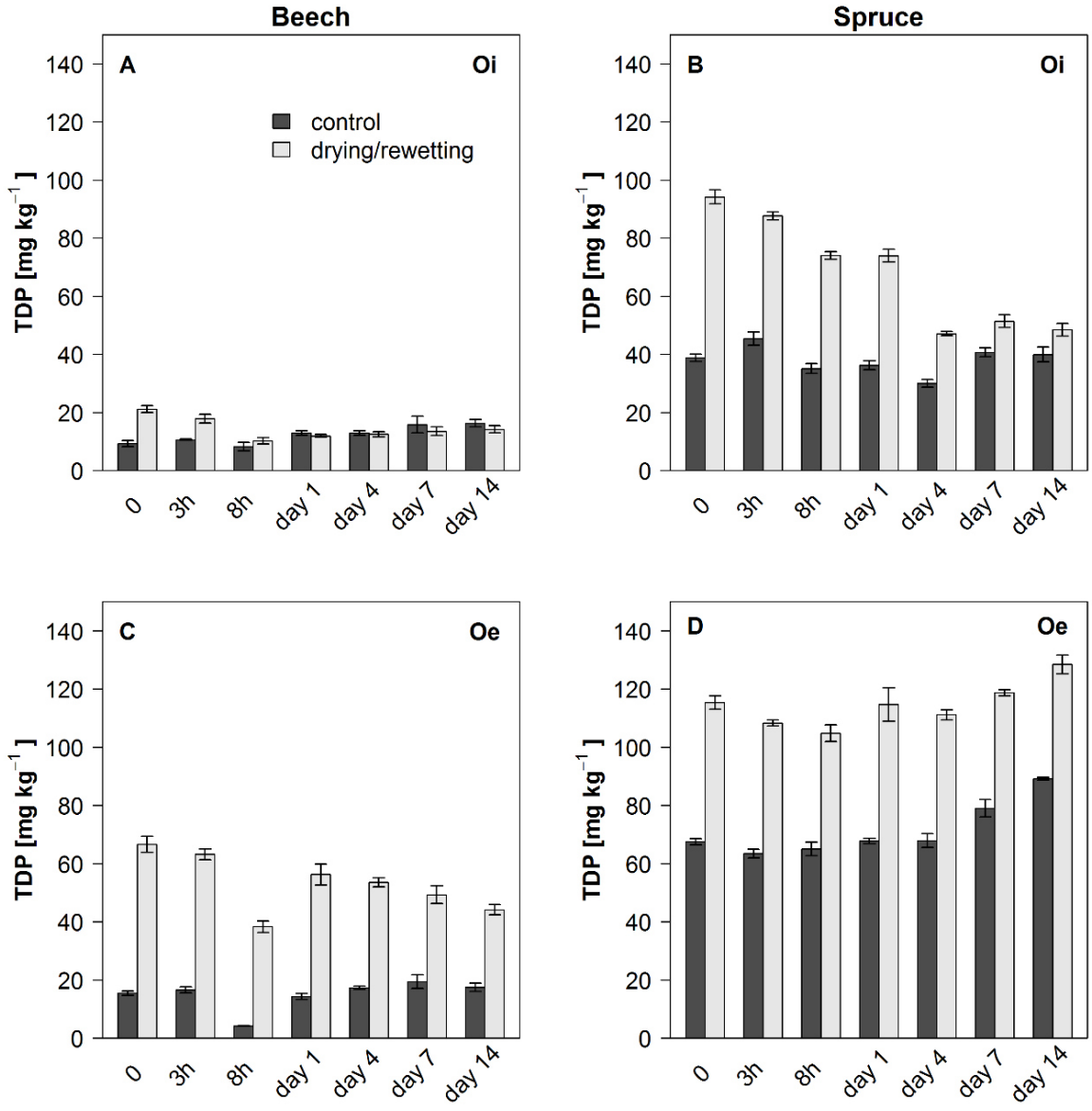

Figure 4. 1. Total dissolved phosphorus (TDP) following drying and rewetting of Oi and Oe layers of beech (A, C) and spruce forest floors (B, D) (Mean ±SEM; n= 4).

