Impact of bacterial biomass on soil particle wettability under various moisture conditions

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SUMMARY

Prolonged droughts render the development of soil water repellency (SWR), which in turn impacts the infiltration and distribution of water through the soil profile, exposing soil microorganisms to water stress. Soil microorganisms (particularly necromass) significantly contribute to soil organic matter (SOM), which is believed to be the source of water repellency in soil. SWR-induced water stress is hypothesized to increase bacterial cell surface hydrophobicity (CSH). Interaction of bacterial cells with soil particles will change the wetting properties of the particles, with stronger impact of stressed cells. Furthermore, variations in soil moisture can lead to increased SWR associated with shifts in microbial abundance and community structure. Growth of all the strains investigated was inhibited by both matric and osmotic stress, and in both growth conditions. However, changes in cell surface hydrophobicity (CSH) varied between different stress types and growth conditions. The CSH of *B. subtilis and P. fluorescens* increased with increasing stress level, *R. erythropolis* and *M. pallens* exhibited generally high but constant contact angle (CA) at all stress levels, while *A. chlorophenolicus A6* and *N. aromaticivorans* exhibited rather inconsistent response to growth conditions and type of stress. In none of the experiments, the CA exceeded 110°. This contact angle thus seems to represent an upper limit for CSH. In an association with quartz minerals, *B. subtilis* and *P. fluorescens* rendered the surface of the minerals hydrophobic. The degree of initial hydrophobicity of the CMAs was significantly higher with osmotically stressed cells. However, the high degree of water repellency did not persist, probably due to biomass loss.

The response of the two soils to fluctuations in water content (WC) varied, depending on water content and the initial level of SWR, with no changes observed in CA and community composition of the initially more hydrophobic soil. Changes were observed in moderately hydrophobic soil, particularly under lower WC. The results reported in this dissertation show the significant role of bacterial surface hydrophobicity in the development of SWR. While stress induced CSH has only a short-term impact on water repellency in soil, Prolonged dry conditions lead to a shift towards more adapted microbial community with higher CSH.

Keywords: Soil water repellency, bacterial cell surface hydrophobicity, water stress, community composition

ZUSAMMENFASSUNG

Lang andauernde Trockenheit fördert die Entwicklung von wasserabweisenden Oberflächen von Bodenpartikeln. Eine verringerte Benetzungsfähigkeit des Bodens beeinflusst die Infiltration und die Bewegung des Wassers durch den Boden, damit werden Bodenmikroorganismen Wasserstress ausgesetzt. Mikroben stellen einen großen Anteil der organischen Bodensubstanz (OBS), was die Ursache für die Entwicklung des Wasserabweisungsvermögens in Boden ist.

Es wird angenommen, dass 1) Wasserabweisungsvermögens -induzierter Wasserstress die bakterielle Oberflächenhydrophobie erhöht, 2) die Interaktion der Bodenpartikeln mit bakteriellen Zellen führt zu reduzierten Benetzbarkeit der Mineralpartikeln und dies wird vor allem bei gestressten Zellen ausgeprägt und 3) Außerdem führt die Variationen der Wasserverfügbarkeit in Boden zu einem erhöhtem Wasserabweisungsvermögens, welches durch die Veränderungen der Zusammensetzung mikrobiellen Gemeinschaft hervorgerufen ist.

Das Wachstum aller untersuchten Bakterien wurde sowohl durch osmotischen als auch durch Matrixstress gehemmt. Die Veränderungen der Oberflächenhydrophobie bakterieller Zellen unterschieden sich zwischen verschiedenen Stresstypen und Wachstumsbedingungen. Der Oberflächenhydrophobie von *B. subtilis* und *P. fluorescens* stieg mit zunehmendem Stresslevel an, *R. erythropolis* und *M. pallens* zeigten unabhängig vom Stresslevel allgemein hohe, aber konstante Kontaktwinkel. *A. chlorophenolicus* A6 und *N. aromaticivorans* haben sehr variabel auf Wachstumsbedingungen und Stresstypen des Wasserstress reagiert. In keinem Fall überstieg der Kontaktwinkel der Zelloberflächen 110°. Dieser Kontaktwinkel scheint daher eine Obergrenze darzustellen. Nach der Zugabe zu Quarz machten *B. subtilis* and *P. fluorescens* die Oberfläche der Mineralpartikel hydrophob. Zu Beginn war die Hydrophobie der Zell-Mineral-Assoziationen bei osmotisch gestressten Zellen höher als bei ungestressten Zellen, trotzdem die hohe Wasserabweisung, die durch den Wasserstress induziert worden war, nahm schnell ab, möglicherweise durch Biomasseverlust.

Die Reaktion die zwei untersuchenden Böden hing ab von der Wasserverfügbarkeit, und dem Ausmaß ihres Wasserabweisungsvermögens. Die durch die Trockenheit hervorgerufenen Veränderungen der Zusammensetzung mikrobiellen Gemeinschaft und Wasserabweisung waren klein, jedoch ausgeprägter im Boden mit gemäßigter Wasserabweisung als im hydrophoberen Boden. Indem Oberflächehydrophobie nur einen kurzfristigen Einfluss auf die Benetzungseigenschaften des Bodens hat, längere

Trockenheit kann zu einer Verschiebung hin zu einer angepassten mikrobiellen Gemeinschaft mit höherem Oberflächehydrophobie führen.

Schlagwörter: Wasserabweisungsvermögen, Boden, Oberflächehydrophobie, bakterielle Zellen, mikrobielle Gemeinschaft

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LIST OF ABBREVIATIONS

ANOVA	Analysis of variance
ASV	Amplicon sequence variant
BAME	Bacterial Acid Methyl Esters
CA	Contact angle
CAL	CAL soil from Altmark region in Saxony-Anhalt
CMA	Cell-mineral association
CSH	Cell surface hydrophobicity
Cy	Cyclopropane fatty acid
DNA	Deoxyribonucleic acid
DSMZ	Deutsche Sammlung von Mikroorganismen und Zellkulturen
EA-C-irMS	Elemental Analyser-Combustion-isotope ratio Mass Spectrometry
EPS	Extracellular polymeric substance
FA	Fatty acids
FAME	Fatty Acid Methyl Esther
G-	Gram-negative bacteria
G+	Gram-positive bacteria
GC-MS	Gas Chromatography Mass Spectrometry
IPCCI	Intergovernmental Panel on Climate Change
LUE	Lüss soil from Lüneburg Heath in Lower Saxony
MAP	Mean annual precipitation
OBS	Organische Bodensubstanz
OD	Optical density
OTU	Operational Taxonomic Unit
PC	Principal component
PCoA	Principal Coordinates Analysis
PCR	Polymerase Chain Reaction
PEG	Polyethylene glycol
PERMANOVA	Permutational multivariate analysis of variance
PLFA	Phospholipid fatty acid
RF	Random Forest
SOC	Soil organic carbon
SOM	Soil organic matter
SWR	Soil water repellency
WC	Water content

WDPT	Water drop penetration time
WHC	Water holding capacity
WR	Water repellency

LIST OF SYMBOLS

A_w	water activity
[°C]	temperature in Celsius
C	molar concentration in mols
i	number of ions
[K]	temperature in Kelvin
M	molecular weight (g mol^{-1})
[MPa]	megapascal
p	vapor pressure (Pascals)
R	gas constant (J mol^{-1} K^{-1})
T	temperature
[º]	contact angle (degrees)
μ [h^{-1}]	growth rate (change in optical density per hour)
θ_M	contact angle under matric stress
θ_O	contact angle under osmotic stress
θ_{OS}	contact angle under osmotic stress in surface growth
Ψ	water potential (MPa)
Ψ_M	matric potential (MPa)
Ψ_O	osmotic potential (MPa)
Ψ_{OS}	osmotic potential of solid growth media (MPa)

1 INTRODUCTION

One of the most vital functions of soils in the global ecosystem is the storage and cycling of water and nutrients for plant growth. Thus, soils are crucial for supporting food security and other ecosystem services. Many ecosystem services depend on soil health and soil biota (Wagg et al., 2014). Soil organic carbon (SOC) quality and quantity play a crucial role in soil health (Lal, 2016). Soil is a main C reservoir, holding more C than the atmosphere and vegetation (Singh et al., 2010).

Soil microbes play critical roles in driving soil carbon cycle (Schimel and Schaeffer, 2012), thus, the ability of the soil to provide these functions depends on its properties, controlling microbial activity (Delgado-Baquerizo et al., 2016; Wagg et al., 2014). One of the most important soil properties, governing microbial activity is the soil water content (WC; Stark and Firestone,1995).

Climate change can affect soil functions via changes in precipitation and moisture regime (Cook et al., 2018; Grillakis, 2019). According to IPCC 2014 reports, the rainfall patterns around the world, including Europe, are changing due to climate change.

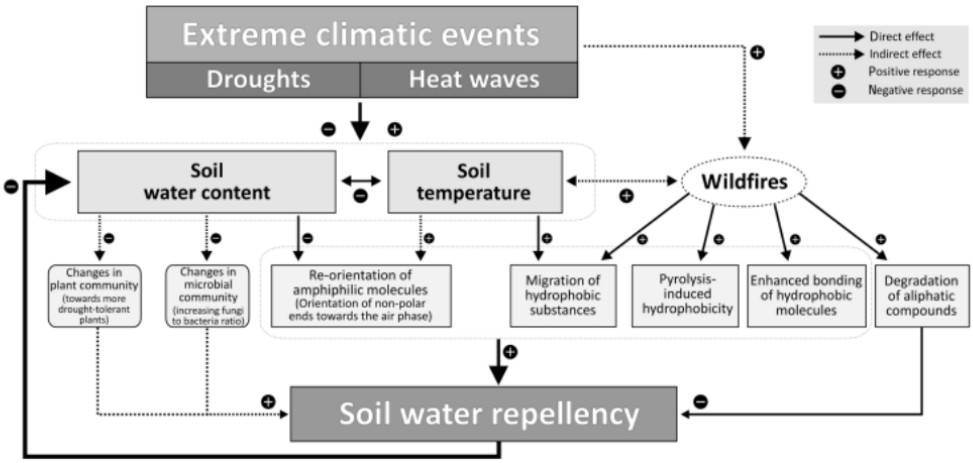

Figure 1 Schematic representation of the role of extreme climatic events in the development of soil water repellency (Goebel et al., 2011)

Changes in precipitation frequency, intensity, as well as temporal distribution will result in increased frequency of droughts during summers and copious rainfalls during winter. As a result, increase in drought affected areas in Europe will be observed (Grillakis, 2019; Samaniego et al., 2018). Reduced and uneven precipitation patterns and

increased temperature will result in reduced soil moisture levels, which plays an essential role in many hydrological, biological and biogeochemical processes in terrestrial ecosystems. Under prolonged periods of drought soils can manifest water repellency (DeBano, 1981; DeBano, 2000).

2 STATE OF THE ART

2.1 Soil water repellency

Soil water repellency (SWR) is a widespread phenomenon. SWR is the reduction in the rate of wetting and retention of water in soil caused by the presence of hydrophobic coatings on soil particles (Hallett, 2007). Naturally occurring water repellent soils have been reported all around the world; in different climatic conditions, under various land covers and a wide range of soil physicochemical properties. Initially being associated with arid and semiarid regions (Doerr et al., 2000), water repellent soils were reported also in temperate and humid regions (DeBano 1981, 2000; DeBano and Krammes, 1966; Jaramillo et al., 2000). Water repellency (WR) is generally observed in sandy soils; however, it was reported in clayey, loamy soils as well (Dekker and Ritsema, 2000).

Water repellent soils are associated with various land covers, such as croplands, pastures and forests (Bachmann et al., 2016; Hall et al., 2010; Šurda et al., 2020).

SWR can hinder infiltration of water into the soil. This in turn will result in preferential flow through the soil profile, thus rendering uneven wetting patterns (Doerr and Ritsema, 2005; Gimbel et al., 2016). Decreased soil water content will cause reduced availability and diffusion of substrates and nutrients, colloidal transport and thus impair the bacterial activity in soil (Papendick and Campbell, 1981). Soil microorganisms are playing a crucial role in C mineralization, nutrient cycling, soil structure stabilization and so on (Delgado-Baquerizo et al., 2016). Thus, understanding the mechanisms of water repellency in soil, and its effects on the soil microbial community is crucial.

It is accepted that SWR is caused by amphiphilic organic compounds coating the surface of soil particles (Doerr et al., 2002; Ellies et al., 2005; Horne and McIntosh, 2000; Leelamanie and Karube, 2007). The SWR occurs as a result of interactions between water molecules and amphiphilic organic coating (Woche et al., 2017). According to (Doerr et al., 2000) in hydrophilic soil those compounds are oriented with their hydrophilic heads towards the water molecule (Figure 2, a). However, as the soil dries the molecules at the mineral surface start to reorient and reorganize (Figure 2, b) and eventually render the mineral surface hydrophobic (Figure 2, c). Those compounds are believed to be mostly of plant origin. While leaf litter (Alanís et al., 2017; Mao et al., 2016) and root exudates (Ahmed et al., 2017; Mao et al., 2014) of plants are believed to be the main source of water repellency, soil microorganisms

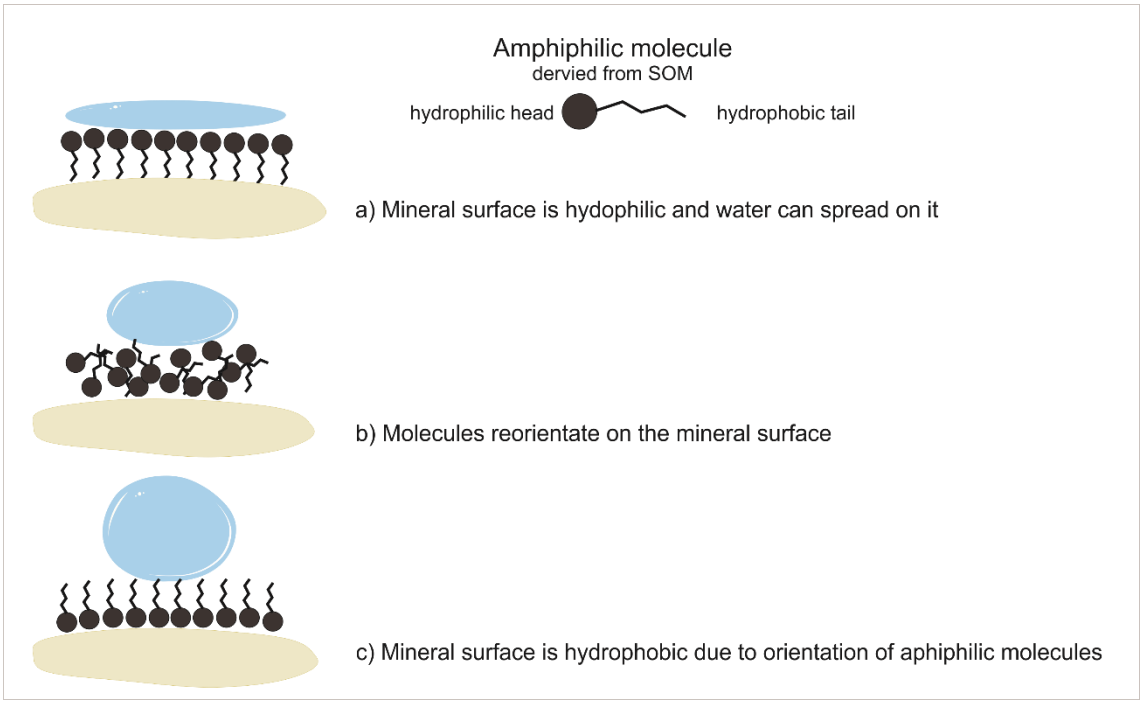

Figure 2 Schematic representation of the orientation of amphiphilic compounds on a a) hydrophilic mineral surface rendering it hydrophobic, b) reorientation due to reduced water availability and c) mineral surface becomes hydrophobic (modified from (Doerr et al., 2000)).

have been also shown to play a role (Achtenhagen et al., 2015; Bond and Harris, 1964; Hallett and Young,1999; Lozano et al., 2014).

Microorganisms can contribute to SWR by releasing hydrophobic compounds (Hallett et al.,2011; Lamparter et al.,2014; White et al.,2000), as a part of soil organic matter (SOM; Kindler et al., 2009; Ludwig et al., 2015; Miltner et al., 2009) or by direct attachment to soil minerals (Achtenhagen et al., 2015). Furthermore, soil aggregate stability, the ability of soil aggregates to withstand external disturbance and regulate the movement and storage of water throughout the soil profile, has been shown to have a positive correlation with SWR (Goebel et al., 2005; Liu et al., 2019). The water repellent soil aggregate surfaces reduce the rate of mineralization of SOM, entrapped in soil aggregates and simultaneously improve the aggregate stability (Goebel et al., 2005) (Leelamanie and Karube, 2014). Studies have shown that soil microorganisms also play an important role in soil aggregate formation and stability (Totsche et al., 2018). Extracellular polymeric substance (EPS) released by bacteria and fungi and hyphal network significantly. contribute to soil particle aggregation and stabilization (Costa et al., 2018; Lehmann et al., 2020; Rillig and Mummey, 2006).

2.2 Contribution of soil bacteria to SOM

SOM is derived from aboveground plant residues, root exudates and microbial exudates (Kögel-Knabner, 2002). The living soil microbial biomass carbon constitutes only 1-2% of total SOC, therefore, the contribution of microbial biomass to SOM was previously neglected. However, in recent years growing number of studies have shown that the contribution of bacteria to SOM was grossly underestimated. Recent data estimate that the microbial necromass can make up to 50% of SOC (Liang et al., 2019). A conceptual model of how bacterial cell envelope fragments stabilize in soil was proposed by (Miltner et al., 2012). The "PATCHY FRAGMENT FORMATION CYCLE'' (Figure 3) suggests that, when facing unfavorable conditions, metabolic activity of soil bacteria will gradually cease, due to reduced substrate availability. With time the cells won't be able to meet the minimal energy and carbon requirements for survival and will eventually die.

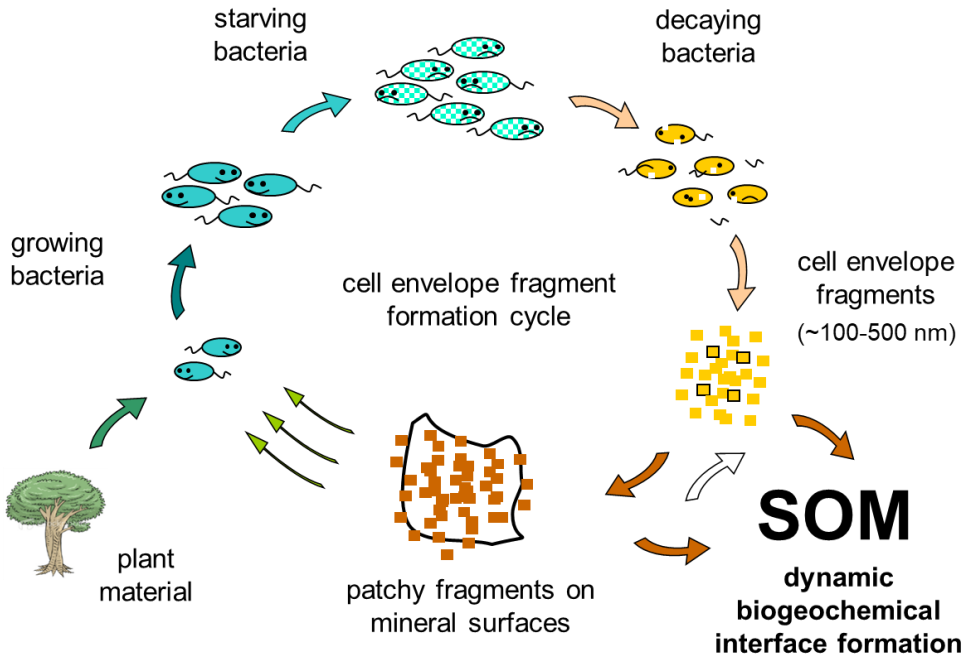

Figure 3 The cycle of patchy fragment formation from microbial necromass (Miltner et al., 2012).

The bacterial biomass will disintegrate, and the fragments will adsorb to soil minerals, stabilize in soil and become a substantial part of SOM. The incorporation and stabilization of microbial cell fragments into SOM is reported in several studies (Kallenbach et al., 2016; Kallenbach et al.,2015; Kindler et al., 2006; Ludwig et al.,

2015; Miltner et al., 2009). Considering the contribution of SOM to SWR formation and contribution of microbes to SOM, the possible direct involvement of bacteria, as a part of SOM or by direct attachment of living cells to soil particles, in the formation of SWR cannot be neglected.

2.3 Impact of SWR on soil bacteria

Soil WC is an important factor controlling microbial activity. Decreased soil water content can result in reduced availability and diffusion of substrates and nutrients, colloidal transport and thus impair the bacterial activity in soil (Csonka and Hanson, 1991; Or et al., 2007; Stark and Firestone, 1995). It is also a determining factor for microbial community structure and diversity (Drenovsky et al., 2004; Treves et al., 2003). SWR reduces water infiltration and affects its distribution in soil profile (Doerr et al., 2000; Goebel et al., 2005; Goebel et al., 2011), exposing soil microorganisms to water stress (Stark and Firestone, 1995). However, the “origin” of the water stress can vary. On one hand, water potential can be reduced due to high concentration of solutes, causing osmotic stress. Alternatively, the low water potential can occur when the soil dries out. In this case the water potential is reduced by the increased surface tension between the water molecules and mineral surface. This results in reduced matric potential, causing matric stress. In saturated non-saline soils water stress is mainly determined by osmotic potential, however, as the soil WC decreases, matric potential becomes the main component of soil total water potential. In order to derive information on the impact of water stress on cell surface hydrophobicity of soil bacteria, the differential in vitro impact of osmotic and matric stress should be studied in close to real conditions. To understand the adaptation mechanism to water stress, most studies assess the bacterial response to osmotic stress caused by permeating solutes used to lower the water potential of the growth medium (Csonka and Hanson, 1991; Hachicho et al., 2017; López et al., 2000; Rojas et al., 2014). However, to reduce the matric potential of the growth media, similar to dry soil, high-molecular-weight polyethylene glycol (PEG) can be used (McAneney et al., 1982; Steuter et al., 1981; McAneney, 1982). Due to the large size, PEG molecules do not penetrate bacterial cell wall, but rather captures the water in their macromolecular structures, making it unavailable for soil microorganisms. In PEG-amended growth medium bacteria are exposed to water stress, similar to matric stress in dry soil. Therefore, in this study “matric” stress/potential will be used to refer to PEG-induced stress in growth media.

2.3.1 Water stress in a single cell

The principal stress response mechanism of bacteria to osmotic and matric stress is similar (Brown, 1990). Bacteria have developed various behavioral and physiological mechanisms to adapt to water stress. To survive water stress bacteria can enter dormancy (Jones and Lennon, 2010; Lennon and Jones, 2011), produce biofilms (Chang et al., 2007; Lennon et al., 2012), synthesize and accumulate compatible solutes (Bremer and Krämer, 2019; Lennon et al., 2012) or alter the composition of cellular membrane phospholipid fatty acids (Mutnuri et al., 2005; de Carvalho et al., 2014; Unell et al., 2007).

In general, different bacterial strains can exhibit rather a wide range of cell surface hydrophobicity (CSH), which plays an essential role in the adhesion of bacteria to surfaces (van Loosdrecht et al.,1987b). He reported a wide range of contact angle (CA) of cells surfaces of 23 different strains (15°-70°). Nevertheless, increase in surface hydrophobicity as a response to stress was reported as well (Baumgarten et al., 2012; de Carvalho et al., 2016b; Hachicho et al., 2017). Baumgarten et al, 2012 showed, that when exposed to osmotic stress *Pseudomanas putida* DOT-T1E releases outer membrane vesicles, which lead to increased surface hydrophobicity. Surface hydrophobicity of *P. putida* mt-2 was shown to also increase under osmotic stress compared to the unstressed control at -2.5MPa water potential (Hachicho et al., 2017), which supports the finding of (Baumgarten et al., 2012). Osmotically-induced increase in CSH was also reported for *Bacillus subtilis (López et al., 2000)* and *Rhodococcus opacus* PWD4 (de Carvalho et al., 2016a).

2.3.2 Water stress on community level

The activity and structure of soil microbial community is affected by various environmental factors. Soil moisture content is considered to be one of the main ones. It is known that exposure to water stress results in reduced growth rate and eventually death of bacterial cells (Potts, 1994). As SWR can affect the water availability in soil, it can indirectly be a source of water stress for soil microorganisms, affecting microbial activity and driving changes in soil microbial community, shifting it towards a more resistant to fast change in water potential(WP; Fierer et al., 2003). When exposed to water stress, different microorganisms exhibit different levels of resistance and resilience. Fungi have been reported to be more tolerant to water stress, due to their ability to accumulate osmolytes, without hindering their metabolism (Brown, 1990) and

their hyphal network, which allows them to transport resources with their hyphae and thereby obtain water and nutrients from distance, if the substrate diffusion is reduced in their surrounding (Bapiri et al., 2010 ; de Vries et al., 2018; De Vries and Shade, 2013). However, fungi do not always thrive in low water potentials. Decrease in fungal biomass under water stress was also reported (Williams, 2007). Bacteria belonging to most abundant phylum in the soil, Proteobacteria, Acidobacteria, Verrucomicrobia, Firmicutes, Actinobacteria, Bacteroidetes, Planctomycetes (Chodak et al., 2015; Sengupta and Dick, 2015) exhibit different levels of resistance and resilience to reduced water potential. Furthermore, the response of soil microbial communities to shifts in soil water potential had been shown to be affected by the prior exposure to drought (Meisner et al., 2018).

Gram-positive (G+) representatives of Actinobacteria and Firmicutes phyla, are reported to be more resistant to drought, compared to Gram-negative (G-) bacteria (Barnard et al., 2013). Number of G+ bacteria, within Firmicutes and Actinobacteria phylum, are known to sporulate, to withstand unfavorable environmental conditions, including low water potential (Fatima et al., 2019; Hutchison et al., 2014). Furthermore, the ability to produce aerial mycelium, similar to fungal hyphae, gives members of Actinobacteria phylum with survival advantage in extreme environmental conditions (Jiang et al., 2006; Kurapova et al., 2012)

In contrast G- bacteria are less resistant to water stress(Manzoni et al., 2012; Uhlířová et al., 2005). Several studies reported decrease in relative abundance of Proteobacteria, Planctomycetes and Bacteroidetes after exposure to drought and wetting cycles, while Actinobacteria and Firmicutes increase in relative abundance (Barnard et al., 2013; Chodak et al., 2015; Lennon et al., 2012). Contradicting results have been reported on the abundance and water stress response of Acidobacteria. While some studies show high level of drought-sensitivity and decrease in relative abundance with reducing water availability (Acosta-Martínez et al., 2014; Barnard et al., 2013), high relative abundance of Acidobacteria in dry soils was also reported (Curiel Yuste et al., 2014). Soil pH appears to be an important factor determining abundance and drought sensitivity of this phylum, with increased abundance and stress tolerance at low pH soils (Chodak et al., 2015).

2.4 Interaction of bacterial cells with soil minerals

Most soil microorganisms live attached to mineral surfaces (Or et al., 2007) and thus the surface properties of minerals (Stotzky, 1985) and bacterial cell walls (van

Loosdrecht et al., 1987a) impact the attachment of bacterial cells (Bos et al., 1999). Bacterial cells in turn can be expected to influence the properties of the formed microaggregates. Soil microorganisms are reported to play a role in soil aggregate formation. As means for survival in fluctuating soil water content, bacterial produce extracellular polysaccharides(EPS; Or et al., 2007), which due to their hygroscopic property maintain the water in the colony microenvironment as water potential declines and helps in survival of microorganisms (Roberson and Firestone, 1992). EPS is also reported to promote soil aggregate formation (Cania et al., 2019) and stabilization (Park et al., 2007; Redmile-Gordon et al., 2020) and increased soil's water-holding capability (Roberson and Firestone, 1992). It has been suggested, that EPS of some microorganisms could exhibit hydrophobic properties (Neu and Poralla, 1988). *Bacillus subtills* was reported to produce hydrophobic biofilm, triggered by osmotic stress (Arnaouteli et al., 2016; Epstein et al., 2011). Furthermore, Achtenhagen et al., 2015 reported that the direct attachment of material cells to pure soil minerals, rendered those hydrophobic. Additionally, when bacteria were exposed to osmotic stress prior to the attachment to minerals, overall hydrophobicity of these cell-mineral associations was significantly higher, compared to association with unstressed cells. The organic matter entrapped in or surrounding the soil microaggregates is reported to be mainly of microbial origin (Plaza et al., 2013), indicating the direct involvement of bacteria in the development of SWR.