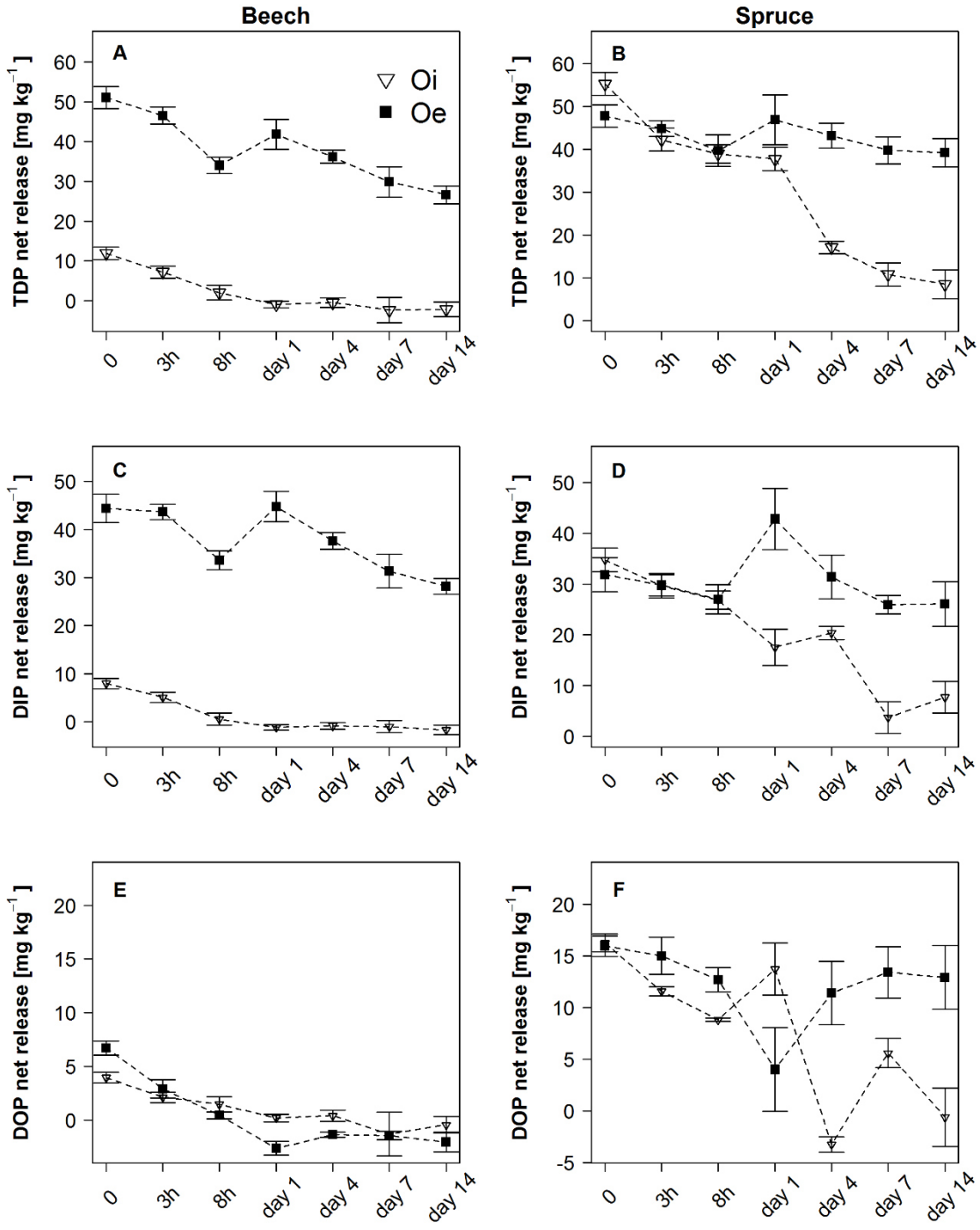

Figure 4. 2. Net release (D/W – controls) of total dissolved P (TDP), dissolved inorganic P (DIP) and dissolved organic P (DOP) after drying and rewetting in Oi and Oe layers of beech (A, C, E) and spruce forest floors (B, D, F) (Mean ±SEM; n= 4).