3 AIMS AND HYPOTHESIS

Though the principal nature of bacterial adaptation mechanisms to osmotic and drought (matric) stress are often similar (osmolyte accumulation, biofilm formation; Brown, 1990), it has been also reported the outer membrane of *P. putida* had differential, concentration dependent response to low water potential, caused by NaCl (permeating) and PEG 8000 (non-permeating) solutes. However, knowledge on the differential effect of low matric and osmotic potentials on bacterial cell surface hydrophobicity is missing. Till now, there are only few studies, examining the impact of osmotic stress on bacterial CSH and to our best knowledge the impact of matric stress has not been yet reported. It has been shown, that bacteria increase cell surface hydrophobicity when exposed to osmotic stress and this response varied between planktonic and surface growth conditions (Baumgarten et al., 2012; Hachicho et al., 2017). Both studies investigated changes in CSH of *P. putida*, a G- soil bacteria, which plays an essential role in carbon and nitrogen cycling. Though G+ bacteria are known to be better adapted to drought (Manzoni et al., 2012), certain phyla of G-bacteria are also relatively abundant in dry soils (Barnard et al., 2013). Due to the structural differences of the cell wall, the response to water stress of G- and G+ bacterial cell surface hydrophobicity can be fundamentally different. However, up to date there are no comprehensive studies available on changes in bacterial CSH of bacteria with different cell wall structures. In soil, bacteria mostly exist on the surface of soil particles, thus being exposed to air/liquid/solid interface. These conditions are extremely different of those in vitro liquid cultures. Thus far, there is only one study comparing the impact of osmotic stress on CSH of *P. putida* in different growth conditions (Hachicho et al., 2017).

Bacterial cells and the cell wall fragments are mostly attached to the surface of soil minerals and have been shown to impact the surface wettability of minerals (Achtenhagen et al.,2015 ; Schurig et al., 2013). Moreover, the impact of bacterial surface properties on the minerals is exacerbated by exposure to osmotic stress (Achtenhagen et al., 2015). However, the impact of bacteria with different cell wall properties on the minerals has not been yet reported. Furthermore, it is not clear whether this impact persists over time, after the stress subsides.

Soil minerals are mostly coated by SOM; consequently, the properties of these organic coatings will determine the surface properties of the minerals (e.g., water repellency). The occurrence and the degree of water repellency is often positively correlated to the amount of the SOM; however, the composition of SOM is also a determining factor

(Blanco-Canqui, 2011; Lal, 2016). It is known that bacterial necromass stabilizes in SOM, thus impacting the composition of SOM (Miltner et al., 2012). Bacterial cells attached to soil minerals and cell wall fragments incorporated to SOM could impact soil wettability. Furthermore, reduced water availability could result in stress-induced increase in bacterial cell surface hydrophobicity.

Numerous studies report on the impact of drought and drying-rewetting on soil microbial community composition (Bapiri et al., 2010; De Vries and Shade, 2013 ; Hueso et al., 2011; Meisner et al., 2018) and changes in degree of SWR (Bachmann et al., 2021; Bayad et al., 2020 ; Vogelmann et al., 2013). It has been also shown, that the microbial communities vary between soils with different levels of water repellency (Lozano et al., 2014). However, to the extent of our knowledge no comprehensive studies are available connecting the development of SWR to stress induced changes in bacterial CSH.

Based on the abovementioned research gaps the aim of the present thesis was to evaluate (1) differential impact of matric and osmotic stress on bacterial cell surface hydrophobicity (2) their impact on the surface wettability of soil minerals and (3) drought driven changes in SWR and microbial community composition. In order to accomplish these aims, the following research hypotheses were tested:

1. Bacterial adaptation to water stress will result in changes in cell surface properties.
    i) Cells of different bacterial species will turn more hydrophobic due to exposure to water stress (osmotic and matric) compared to unstressed cells.
    ii) The response degree of cell surface hydrophobicity to osmotic stress will be different, compared to matric stress.
    iii) Bacterial cells will exhibit different levels of susceptibility depending on growth conditions (surface and submersed).

The impact of water stress (osmotic and matric) on bacterial growth and cell surface hydrophobicity was assessed. Furthermore, bacterial growth inhibition and changes in CSH due to osmotic stress were assessed in different growth conditions (surface and submersed). Changes in surface hydrophobicity were analyzed by means of contact angle measurements.

2. Bacterial cell surface properties will affect the wetting properties of soil minerals.
    i) Changes in bacterial cell surface properties due to exposure to osmotic stress will impact the surface properties of cell-mineral associations.

    ii) Bacteria-induced surface hydrophobicity of cell-mineral associations will persist over time.

The influence of bacteria and their cell wall fragments on the wettability of medium sized quartz (0.2 - 0.8 mm) was tested. To investigate the effect of osmotically stressed bacteria with different cell wall structures on the wettability of soil mineral, cells of *B. subtilis* and *P. fluorescens* were mixed with quartz and incubated for 2 months. Changes in surface hydrophobicity were monitored throughout the incubation. Additionally, the amount of total phospholipid fatty acids (PLFA) was analyzed as a measure of living bacterial biomass (Frostegård and Bååth, 1996).

3. Drought and drying-rewetting cycles will affect soil microbial community in soils with different levels of water repellency and drought history.
    i) Exposure to water stress (constant or fluctuating) will increase soil water repellency.
    ii) Soil microbial community will shift to a more drought resistant community.
    iii) Impact of drought on community composition and soil wettability will be less pronounced in the soil with drought history, due to adaptation.

To investigate the impact of different WC in soil on the bacteria-induced soil water repellency, two soils with different levels of water repellency were incubated under 3 different water regimes. Changes in soil water repellency under these water regimes were monitored via contact angle measurements. Furthermore, changes in soil microbial community composition were investigated via PLFA analysis and high-throughput sequencing of 16S rRNA gene. Finally, we used Random Forest (RF) machine learning algorithm to identify bacteria possibly associated with SWR in these soils.

4 MATERIALS AND METHODS

4.1 Culture conditions and microorganisms

To understand the impact of water stress on bacterial cell surface properties pure culture experiments were performed using cultures of 6 different soil bacteria (Table 1). The typical soil bacterial strains were selected to cover a wide range of environmental conditions and cell wall structures. *B. subtilis* and *A. chlorophenolicus*, were chosen as representatives of G+ bacteria and *P. fluorescens* and *N. aromaticivorans* as G-. Furthermore, G+ bacteria *M. pallens* and *R. erythropolis* were selected due to their extremely hydrophobic cell surfaces, caused by the presence of mycolic acids in their cell wall. This impacts the permeability to nutrients and other hydrophilic substances through the cell wall (Jarlier and Nikaido, 1990).

Table 1 List of microorganisms used in stress experiment

Microorganism	Strain	Gram stain	Origin
Bacillus subtilis	DSM 10, Marburg^T	+	unknown
Arthrobacter chlorophenolicus	DSM 12829, A6^T	+	Soil
Pseudomonas fluorescens	DSM 50090^T	-	Pre-filter tank
Novosphingobium aromaticivorans	DSM 12444, F199^T	-	Deep terrestrial subsurface sediments
Rhodococcus erythropolis	DSM 43066^T	-	Soil
Mycobacterium pallens	DSM 45404, czh-8^T	-	Hawaiian soil

*All the strains were obtained from The Leibniz Institute DSMZ-German Collection of Microorganisms and Cell Cultures (T- type strain)

Pure cultures were propagated in adequate medium recommended for each strain by DSMZ, transferred to cryovials and stored at -20°C until further use. Prior to the experiments, pre-cultures were prepared by inoculating 50 ml sterile mineral salt medium (Table 2), supplemented with 4 g/L sodium succinate and 1 g/L yeast extract

as carbon source (Hachicho et al., 2017). The overnight cultures were incubated at 30°C on an orbital shaker (160 rpm) and used as inoculants in growth inhibition experiments.

Table 2 Mineral medium composition

Compound	Concentration
Na_2HPO_4	7 g/L
KH_2PO_4	2.8 g/L
NaCl	0.5 g/L
NH_4Cl	1 g/L
$MgSO_4 \cdot 7H_2O$	0.1 g/L
$FeSO_4 \cdot 7H_2O$	0.01 g/L
$MnSO_4 \cdot H_20$	5 mg/L
$ZnCl_2$	6.4 mg/L
of $CaCl_2$ $6H_2O$	1 mg/L
$BaCl_2$	0.6 mg/L
$CuSO_4 \cdot 5H_2O$	0.4 mg/L
$CoCl \cdot 6H_20$	0.4 mg/L
H_3BO_3	6.5 mg/L
EDTA;	10 mg/L
HCl (37%)	146 µl/L

4.1.1 Adjustment of osmotic $\Delta\Psi_O$ and matric $\Delta\Psi_M$ potentials of growth media

Sodium chloride and PEG 8000 were used to change the osmotic and matric potentials of mineral medium by -0.5, -1.5, -2.5 and -3.5MPa. High molecular weight PEGs are used to reduce the matric potential in a growth medium, as they are too large to penetrate cell walls, but rather reduce the water potential by retaining the water in their macromolecular structure. Thus, they can lower the matric potential in the growth medium, mimicking the conditions in dry soil (Steuter et al., 1981). The amount of PEG 8000 and NaCl to be added were calculated based on the equation 1(Michel and Kaufmann,1973), and equation 2, respectively:

$$[PEG] = 4 - (5.16 * \Psi * T - 560 * \Psi + 16)^{0.5}/(2.58 * T - 280) \quad (1)$$

$$\Psi = -iCRT \quad (2)$$

where [PEG] amount (g) was calculated based on Ψ water potential (Pa), and T is the air temperature (K), i- the number of ions formed due to dissociation of NaCl, C is the molar concentration of NaCl used and R is the gas constant (8.31 J mol^{-1} K^{-1}).

To verify the achieved water potentials of the samples, water activity (A_w) of the liquid and solid media were measured with the LabMaster®-aw instrument (Novasina AG, Switzerland). For A_w measurement 5 ml of liquid and solid media was filled into dry sample cup (Ø 40 mm, 12 mm, polypropylene, ePW sample cups, Novasina). System parameters were set at 5 min stabile observation time for temperature and water activity. The measurements were performed at 30°C. Measured A_w values were converted to osmotic and matric water potentials using equation 3 (Campbell and Gardner,1971):

$$\Psi = R * T * lnAw \quad (3)$$

where Ψ is the water potential (Pa), R is the gas constant (8.31 J mol^{-1} K^{-1}), and T is the air temperature (K).

Water potentials values, derived from Aw measurements were used for presentation and interpretation of the obtained results, rather than the calculated values presented in Table 3.

Table 3 Used concentrations of NaCl and PEG 8000 and corresponding osmotic and matric potential values of liquid and solid mineral salt media.

	Measured shift in water potential of the media [MPa]				
		Osmotic potential		**Matric potential**	
intended shift in water potential	NaCl (g/L)	Ψ_O	Ψ_{OS}	PEG 8000 (g/L)	Ψ_M
0	0	0.0	0.0	0	0.0
Δ 0.5	6	- 0.6	- 0.5	208	- 0.9
Δ 1.5	18	- 2.0	- 1.5	384	- 2.5
Δ 2.5	29	- 2.9	- 2.7	500	- 3.7
Δ 3.5	41	- 4.6	- 3.8	592	- 4.6

4.1.2 Growth inhibition in submersed culture

For growth inhibition experiments in submersed culture 50 ml of sterile mineral salt media supplemented with different amounts of NaCl or PEG 8000 (Table 3) was inoculated with overnight cultures, to obtain initial optical density (OD_{560}) of ~ 0.05 in 250 ml glass vials. The vials were incubated at 30°C on an orbital shaker (160 rpm). The duration of incubation varied between strains, due to species specific growth rates and are presented in Table 7 (Appendix).

Cell growth was monitored by optical density measurement using a Perkin Elmer UV/VIS Spectrophotometer (UV-Vis Spektrometer: Lambda2S, Perkin/Elmer, Waltham, USA). Cells were harvested in the exponential growth phase by centrifugation at 11,000 g for 15 min (Hermle Z383K), resuspended in 2 ml KNO_3 (10 mM, pH 7.0) and transferred to 2 ml reaction tubes. Each sample was washed twice with 2 ml KNO_3 followed by 1 min centrifugation at 10,000 g. The washed biomass was resuspended in 1 ml KNO_3 and stored overnight at 5°C until contact angle analysis.

4.1.3 Growth inhibition in surface culture

Five ml of solid mineral salt media was inoculated with a 5 µl-drop of the overnight culture in the center and incubated at 30°C. For OD_{560} measurements the colonies were resuspended in 2 ml KNO_3 by vigorous pipetting. At the end of incubation, the biomass was washed twice as described in section 4.1.2 and stored till further analysis. The growth inhibition is presented as percent of growth rate of the corresponding unstressed controls. The growth rate µ [h^{-1}] of each culture (surface and submersed) in the exponential growth phase was calculated based on the equation 4 (Keweloh et al.,1989):

$$\mu\ [h^{-1}]\ \frac{ln\, x_{t_2} - ln\, x_{t_1}}{t_2 - t_1} \qquad (4)$$

where x_{t1} and $x_{t2\ are}$ the optical density of the samples at the beginning (t_1) and end (t_2) of the incubation, respectively.

4.2 Incubation experiment with bacterial cell-mineral associations (CMA)

4.2.1 Microorganisms and growth conditions for CMAs

For CMA incubation experiment *B. subtilis* and *P. fluorescens* were grown as described in section 4.1.2, with glucose being used as C source, instead of sodium succinate. Both strains were grown in mineral salt media without or with addition of 0.5 M NaCl. Growth of the strains was monitored via optical density measurements. Cell number was estimated using a coulter counter Multisizer 3 (Beckman Coulter, High Wycombe, UK). The cells were harvested by centrifugation (10,000 g for 10 min) and washed twice with 10 mM KNO_3 solution and stored overnight at 5°C.

4.2.2 Cell-mineral association preparation and incubation conditions

Unstressed and osmotically stressed cells of *B. subtilis* and *P. fluorescens* were suspended in 10 mM KNO_3 solution and mixed with washed and calcined medium-grained quartz (0.2-0.8 mm; Merck KGaA, Darmstadt, Germany), with concentration of 10^9 cells g^{-1} quartz. The cell mineral associations were supplemented with soil extract. Briefly, 5 g of soil per 180 g of dry quartz, obtained from Calvörde (CAL), located in the Altmark region in Saxony-Anhalt (52°22.82' N, 11°17.41 ± ' E), were shaken for 2 hours at an average speed in 20 ml 10mM $CaCl_2$, centrifuged for 10 mins at 2000 g. Supernatant was transferred into a new tube and centrifuged for 20 mins at 10000 g. The pallet was suspended in 10 ml $CaCl_2$ and added to the CMAs. Minerals supplemented with only the bacteria extracted from CAL soil were used as control. The samples were well mixed to ensure homogenous distribution in 1 l Duran glass bottle with a rubber sealed cap. Water content of the samples was adjusted to 50% of the water holding capacity (WHC) of quartz. A glass vial containing 10 ml 2M NaOH was placed in the bottle to trap CO_2 produced as a result of microbial respiration and was replaced with every sampling.

The cell mineral associations were incubated under controlled laboratory conditions in the darkness and a constant temperature of 20 ± 2°C. The samples were opened after 2, 4, 8, 16, 32, 50 and 80 days, 10 g of sample was removed for future analysis. CMAs with *B. subtilis* were incubated for 80 days and the ones with *P. fluorescens* for 50 days.

4.3 Incubation experiment with natural soil

4.3.1 Site description and soil sampling

For the soil incubation experiment soil was collected from two different beech (Fagus sylvatica L.) forests, located in northern Germany, characterized by contrasting hydroclimatic conditions (Goebel et al, 2019 (unpublished)).
Lüss (LUE), located in the Lüneburg Heath in Lower Saxony (52°49.83' N, 10°18.99' E) at 117 m a.s.l, is characterized by mean annual temperature of 8.7°C and mean annual precipitation (MAP) of 816 mm (Meier et al.,2018). In contrast, CAL site is located in the Altmark region in Saxony-Anhalt (52°22.82' N, 11°17.41' E) at 105 m a.s.l. and is characterized by a mean annual temperature of 9.3 °C and a mean annual precipitation of 594 mm (Meier et al., 2018).

Table 4 Physico-chemical properties of CAL and LUE soils

	soil	
	CAL	LUE
pH	2.9 ± 0.0	3.3 ± 0.0
C (%)	8.2 ± 0.2	6.2 ± 0.0
N (%)	0.4 ± 0.0	0.3 ± 0.0
C/N	21.1	18.6
Clay (%)	5.2	5.5
Silt (%)	10.9	26.1
Sand (%)	83.9	68.3
Gravimetric WC at pF2.5 (%)	23.2 ± 0.6	29.4 ± 0.4
Gravimetric WC at pF4.2 (%)	16.3 ± 0.2	15.6 ± 1.9
Contact angle (°)	128 ± 6	82 ± 7

Both soils were classified as podzolic Umbrisols (spodic Dystrudepts) developed on fluvioglacial sandy deposits from the penultimate Ice Age (Saale) (Knutzen et al., 2015). Soil samples were collected from the A horizon (0-2 cm) at both sites in November 2018, sieved < 5 mm to remove larger roots and stones and stored at 4°C until further use. The physico-chemical properties of both soils were analyzed after

collecting from the field and are presented in Table 4(Goebel et al., 2019(unpublished)).

4.3.2 Soil texture and water retention properties

Particle size distribution of material <2 mm was determined by wet-sieving and sedimentation analysis (Gee and Bauder, 1986) after organic matter destruction with 35% H_2O_2 and chemical dispersion with 0.05 M $Na_4P_2O_7$. Water retention properties were determined by measuring soil water content on material adjusted to -32 kPa (pF2.5) and -1500 kPa (pF4.2) in a pressure chamber (Eijkelkamp Soil & Water, Giesbeek, The Netherlands).

4.3.3 Sample preparation and incubation modes in soil incubation experiment

To adjust soil water content to water potentials of pF2.5 and pF4.2 the soils were adjusted to 45% water content, by adding deionized water, then air-dried at 20°C until the respective pF values were reached. Both soils (CAL and LUE) were incubated in three incubation modes: “wet” mode, in which the soil moisture was maintained constant at its field capacity (pF2.5), “dry” mode at constant water potential of pF4.2 (at permanent wilting point) and “intermittent” mode, with varying water potential between pF2.5 and pF4.2. In the “intermittent” incubation mode, samples were initially adjusted to pF2.5 and left to dry for 30 days. After 30 days samples were taken for contact angle and chemical analysis and the soil was rewetted by addition of deionized water to reach pF2.5.

Additionally, to accentuate the contribution of microbial biomass to soil wettability, microbial growth was induced by the addition of an easily degradable substrate. For this, one half of each soil was amended with a mixture containing 84.2 mass-% glucose ($C_6H_{12}O_6$), 13.7 mass-% diammonium sulfate ($[NH_4]_2SO_4$), and 2.1 mass-% potassium dihydrogenphosphate (KH_2PO_4; ISO-17155:2012,2012). Of this substrate, an amount of 0.4 g g^{-1} soil organic C was added to the soil, corresponding to 32.7 and 25.0 g kg^{-1} dry soil for CAL and LUE, respectively. Overall, 27 samples per soil variant were prepared to allow sampling at three time points of three physical replicates incubated in three different modes.

For each sample 10 g dry soil was filled into a sterile glass vial (5 cm height; 2.75 cm

inner diameter; baked out at 300°C) and adjusted to a bulk density of about 0.8 g cm^{-3} by pressing. Soil samples were placed in sealable acrylic glass boxes, containing vessels with polyethylene glycol (PEG) solutions (PEG 1000, Sigma Aldrich) to keep the soil water potential constant. The respective concentration of the PEG solution was calculated by Raoult's law (equation 5; Wheeler et al., 2012) and equation 6 (Campbell and Gardner, 1971):

$$\frac{p}{p_{\circ}} = \frac{1}{(1+\frac{xMw}{1-xMp})}, \quad (5)$$

$$\Psi = \frac{RTa}{Mw} ln \frac{p}{p_{\circ}} \quad (6)$$

where p and p_0 are actual and saturated vapor pressure (Pa), respectively, x is the mass fraction of PEG, M_w and M_p are the molecular weights of water (0.018kg mol^{-1}) and PEG (1kg mol^{-1}), respectively, Ψ is the water potential (Pa), R is the gas constant (8.31 J mol^{-1} K^{-1}), and T_a is the air temperature (K). For 20°C (T_a = 293.15 K) this yielded PEG mass fractions of 0.013 and 0.396 for water potentials of -32 kPa (pF2.5) and -1500 kPa (pF4.2), respectively. The incubation boxes were additionally sealed with Parafilm® M (Bemis Company, Inc., Oshkosh, USA) and plastic covers to ensure air tightness. The incubation was carried out in a thermostatic cabinet (Lovibond TC 255 S, Tintometer GmbH, Dortmund, Germany) at 20 ± 0.2°C in the dark. The position of the boxes within the cabinet was regularly changed to minimize potential thermal stratification effects. After incubation times of 29, 60, and 88 days three replicate samples of each incubation mode were taken for further analysis.

4.4 Chemical analyses

4.4.1 CO_2 measurement

Microbial respiration was determined by quantifying the carbon dioxide (CO_2) released from CMAs during the incubation at 20°C. The CO_2 trapped in 2 M NaOH solution was measured TOC-5050, Duisburg, Germany). To quantify the CO_2 concentration for the samples the CO_2 concentration of the control with no addition of bacteria was subtracted. Cumulative concentration of CO_2-C was calculated and is presented as percent of initially added C (Miltner et al., 2005).

4.4.2 Total bacterial biomass C

For total bacterial biomass derived organic C measurements 30-50 mg, ground to fine powder, CMAs were weight into 10x10 mm tin capsules (Hekatech GmbH, Germany) and analyzed on elemental analyzer-combustion-isotope ratio mass spectrometer (EA-C-irMS; Finnigan MAT 253, Thermo Electron, Bremen, Germany (Girardi et al., 2013); coupled to Flash EA 2000 (ThermoFinnigan). Temperature in the oxidation reactor was set to 1020°C and the reduction reactor was 650°C. Acetanilide (Hekatech GmbH, Germany) standard curve was used for C concentration calculation in the samples(Adam et al., 2015).

4.4.3 Phospholipid fatty acid analysis

PLFA are known to be present only in intact living bacterial cells and degrade fast once the cell is dead (Jenkinson and Ladd,1981), therefore they are used for estimation of living microbial biomass and microbial community composition (Frostegård and Bååth, 1996). To analyze the total living bacterial biomass and the relative abundance of different microbial groups phospholipid fatty acids were extracted both from soil samples and CMAs. For the extraction 1 g of soil and 2 g of CMA were used ((Bligh and Dyer, 1959), modified by (Miltner et al., 2005)). To each sample 2 ml phosphor buffer, 5 ml methanol and 2.5 ml chloroform were added, and the mixture was shaken for 2 h on a rotary shaker (Infors HT, Bottmingen, Switzerland). After shaking, 2.5 ml of deionized water and 2.5 ml of chloroform was added and left overnight, to achieve phase separation. Following the phase separation, the bottom chloroform phase was collected and dried over sodium sulfate and evaporated under N_2. This phase was then fractionated into neutral lipids, glycolipids and PLFA according to (Miltner et al., 2005) by eluting the sample over silica columns (Unisil) previously washed with ammonium acetate (0.02 M). The total lipids were eluted to neutral lipids, glycolipids and PLFA by subsequent addition of 5 ml chloroform, 5 ml acetone and 15 ml methanol. The methanol phase, containing the PLFA, was collected and evaporated under N_2. The PLFA were resuspended in the mixture of methanol/Trimethylchlorosilane [(TMCS), 9:1; v: v] and methylated at 60°C for 2 h and dried under N_2 (Thiel et al., 2001). Prior to the injection into the GC/MS with BPX-5 column, the samples were dissolved in hexane and heneicosanoate methyl ester (BAME 21:0) was added to each sample and in internal standard. The following program was used for the analysis: initial temperature 50 °C (hold for 1 min), heat to 250°C (0 min) at 4°C/min and finally to

300°C (10 min) at 2°C/min. The injector was set to 280°C and the samples were injected splitless. The transfer line was held at 300°C and the helium flow was set to 2.5 ml per minute.

Table 5 Phospholipid fatty acids (PLFAs) used as biomarkers in this study, with their shorthand (part 1)

PLFA groups	**PLFA markers**	**PLFA biomarker**
Sum of all PLFA		total living microbial biomass (Frostegård and Bååth,1996)
Saturated fatty acids		
Straight chain	14:0, 15:0, 16:0, 17:0, 18:0, 19:0, 20:0	general bacteria
Terminally branched	i15:0, a15:0, i16:0, i17:0, a17:0	Gram positive bacteria (Zelles, 1997)
Mid chain branched	10me16:0, 12me16:0, 10me17:0, 10me18:0	Actinobacteria (Kroppenstedt, 1985)
Cyclopropane	Cy 17:0, Cy 19:0	Environmental stress in G-bacteria (Ramos et al., 2001)
Unsaturated fatty acids		
Monounsaturated	16;1ω9, 16:1ω9, 16:1ω7, 18:1ω9, 18:1ω7, 18:1ω5	G- bacteria (Unell et al., 2007)
Polyunsaturated	18:2ω6,9	Fungi (Frostegård and Bååth,1996)
Fungi/ bacteria	Polyunsaturated/ monounsaturated+ terminally branched+ mid branch chained+ cyclopropane fatty acids	Reduced C and nutrient availability (Wang et al.,2020b)

Table 5 Phospholipid fatty acids (PLFAs) used as biomarkers in this study, with their shorthand (part 2)

Microbial stress indicators		
G+/G-	Branched/Monounsaturated	Reduced C and nutrient availability (Wang et al.,2020b)
cy/pre	cy17:0+ cy19:0/ 16:1ω7+ 18:1ω7	Slow metabolic activity due to environmental stress
Iso/antiiso	i15:0+ i16:0+ i17:0/ a15:0 + a17:0	Stress in G+ bacteria
trans/cis	16:1ω7t+18:1ω7t/ 16:1ω7c+ 18:1ω7c	Stress in bacteria of the genera Pseudomonas and Vibrio (Heipieper et al.,2003)

Fatty acid peaks were identified based on the retention time and comparison of mass spectrum with standard Bacterial Acid Methyl Esters (BAME). Standard nomenclature is used to describe FAMEs. They are designated by A: B ω C, where A indicated the total number of carbon atoms, B is the number of double bonds and C indicates the position of the double bond from the methyl end of the molecule. The prefixes “a” and “i” refer to anteiso-and iso-branched fatty acids. The prefix “xMe” indicates a presence and the position of methyl group, relative to the carboxyl end of the molecule, and “cy” indicates cyclopropane fatty acids. Fatty acids were grouped based on their structural characteristics and their use as biomarkers for broad taxonomic microbial groups or as stress indicators and are presented in the Table 5. PLFA concentrations are presented as nmol g^{-1} of soil. The abundance of specific fatty acid groups is presented as percent of total PLFA. The sum of all the PLFA represent total viable microbial biomass.

Polyunsaturated fatty acid 18:2ω6 was used as an indicator of fungal biomass (Frostegård and Bååth, 1996). Gram positive bacteria were indicated by terminally branched fatty acids (i15:0, a15:0, i16:0, i17:0, a17:0), Gram-negative bacteria by monounsaturated PLFAs (16;1ω9,16:1ω9, 16:1ω7, 18:1ω9, 18:1ω7, 18:1ω5, cy17:0, cy19:0; Wilkinson and Ratledge, 1988; Zelles, 1997) and Actinobacteria biomass by mid chain branch fatty acids (10me16:0, 12me16:0, 10me17:0, 10me18:0; Kroppenstedt, 1985; Lechevalier, 1977). Detected saturated fatty acids (14:0, 15:0, 16:0, 17:0, 18:0, 19:0, 20:0, br16:0) and branched monounsaturated fatty acids (br16:1

and br17:1) were not attributed to a specific microbial group but were included into the calculations of total PLFA.