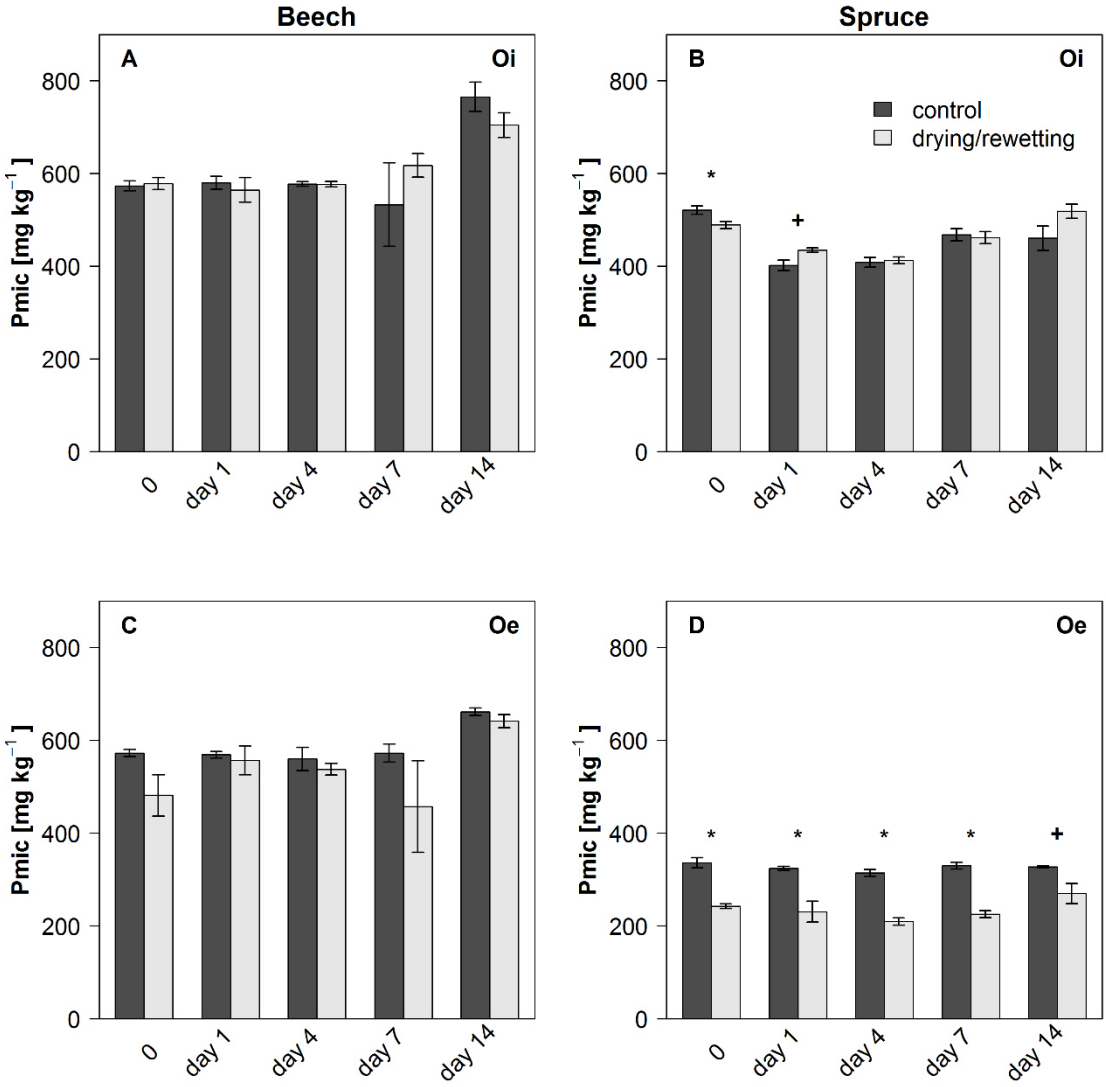

Figure 4. 3. Microbial biomass phosphorus (Pmic) following drying and rewetting of Oi and Oe layers of beech (A, C) and spruce forest floors (B, D) (Mean ±SEM; n= 4, *: $p < 0.05$; +: $p < 0.1$).

5. Appendix

Contributions to the included manuscripts

Table 5. 1. Contributions [%] of each author to the manuscripts.

With respect to:

a: concept and experimental design

b: field and laboratory work

c: data evolution and statistical analysis

d: discussion and interpretation of results

e: manuscript preparation.

manuscript	author	a	b	c	d	e
Drying-rewetting cycles release phosphorus from forest soils	M-V. Dinh	30	70	50	50	40
	T. Schramm	0	30	0	0	0
Published in Journal of Plant Nutrient and Soil Science (2016), 179, 670-678.	M. Spohn	30	0	20	20	20
	E. Matzner	40	0	30	30	40
Release of phosphorus from soil bacterial and fungal biomass following drying/rewetting	M-V. Dinh	30	100	40	50	45
	A. Guhr	15	0	20	15	15
Published in Soil Biology & Biochemistry (2017), 110, 1-7	M. Spohn	15	0	10	10	10
	E. Matzner	40	0	30	25	30
Drying and rewetting of floors: Dynamics of soluble phosphorus, microbial biomass	M-V. Dinh	40	100	50	50	55
	A. Guhr	20	0	20	20	25
In preparation (2017)	E. Matzner	40	0	30	30	20

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Publications

Mai-Van Dinh, Thilo Schramm, Marie Spohn and Egbert Matzner (2016):

Drying–rewetting cycles release phosphorus from forest soils.

Journal of Plant Nutrient and Soil Science 179, 670-678.

DOI: 10.1002/jpln.201500577

Mai-Van Dinh, Alexander Guhr, Marie Spohn and Egbert Matzner (2017):

Release of phosphorus from soil bacterial and fungal biomass following drying/rewetting

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DOI: 10.1016/j.soilbio.2007.02.014

Mai-Van Dinh, Alexander Guhr and Egbert Matzner (2017):

Drying and rewetting of forest floors: Dynamics of soluble phosphorus, microbial biomass phosphorus.

In preparation.

Versicherungen und Erklärungen (Declarations)

(§ 5 Nr. 4 PromO)

Hiermit erkläre ich, dass keine Tatsachen vorliegen, die mich nach den gesetzlichen Bestimmungen über die Führung akademischer Grade zur Führung eines Doktorgades unwürdig erscheinen lassen.

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Hiermit erkläre ich mich damit einverstanden, dass die elektronische Fassung meiner Dissertation unter Wahrung meiner Urheberrechte und des Datenschutzes einer gesonderten Überprüfung hinsichtlich der eigenständigen Anfertigung der Dissertation unterzogen werden kann.

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