Additionally, ratios of fungal to bacterial PLFA, G+/G- and cyclopropane fatty acids to their precursors were calculated. Ratio of fungal to bacterial and G+/G- bacterial PLFA are associated with increase in microbial community structure resistance to nutrition stress (Wang et al., 2020b). G+ bacteria and fungi considered K-strategists, meaning that they exhibit higher tolerance to stress and are able to grow more slowly on substrate-limited environments (De Vries and Shade, 2013). Another stress indicator in G+ bacteria is the change in the iso/anteiso ratio, which has been shown to occur as result of environmental stress (Unell et al., 2007). Increase in ratios of trans to cis monoenoic and cyclopropane fatty acids to its monoenoic precursors are used as stress indicators for G- bacteria, therefore were also calculated in this study.

4.4.4 Organic C and N content

To determine the organic C and N content of the soils were determined in duplicate by dry combustion and infrared detection of CO_2 and thermal conductivity measurement of N_2 using a Vario EL III Elemental Analyzer (Elementar Analysensysteme GmbH, Hanau, Germany) on oven-dried (105°C) powdered (ball-milled) material.

4.5 Determination of surface hydrophobicity

4.5.1 Preparation of bacterial lawns for water contact angle measurements

To obtain information about bacterial cells surface hydrophobicity of pure cultures, contact angle of the samples obtained during growth inhibition experiments were measured. Depending on the OD of the samples, 100-500 µl of the biomass was suspended in 20 ml KNO_3 and the suspension was filtered through a cellulose nitrate membrane filter (pore size 0.45 µm, Ø 25 mm, NC 45, Whatman) until the passthrough was ceased, to produce a homogenously covered filter surface. The filters were mounted to a microscope slide with double-sided adhesive tape and dried for two hours. For each sample 2 filters were prepared, and 4 measurements were made at ambient laboratory air temperature and humidity per filter at different spots and the average for θ [°] was calculated.

4.5.2 Preparation of CMAs and soil samples for water contact angle measurements

To measure the water contact angle of CMAs and soil samples used in incubation experiment the samples were prepared according to (Bachmann et al., 2003). Briefly, well homogenized samples were sprinkled on a glass slide covered with double-sided adhesive tape and gently pressed, to get a thin homogeneous layer of soil particles. After preparation the glass slides were left for 2-3 h at room temperature to dry.

4.5.3 Contact angle measurements

As a measure for cell surface hydrophobicity, the contact angle of pure cultures, CMAs and soil samples were measured. The CA of the bacteria was measured with the sessile drop method as described by van Loosdrecht et al. (1987, a). The CA of the CMAs and soil samples were determined using the sessile drop method as described by Bachmann et al. (2000). CA measurements of pure cultures and CMAs were performed with drop shape analysis system (DSA 100, Krüss GmbH, Germany). A single water droplet (3 µl volume at a flow rate of 40 µl/s) was placed on the sample surface and the contact angle θ [°] was determined by image analysis with the DSA software. Contact angle of soil samples was measured using a contact angle microscope (OCA 15, DataPhysics, Filderstadt, Germany). Immediately after preparation initial contact angle was measured by placing 1 µl of deionized water (instead of 3 µl used for measuring CA of bacteria and CMAs) and was evaluated at the intersections of the drop contour line with the solid surface by automatic drop shape analysis using the software SCA20 (DataPhysics, Filderstadt, Germany). CA values between 0° to 90° show reduced wettability, values greater than 90° were considered extremely hydrophobic (Goebel et al.,2011).

4.6 Microbial community structure analysis

4.6.1 DNA extraction and sequencing

The total genomic DNA was extracted from 0.5 g of soil sample, taken at the beginning and end of the incubation, with ‘NucleoSpin Soil’ kit (Macherey-Nagel GmbH & Co. KG, Düren, Germany) according to the manufacturer’s protocol using buffer SL2 with

10 uL enhancer SX. The quality of the DNA was checked by 1% agarose gel electrophoresis and DNA concentration was measured via a NanoDrop ND-1000 UV/VIS spectral photometer (PeqLab, Germany). The 16S rRNA genes were further amplified and sequenced via Illumina MiSeq. The variable regions V3-V4 of the bacterial 16S rRNA gene fragments were amplified (Forward primer-5'TCGTCGGCAGCGTCAGA TGTGTAT AAGAGACAGCCTACGGGNGGCWGCAG and Reverse primer-5 'GTCTCGTGG GC TCGGAGATGTGTATAAGAGACAGGACTACHVGGGTATCTAATCC) (Vasileiadis et al.,2012). The PCR products were further purified with AMPure XP beads via magnetic stand. Index PCR with the purified PCR products was applied with the Nextera XT Index kit to attach dual indices and Illumina sequencing adapters.

4.6.2 Data analysis

Amplicon sequence variant (ASV) was used in this study instead of operational taxonomic units (OTUs) to determine the α-and β-diversities. Unlike OTUs, which are clustered based on certain dissimilarity threshold (commonly used 3%) (Westcott and Schloss,2015), ASVs allow to distinguish sequence variants differing by one nucleotide (Callahan et al.,2017).

Shannon index and ASV counts (α-diversity) were determined using the R package phyloseq (McMurdie and Holmes,2013). Differences in bacterial community composition (β-diversity) were calculated using Bray-Curtis dissimilarity index based on rarefied (122020 ASV counts per sample) ASV abundances. Permutational multivariate analysis of variance (PERMANOVA) were calculated by “adonis2” function in “vegan” R package using permutations to determine whether time, incubation modes and substrate addition were significant factors driving the shifts in ASV abundance. Further, ASVs which could be used to classify the difference between moisture treatments were identified via Random Forest (RF) analysis and hereafter will be referred to as “bioindicators” (classifiers). Random Forests is a data mining method assembles of many decision trees with binary divisions (Breiman,2001; Wei et al.,2018). In RA operates by constructing a multitude of decision trees trained with an independent bootstrap sample, by randomly drawing some ASVs multiple times, while others may not be drawn. The points not drawn into a bootstrap sample are the “out of the bag” (OOB) samples, an accuracy predictor.

In order to determine bioindicators, the analyzes were conducted with three steps (Rosado et al.,2019). Firstly, a RF constituted of 2000 trees were computed using the

default settings of the “randomForest” function implemented in the “randomForest” R package (Liaw and Wiener,2002). To assess the prediction accuracy, confusion matrixes of OOB error were generated, using the rarified relative abundance of ASVs with “Time” and “Time + Water regime” as factors. Due to abundance of shared ASVs between the “wet” and “dry” incubation modes at the beginning of the incubation and between “wet” and “intermittent” modes at the end of the incubation, the OOB error for classification of ASVs using “Time + Water regime” as factor was high. To overcome this problem, the ASVs were grouped into new groups. All the ASVs identified in “wet” and “dry” soils at the beginning of the incubation were combined into one group. ASVs identified in the soils at “wet” and “intermittent” at the end of the incubation into second group, thus leaving the soil at “dry” at the end of the incubation as a separate group.
The variable importance of ASVs was calculated via Mean Decrease Gini for “Time” and “Time + Water regime” factors. Mean Decrease Gini is a measure of how important a variable is for classifying the data across all trees making up the forest. A higher Mean Decrease in Gini indicates higher variable importance, meaning higher loss of accuracy if the variable is excluded. Optimal breakpoints, below which the importance of a variable in the model is insignificant, were estimated using the “breakpoints” function included in the “strucchange” R package. ASVs with Mean Decrease Gini values above the breakpoint curve were chosen to be part of the classifier (approx. 30 ASVs for each factor). Afterwards the potential bioindicator ASVs important for only Water regime were identified by deducting the ASVs that were relevant for both Time and Water regime. Finally, the obtained bioindicators were checked for their significance between the water regimes via the LSMEANS test with pair-wise methods adjusted by false discovery rate (FDR) correction (Lenth,2016). About the same number of ASVs were selected as bioindicators for both soils, 13 ASV for CAL and 15 ASV for LUE soil. To highlight the ASVs which were significantly pairwise associated with incubation modes and sampling time ($P < 0.05$), ASVs were divided into three groups: beginning “wet” + “dry” vs. end “wet” + “intermittent” (group 1), beginning “wet” + “dry” vs. end “dry” (group 2) and beginning “wet” + “intermittent” vs. end “dry” (group 3).

4.7 Statistical analysis

All statistical analysis was performed by means of RStudio software. The significance of the differences between treatments in stress experiments with pure cultures and CMA incubation experiment was determined by two-way ANOVA followed by a

pairwise t-test comparison with Bonferroni adjustment. Relative importance of identified PLFA groups between different incubation modes and substrate amendments in the soil incubation experiment were analyzed by Repeated Measures ANOVA, using time as a within-subjects' factor. The data are presented as arithmetic mean ± standard error.

5 RESULTS

5.1 Bacterial growth in pure culture experiment under water stress

To investigate the differential impact of osmotic and matric stress on the growth and surface hydrophobicity of bacteria, pure culture experiments were performed in liquid mineral media, where 6 different bacterial strains were grown in presence of NaCl-induced osmotic and PEG 8000-induced matric stress. The growth inhibition and changes in cell surface contact angle were measured. Furthermore, the impact of osmotic stress was assessed in surface growth, to apprehend information on the response to osmotic stress in conditions more like ones in soil. The impact of matric stress in surface growth was not investigated, due to shortcomings of the plate preparation method. Growth of the strains was monitored by OD_{560} measurements, and the results are presented in Figure 4 and Table 6.

Table 6 Growth rates of bacteria in mineral salt medium expressed as change in OD_{560} per hour

Stress type	**osmotic**		**matric**
Growth condition	**submersed**	**surface**	**submersed**
A. chlorophenolicus A6	0.43 ± 0.04	0.09 ± 0.02	0.44 ± 0.04
B. subtilis	0.37 ± 0.04	0.15 ± 0.01	0.39 ± 0.07
M. pallens	0.1 ± 0.02	0.05 ± 0.02	0.11 ± 0.02
N. aromaticivorans	0.11 ± 0.1	0.14 ± 0	0.15 ± 0.02
P. fluorescens	0.45 ± 0.05	0.2 ± 0.02	0.47 ± 0.07
R. erythropolis	0.24 ± 0.04	0.18 ± 0.02	0.25 ± 0.02

The results showed, that the unstressed cultures of *B. subtilis*, *A. chlorophenolicus* and *P. fluorescens* exhibited higher growth rates, compared to *M. pallens*, *N. aromaticivorans* and *R. erythropolis* during submersed growth. However, during surface growth, the growth rates of the slow growing strains were not affected, while

the growth rates of the fast-growing strains (*B. subtilis*, *P. fluorescens* and *A. chlorophenolicus A6*) significantly lowered.

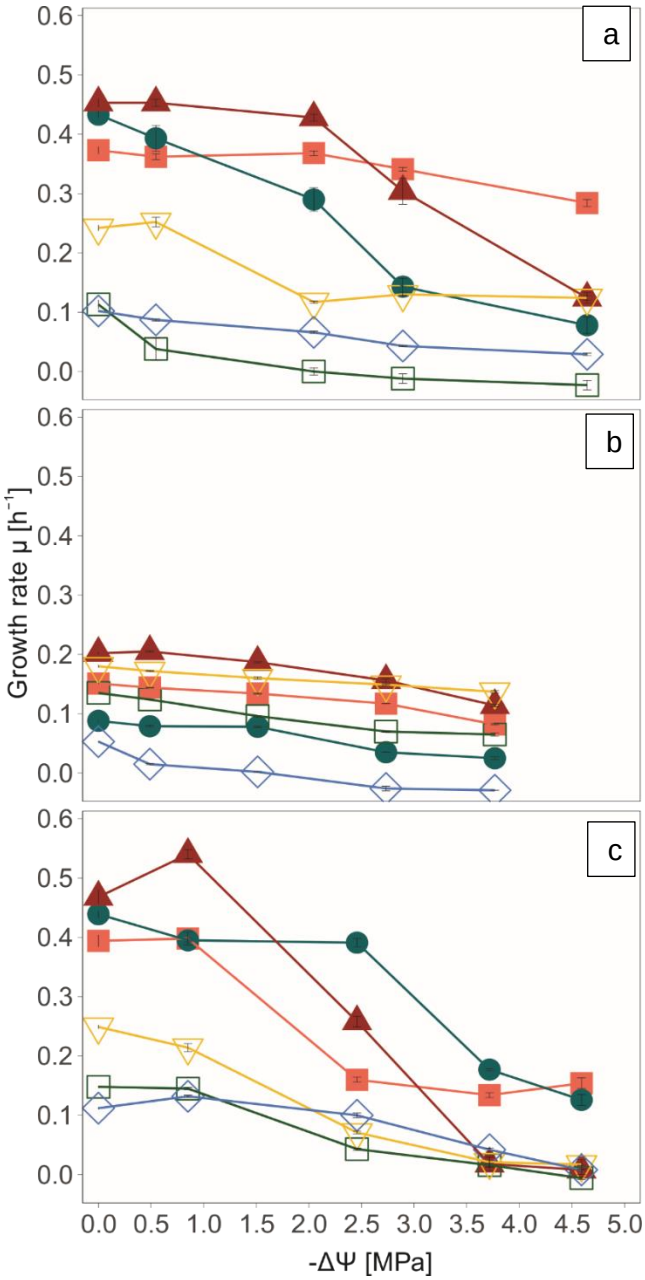

Figure 4 Effect of osmotic and matric stress on growth rate of ▲ *P. fluoresecens*, ● *A. chlorophenolicus*, ■ *B. subtilis*, □ *N. aromaticivorans*, ◊ M. pallens, ▽ *R. erythropolis* exposed to a) osmotic stress in submersed growth, b) osmotic stress in surface growth and c) matric stress in submersed growth. The values are arithmetic mean of n = 3 measurements with the error bars indicating the standard error.

Furthermore, bacterial growth inhibition as a result of both osmotic and matric stress in submersed and surface growth conditions was observed. G+ strains, with exception of *R. erythropolis*, were more susceptible to osmotic stress during surface growth, while the opposite was observed in case of G- bacteria. When grown on the surface, *N. aromaticivorans*, *P. fluorescens*, *R. erythropolis* exhibited higher tolerance to osmotic stress, compared to submersed growth, with the least susceptible being *R. erythropolis* (growth inhibition of 24 ± 0.35%), and most affected being *N. aromaticivorans* (growth inhibition of 52 ± 2%). Growth of *B. subtilis* and *M. pallens* was more affected by low osmotic potential on surface growth compared to submersed growth. *A. chlorophenolicus* was similarly susceptible to osmotic stress under both growth conditions.

5.1.1 Bacterial growth inhibition under osmotic and matric stress

Impacts of different levels of matric and osmotic stress on the growth of the abovementioned strains were monitored, and the growth inhibition relative to the unstressed cultures at each stress level is presented in Figure 5. The water potential of the liquid growth media was -1.0 ± 0.1MPa (corresponding to pF4.0 ± 0.1) and was reduced by addition of different amounts of NaCl and PEG 8000 to lowest water potential of -5.7 ± 0.1 MPa (corresponding to pF4.7 ± 0.0) and -6.0 ± 0.1 MPa (corresponding to pF4.8 ± 0.0), respectively. The values in Figure 5 are presented as change in water potential relative to the control treatment (mineral media without NaCl or PEG 8000).

For most strains significant growth inhibition was observed when the water potential of the growth media was below -2 ± 0.1 MPa. At lower water potential values 4 out of 6 strains were more susceptible to matric stress, with >90% growth inhibition at Ψ_M= -4.6 ± 0.1 MPa, with exception of *A. chlorophenolicus A6* (71 ± 2%) and *B. subtilis* (46 ± 0.5%).

No growth inhibition was observed at higher water potential values for *B.subtilis* in case of both, matric (Ψ_M=-0.9 ± 0.1 MPa) and osmotic (Ψ_O = -0.5 ± 0.1 MPa) stress. However, when exposed to matric stress, the growth rate was inhibited by 60 ± 0% already at Ψ_M=-2.5 ± 0.2 MPa. Osmotic stress had only slight impact on the growth of *B. subtilis* at water potential of Ψ_O = -2.9 ± 0.2 MPa with maximal growth inhibition of only 24 ± 1.2% at Ψ_O = -4.6 ± 0.1 MPa.

Growth of *P. fluorescens*, *R. erythropolis* and *A. chlorophenolicus A6* were inhibited under both stress conditions. Slight increase in matric potential (116 ± 5% at Ψ_M= -0.9

± 0.1 MPa) induced the growth of *P. fluorescens,* however, further reduction resulted in drastic growth inhibition (55 ± 3% at Ψ_M=-2.5 ± 0.2 MPa), with full growth inhibition at Ψ_M = -3.7 ± 0.2 MPa.

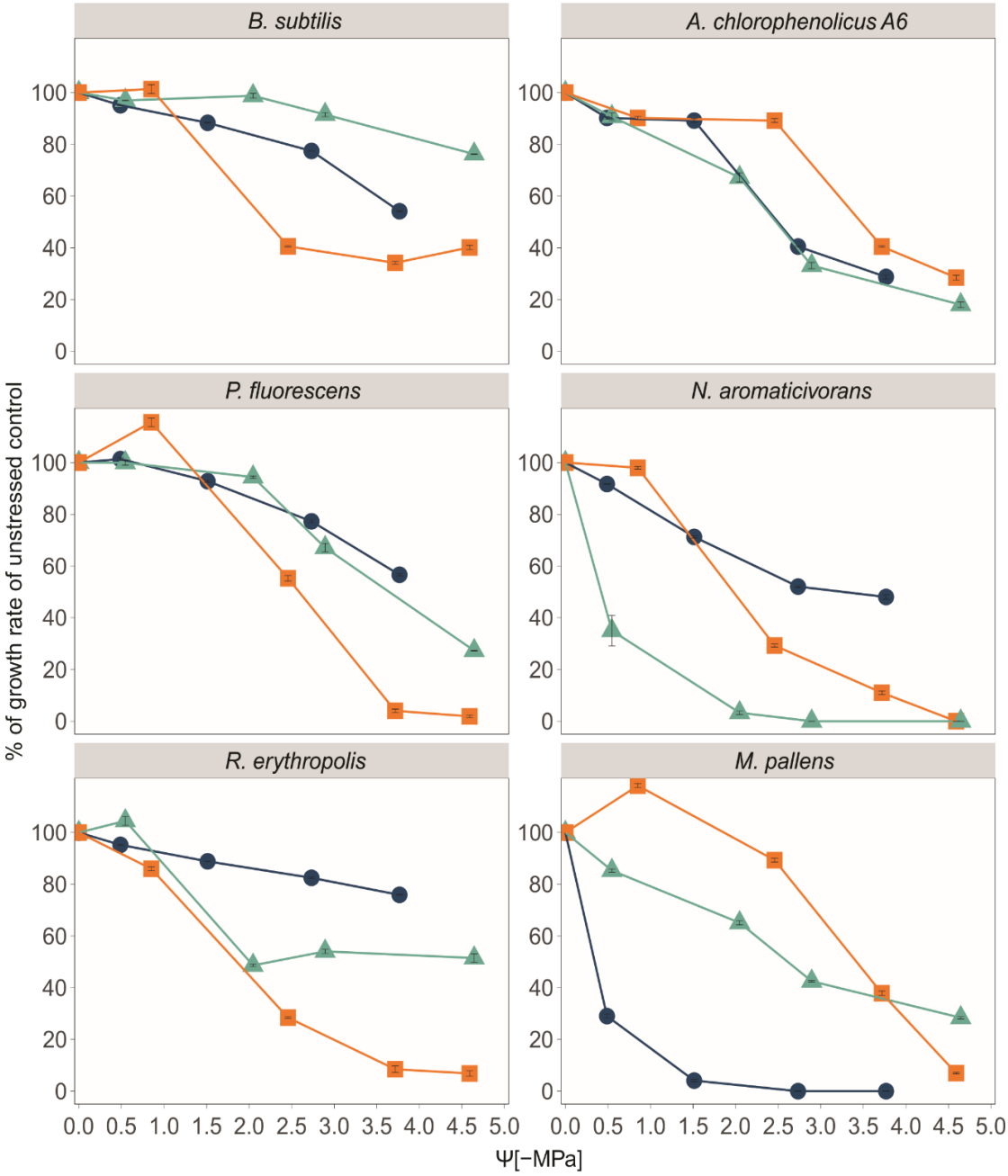

Figure 5 Growth inhibition of bacterial strains, expressed as % of unstressed culture, in different growth conditions: ▲- growth inhibition by osmotic stress submersed growth, ●- growth inhibition by osmotic stress surface growth and ■ - matric stress in submersed growth.

In the presence of osmotic stress, no growth inhibition was observed until the water potential of Ψ_O = -2.0 ± 0.1 MPa, followed by gradual growth inhibition up to 73 ± 1% at Ψ_O = -4.6 ± 0.1 MPa. Exposure of *R. erythropolis* to matric stress resulted in gradual

growth inhibition with decreasing water potential, with full growth inhibition at Ψ_M= -3.7 ± 0.2 MPa. Growth inhibition to 51 ± 1% was observed due to osmotic stress at Ψ_O = -2.5 ± 0.1 MPa, with no further impact of reduced osmotic potential.

The opposite pattern was observed with *A. chlorophenolicus* A6: exposure to osmotic stress resulted in gradual growth inhibition with reduced water potential, with maximal inhibition of 82 ± 2.1% at Ψ_O = -4.6 ± 0.1 MPa. Up to matric potential of Ψ_O = -2.5 ± 0.2 MPa no growth inhibition of *A. chlorophenoilicus A6* was observed. However, further reduction of matric potential resulted in significant growth inhibition, with 71 = -4.6 ± 0.1 MPa.

Growth of *N. aromaticivornas* was inhibited by 65 ± 15% already at water potential of Ψ_O = -0.5 ± 0.1 MPa when exposed to osmotic stress, followed by full inhibition at Ψ_O = -2.0 ± 0.1 MPa. The strain was slightly more tolerant to matric stress. No growth inhibition was observed at Ψ_M= -0.9 ± 0.1 MPa. Further reduction resulted in gradual growth inhibition, reaching 70 ± 5% inhibition at Ψ_M = -2.5 ± 0.1 MPa. Full growth inhibition of *N. aromaticivornats* was observed only at Ψ_O = -4.6 ± 0.1 MPa.

The growth of *M. pallens* was slowly inhibited with decreasing osmotic potential, reaching up to 72 ± 2.9% at osmotic potential Ψ_O = -4.6 ± 0.1 MPa. Significant increase in the growth of *M. pallens* was observed at matric potential of Ψ_M = -0.9 ± 0.1 MPa. However, further reduction had no impact. Growth inhibition was observed starting at Ψ_M= -3.7 ± 0.2 MPa matric potential, with maximal of 93 ± 2.8% growth inhibition at Ψ_M= -4.6 ± 0.1 MPa.

5.1.2 Differential impact of osmotic stress on bacterial growth in surface and submersed growth

Most of the strains responded to osmotic stress differently in submersed growth compared to surface growth (Figure 5), except for. *P. fluorescens* and *A. chlorophenolicus*. Their growth inhibition followed the same trend during surface and submersed growths. Growth of *P. fluorescens* was similarly inhibited in both growth conditions until Ψ_O = -2.9 ± 0.2 MPa osmotic potential in submersed (32 ± 5%) and Ψ_{OS}= -2.7 ± 0.2 MPa in surface growth (23 ± 1.6%), respectively. Further reduction of water potential of the growth media resulted in growth inhibition of 73 ± 0% at Ψ_O = -4.6 ± 0.1 MPa in submersed growth and 43 ± 1% at Ψ_{OS}= -3.7 ± 0.2 MPa in surface growth. Growth of *A. chlorophenolicus* A6 was similarly inhibited in both growth conditions, with highest inhibition of 82 ± 2% and 72 ± 0% at Ψ_O = -4.6 ± 0.1 MPa and Ψ_{OS} = -3.7 ± 0.2 MPa, in submersed and surface growth, respectively.

In submersed growth *B. subtilis* was more resistant to increased osmotic stress, compared to surface growth. The growth was only slightly inhibited (8 ± 0%) in submersed growth until Ψ_O = -2.9 ± 0.2 MPa, while in surface growth at Ψ_{OS}= -2.7 ± 0.2 the growth was already 23 ± 0% inhibited. *B. subtilis* showed maximal inhibition of 45 ± 0.15% at Ψ_{OS}= -3.7 ± 0.2 MPa in surface growth, compared to only 24% at Ψ_O = -4.6 ± 0.1 MPa in submersed growth.

R. erythropolis and *N. aromaticivorans* exhibited higher tolerance towards osmotic stress in surface growth, compared to submersed growth. In surface growth the growth of *R. erythropolis* gradually reduced with increasing osmotic stress, reaching a maximal growth inhibition of 25 ± 0% at Ψ_{OS}=-3.7 ± 0.2 MPa. In submersed growth 50 ± 2% growth inhibition already at Ψ_O = -2 ± 0.1 MPa (Figure 5), however, further increase in osmotic stress did not affect the growth of *R. erythropolis*. Full growth inhibition of *N. aromaticivorans* was observed already at Ψ_O = -2.8 ± 0.2 MPa in submersed growth, while maximal growth inhibition of 48 ± 0% was observed in surface growth already at Ψ_{OS} = -2.7 ± 0.2 MPa, with no further impact. Only the growth of *M. pallens* was fully inhibited during surface growth already at Ψ_{OS} = -1.5 MPa water potential. In submersed growth, increased osmotic stress resulted in gradual decrease in growth, with 72 ± 2.9% growth inhibition at Ψ_O = -4.6 ± 0.1 MPa.

In the stress exposure experiment growth inhibition of all strains has been observed under both stress types (matric and osmotic), with matric stress having a stronger impact at lower water potentials, compared to osmotic stress. Furthermore, the impact of osmotic stress on growth inhibition was observed in both growth conditions (submersed and surface). However, no consistent trends were observed in growth inhibition. Bacterial response to water stress varied depending on the strain, growth condition and stress type.

5.2 Bacterial cell surface hydrophobicity

Changes in cell surface hydrophobicity due to exposure to osmotic stress were measured through contact angle measurements, which is one of the most commonly used method to measure the level of hydrophobicity of a surface. Contact angle values of unstressed controls ranged between 35° and 110° degrees, depending on the strain. The impact of osmotic and matric stress on surface hydrophobicity has been studied. As a response to reduced water potential, an increase in cell surface hydrophobicity was observed for most of the strains for one or both stress conditions. Increase in contact angle already occurred at water potential values of Ψ_O = -0.55 ± 0.1 MPa when

exposed to osmotic stress, and Ψ_M= -0.85 ± 0.1 MPa in case of matric stress, for most strains, with exception of *N. aromaticivorns* in presence of matric stress.
No significant difference was observed in the unstressed controls between surface and submersed growth, with exception of *A. chlorophenolicus A6,* which exhibited significantly higher CSH during surface growth (70 ± 2°), compared to submersed (45 ± 0°) growth.

5.2.1 Differential impact of osmotic and matric stress on the cell surface hydrophobicity

Different patterns of stress response were observed when comparing the impact of matric and osmotic stress on contact angle of studied strains (Figure 6). Results indicate that *B. subtilis* was affected by both matric and osmotic stress, reaching contact angle of $\theta_{O,\,M}$ = 77 ± 2°, in both stress conditions. Increase in contact angle was also observed for *P. fluorescens* when exposed to both, osmotic and matric stress, reaching maximal contact angle values of θ_O=84 ± 4.4° and θ_M =91 ± 6.8°, at water potential values of Ψ_O = -2.9 ± 0.2 MPa and Ψ_M =-3.7 ± 0.2 MPa.
R. erythropolis exhibited high contact angle already in the unstressed controls (θ_M = 104 ± 4.3° and θ_O = 109 ± 3.2°), with no further significant impact of neither matric, nor osmotic stress.
Matric stress had no impact on the surface hydrophobicity of *A.chlorophenolicus*. Contrary to this, with reducing osmotic potential the contact angle of the strain increased to θ_O= 82 ± 5° at water potential of Ψ_O =-4.6 ± 0.1 MPa, compared to unstressed control θ_O = 44 ± 5°.
When exposed to matric stress, *N. aromaticivorans* exhibited reduced contact angles (θ_M =47 ± 3°), compared to the unstressed control (θo = 61 ± 5°) at water potential of Ψ_M= -0.85 ± 0.1 MPa, with slight increase at lower water potentials, reaching contact angles of θ_M = 77 ± 5° at Ψ_M= -2.5 ± 0.2 MPa. Osmotic stress had no significant impact on surface hydrophobicity of *N. aromaticivorans.* Exposing *M. pallens* to osmotic stress resulted in slight increase of contact angle to θ_O= 91 ± 3° at water potential of Ψ_O = -0.54 ± 0.1 MPa, compared to the unstressed control (θ_O= 82 ± 4°), with no further impact of reduced water potential. On the other hand, when exposed to matric stress, contact angle of *M. pallens* increased to θ_M= 110 ± 1.9° at water potential of Ψ_M = -0.85 ± 0.1 MPa. Further reduction of matric potential, led to reduced contact angle (θ_M

= 92° ± 5.1 at Ψ_M=-2.5 ± 0.2 MPa). Due to lack of biomass for CA measurements, no values were obtained for *M. pallens* and *N. aromaticivornas* at low water potentials.

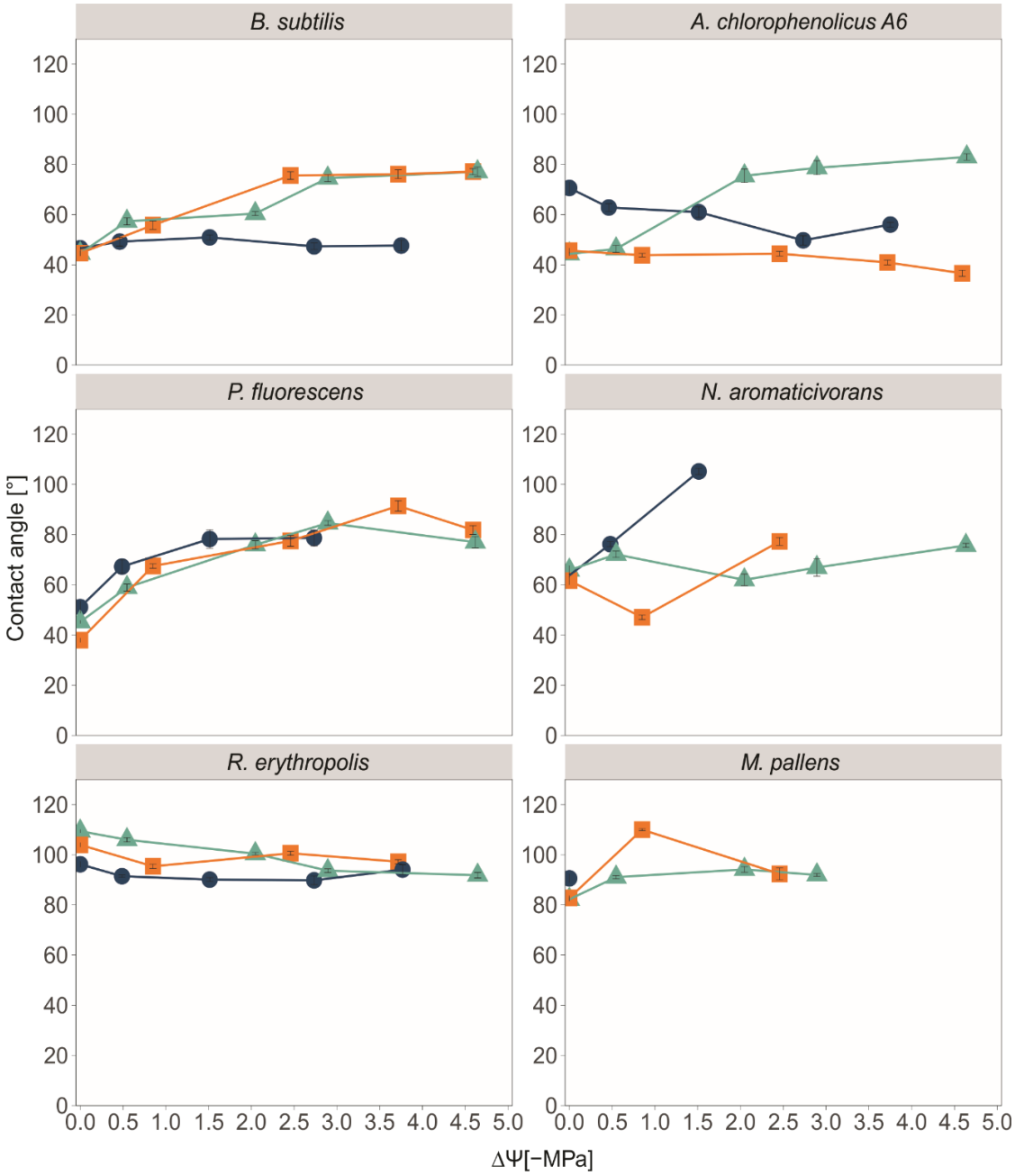

Figure 6 Bacterial cell surface hydrophobicity, expressed as contact angle, in different growth conditions: ▲ - growth inhibition by osmotic stress submersed growth, ● - growth inhibition by osmotic stress surface growth and ■ - matric stress in submersed growth.

5.2.2 Differential impact of osmotic stress on the cell surface hydrophobicity under surface and submersed growth

In surface growth, contact angles of unstressed controls of all strains, with exception of *A. chlorophenolicus A6*, were similar to those of grown in submersed conditions. No impact on *B. subtilis* and *R. erythropolis* surface properties occurred as a result of reduced water potential in surface growth (Figure 6).

No significant difference between unstressed controls of *P. fluorescens* was observed during submersed (θ_O = 45 ± 3°) and surface growth (θo_S= 51 ± 4°). During surface growth the impact of osmotic stress on *P. fluorescens* followed the same pattern as in submersed growth. Contact angles increased with reduction of water potential, reaching θ_{OS} =78 ± 3° at water potential of Ψ_{OS}= -1.5 ± 0.1 MPa, and stayed unchanged at lower values.

Higher contact angle values of unstressed control during surface growth, compared to submersed growth (θ_O = 44 ± 0° and θ_{OS} = 70 ± 2°) were observed for *A. chlorophenolicus A6*. As a result of osmotic stress in surface growth the contact angle of the strain decreased to θ_{OS} = 56 ± 3° at water potential of Ψ_{OS}=-3.7 ± 0.2 MPa, while in submersed growth it increased to θ_O= 82 ± 5° at water potental of Ψ_O = - 4.6 ± 0.1 MPa.

N. aromaticivorans showed increase in surface hydrophobicity during surface growth as a result of stress, increasing the contact angle to θ_{OS} = 105 ± 3.9° at water potential of Ψ_O = -1.5 ± 0.1 MPa.

M. pallens (Figure 6) showed initially high contact angle values, but no significant difference between surface and submersed growth (θ_O = 82 ± 4° and θ_{OS} = 90 ± 5°). Due to slow growth rate of this strain and growth inhibition as a result of osmotic stress no data could be obtained on the impact of osmotic stress on surface hydrophobicity of *M. pallens* during surface growth.

In general, increase in CSH have been observed for most strains in one or more stress type and growth condition. As the growth inhibition, changes in CSH varied depending on the stress type and growth conditions. However, in case of CSH three main trends were observed: 1) increase of SCH with increasing water stress under all conditions investigated, 2) no changes in CSH in initially hydrophobic strains (CA > 80°) and 3) differential response depending on the growth condition and stress type. During surface growth increase in osmotically induced surface hydrophobicity was observed only for G- strains.

5.3 Impact of bacteria on cell-mineral associations

5.3.1 Surface hydrophobicity of cell mineral associations

An experiment was conducted to assess the impact of bacterial attachment on the surface wettability of soil particles and whether the prior exposure of bacteria to water stress will play a differential role in the degree of the impact. Two strains, which were shown to be the most susceptible to osmotic and matric stress in pure culture experiments, *B. sublitis* and *P. fluorescens*, were used. Changes in surface hydrophobicity of this cell-mineral associations over time were monitored via contact angle measurements. Addition of *B. subtilis* cells (10^9 cells g^{-1} quartz) to completely wettable quartz increased the contact angle of the CMAs to θ = 80 ± 6º and θ = 96 ± 8º degrees in case of unstressed and stressed cells, respectively (Figure 7).

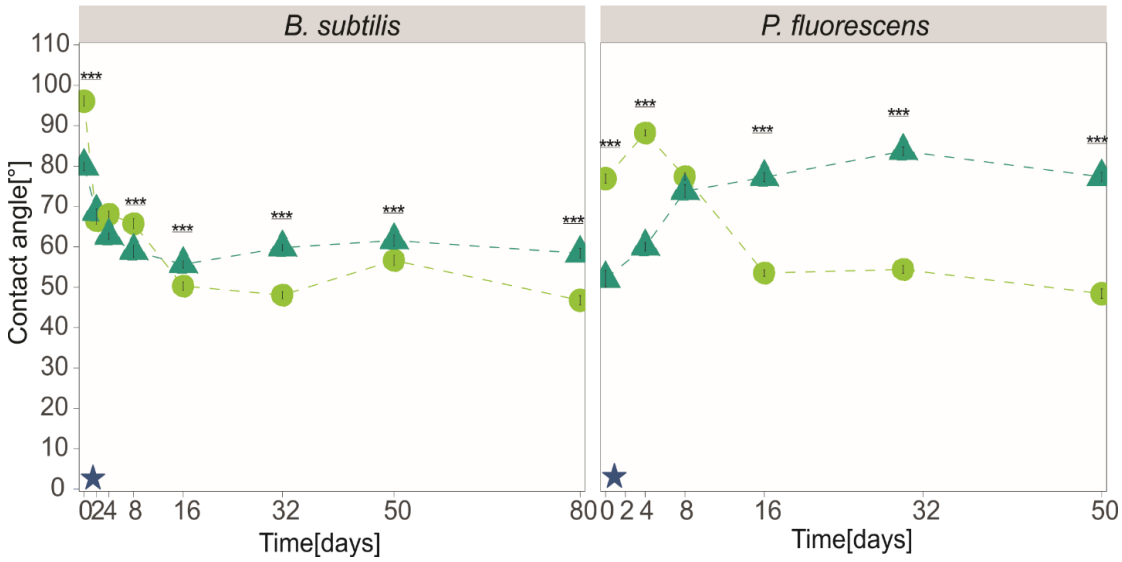

Figure 7. Contact angle of cell-mineral associations of a) *B. subtilis* and b) *P. fluorescens*, and medium-grained quartz (▲ -unstressed cells, ● -osmotically stressed cells and ★ –quartz with no addition of bacteria) over the incubation period of 80 and 50 days. Asterisks indicate significant difference between unstressed and stressed CMAs on a given day. The values are arithmetic mean of eighteen measurements with the error bars indicating the standard error.

During the incubation the contact angle values for both treatments decreased to θ = 55 ± 5º for the unstressed and θ = 49 ± 5º for stressed cells by day 16 and stayed relatively constant (θ = 57 ± 6º for the unstressed and θ = 48 ± 5º stressed) till the end of the experiment.

Addition of *P. fluorecens* to quartz also increased the CA of the CMAs to 52 ± 2º in unstressed CMA and to 77 ± 5º in osmotically stressed CMA at the beginning of

incubation. After 4 days of incubation CA value increased in both treatments. It continued increasing in unstressed treatments but reduced in CMA with stressed cells. In CMA with unstressed *P. fluorescens* cells the CA value reached 74 ± 7^{o} after 8 days of incubation and stayed stable. In stressed CMA CA value reached 53 ± 4^{o} after 16 days and stayed stable until the end of incubation.

5.3.2 Carbon mineralization

Microbial activity in CMAs was monitored by assessing the amount of CO2 mineralized during microbial respiration. Furthermore, the amount of initial and residual bacteria-derived C was monitored. The mineralization of C ceased in both, unstressed and stressed, CMAs for both strains after 16 days (Figure 8). In the CMAs with both strains no difference between mineralization was observed when comparing stressed and unstressed treatments. In the CMAs with addition of *B. subtilis* 12 ± 1% in unstressed and 14 ± 0% in stressed CMAs of the bacteria-derived C was mineralized during the incubation. Furthermore, after 80 days only about 60% of initially added *B. subtilis*-derived C was recovered.

In CMAs with both, unstressed and stressed *P. fluorescens* cells, only 4 ± 0% of bacteria-derived C was mineralized during the incubation. The C loss from the *P. fluorescens* CMAs was very low, with almost 90% of bacterial-derived C being recovered at the end of the incubation.

The high levels of loss are probably due to measurement error. Due to considerably low concentration of C in the analyzed samples the size of the probes had to be significantly increased, leading to technical errors during the analysis.

In summary, the stressed bacterial cells hindered quartz particles water repellent in CMAs with both strains. However, *B. subtilis* had stronger impact, compared to *P. fluorecens*. CMAs with *B. subtilis* had significantly higher CA, compared to the CA of the pure culture, in both, stressed and unstressed CMAs, while the initial CA of CMAs with *P. fluorescens* cells were similar to the CA of the unstressed and stressed pure culture. Nevertheless, within the first 7 days of incubation the CA of *B. subtilis* CMAs decreased, while increase was observed in *P. fluorescens* CMAs, followed by reduction in the CMA with stressed *P. fluorescens* cells. These changes in surface wettability of CMAs were accompanied by reducing respiration rates, which completely ceased after 7 days in CMAs with both strains.

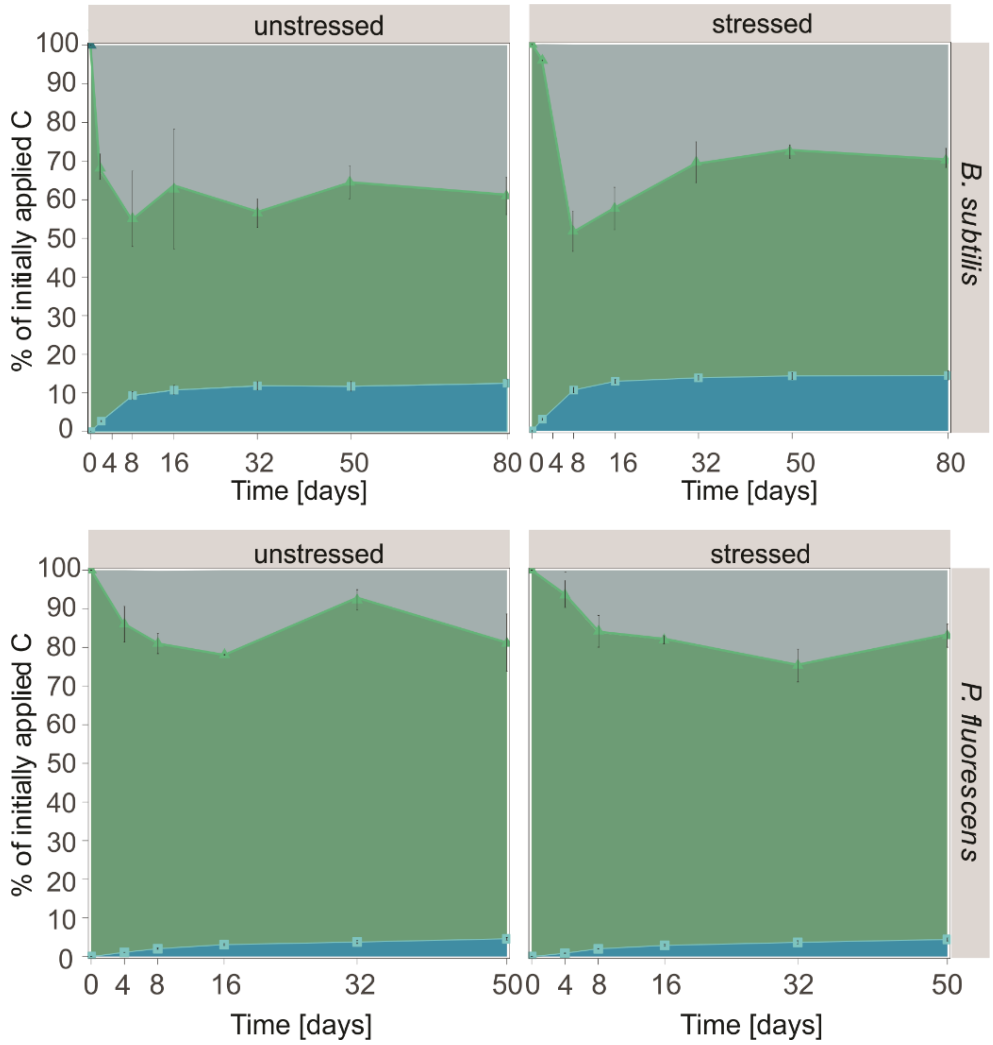

Figure 8 Carbon mass balance for unstressed *B. subtilis* CMAs, stressed *B. subtilis* CMAs, unstressed *P. fluorescens* CMAs, and stressed *P. fluorescens* CMAs, where blue indicates mineralized C, green –*B. subtilis/P. fluorescens* derived C and grey –C loss from the system. The values are arithmetic means of n = 3 measurements with the error bars indicating the standard error.

5.3.3 PLFA composition of *B. subtilis* CMAs

Abundance of PLFA were used as a proxy to describe the microbial community structure and living microbial biomass in the CMAs, containing osmotically stressed and unstressed *B. subtilis* or *P. fluorescens* cells, control containing only medium grained quartz and bacterial extract from CAL soil.

At the beginning of the experiment, the total amount of PLFA was slightly higher in the CMAs with unstressed *B. subtilis* cells (646 ± 27 nmol·g^{-1}), compared to the osmotically stressed ones (562 ± 25 nmol·g^{-1}'; Figure 9; Table 8, Appendix).

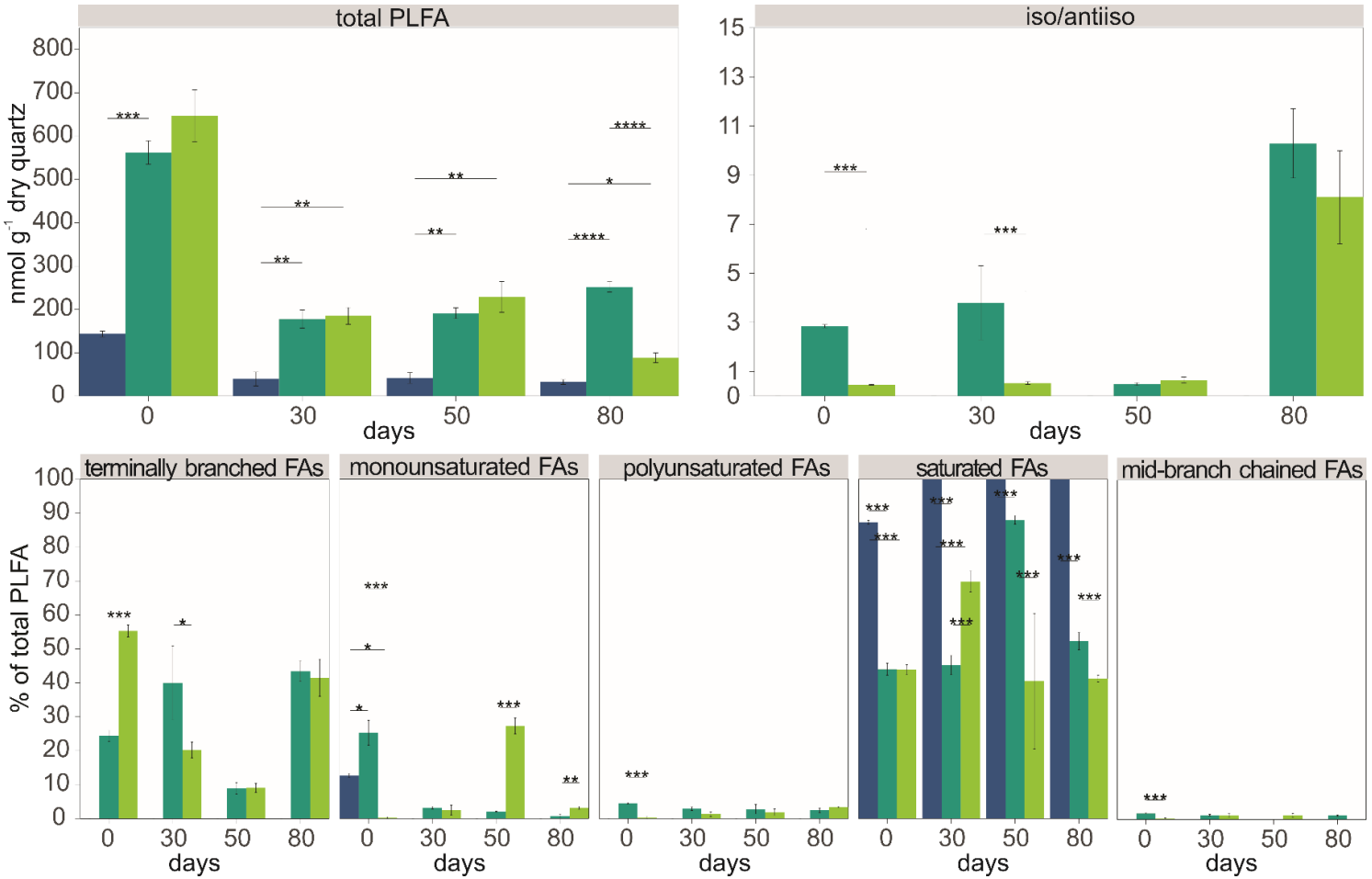

Figure 9 PLFA composition of B. subtilis CMAs. Dark blue indicates the control treatment (no *B. subtilis* cells), dark green –CMAs with stressed *B. subtilis* cells and light green –unstressed *B. subtilis* cells. The values are arithmetic mean of n = 3 measurements with the error bars indicating the standard error. Asterisks show statistically significant differences between control, stressed and unstressed treatments.

After 30 days significant decrease in the abundance of PLFA was observed in both CMAs. No difference was observed between the treatments. The amount of total PLFA in CMAs with osmotically stressed *B. subtilis* cells stayed relatively stable until 50 days of incubation, with slight increase by the end of the experiment. In the CMA with unstressed *B. subtilis* total amount of PLFA decreased at the end of the experiment. In the control treatment amount of total PLFA decreased already at day 30 and stayed stable later.

Branched fatty acids, particularly i15:0 and a15:0 are the major and characteristic component of lipid membrane of *B. subtilis.* Changes in the iso/anteiso ratio have been shown to occur as a result of hyperosmotic stress, thus are calculated in our study, as stress indicator. At the beginning of the incubation a significant difference has been observed in iso/anteiso ratio between unstressed (0.5 ± 0.02)) and osmotically stressed (2.8 ± 0.1)) CMAs. Though, the differences were observed at the end of the incubation between the treatments, the ratio was significantly higher in both, compared to the beginning of the incubation. In both CMAs, iso/anteiso ratio increased to 8.1 ± 2.7 in unstressed and 10.3 ± 2.0 in osmotically stressed CMAs. In all three treatments decrease in overall PLFA concentration and the relative amounts of all PLFAs was detected (due to bacterial death), with exception of 18:2ω9, i16:0, 18:1ω9 18:0 and 15:0 in CMAs amended with unstressed *B. subtilis*, which decreased in concentration but increased the relative abundance.

5.3.4 PLFA composition of *P. fluorescens* CMAs

As in CMAs with *B. subtilis*, total amount of PLFA in *P. fluorescens* CMAs decreased during the incubation in both unstressed and osmotically stressed treatments. No difference was observed as a result of exposure to osmotic stress. In both treatments total PLFA reduced from approximately 250 nmol·g^{-1} to 60 nmol·g^{-1}, throughout the incubation (Figure 10; Table 9, Appendix). In both, unstressed and osmotically stressed *P. fluorescens* CMAs saturated fatty acids 16:0 and 18:0, and monounsaturated fatty acids 16:1ω7t, 16:1ω7c, 18:1ω7t, 18:1ω7c, 17:0 cyclic and 19:0 cyclic were identified. At the beginning of the incubation no significant differences were observed between any of the PLFA groups between stressed and unstressed CMAs. Monounsaturated fatty acids were significantly lower in the unstressed CMA after 30 days (9 ± 2%), compared to the osmotically stressed treatment (22 ± 6%) until the end of incubation.

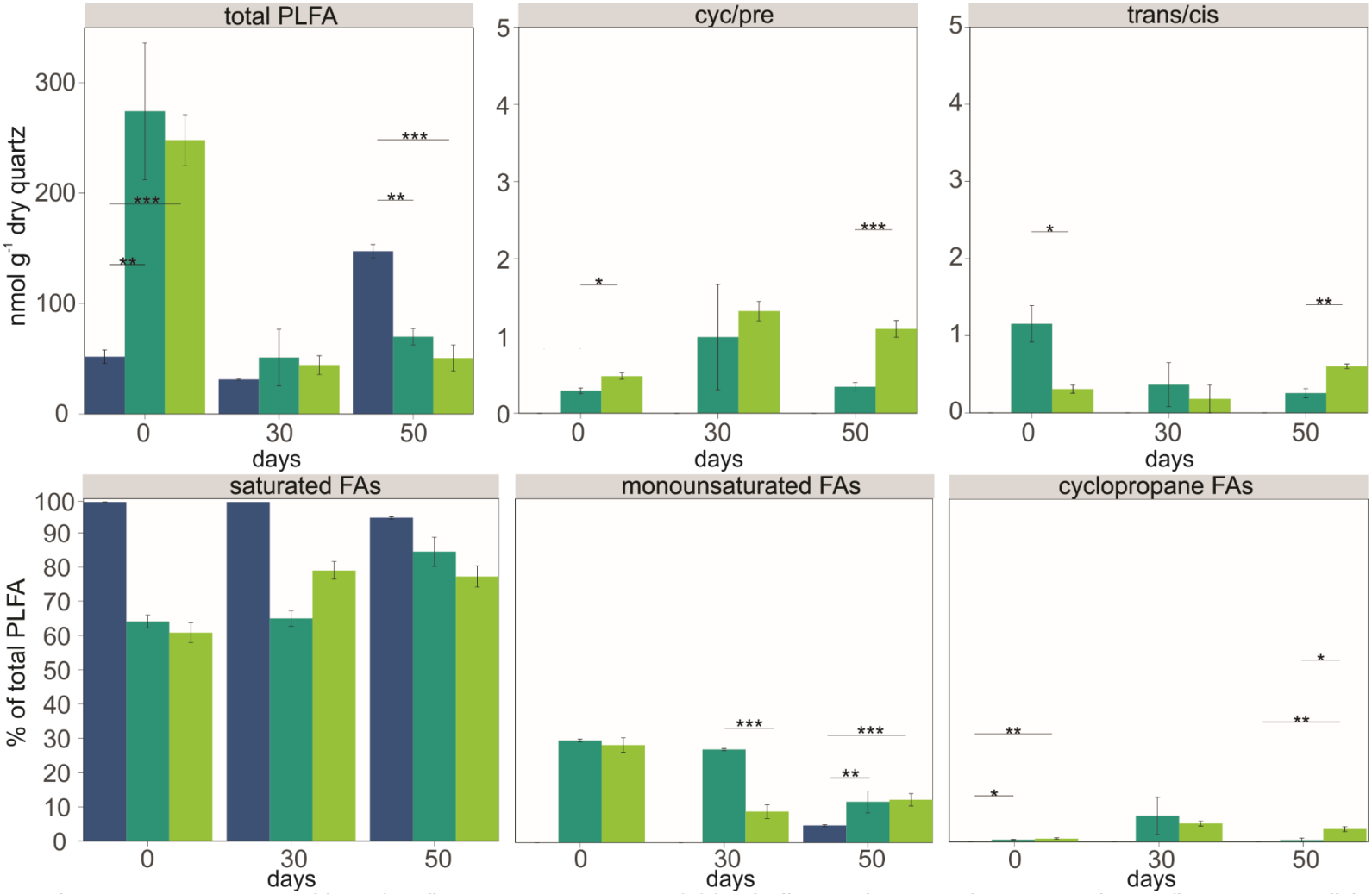

Figure 10 PLFA composition of *P. fluorescens* CMAs. Dark blue indicates the control treatment (no *P. fluorescens* cells), dark green –CMAs with stressed *P. fluorescens* cells and light green –unstressed *P. fluorescens* cells. The values are arithmetic mean of n = 3 measurements with the error bars indicating the standard error. Asterisks show statistically significant differences between control, stressed and unstressed treatments.

However, due to reduction in relative abundance of monounsaturated fatty acids in stressed CMAs, no significant differences were observed between unstressed (13 ± 2%) and stressed (12 ± 3%) CMAs at the end of the incubation. Trans/cis ratio was higher at the beginning in osmotically stressed CMAs (1.2 ± 0.3), compared to unstressed ones (0.3 ± 0.07). During the incubation the trans/cis ratio increased in unstressed CMA from 0.3 ± 0.1 to 0.6 ± 0.0 and decreased in osmotically stressed CMAs from 1.2 ± 0.28 to 0.2 ± 0.1. The ratio of cyclopropyl fatty acids to their precursors was significantly higher in the unstressed CMAs (0.5 ± 0.0) compared with osmotically stressed CMAs (0.3 ± 0.0) at the beginning of the incubation.

Overall, reduction in the total amount of PLFA during the incubation was observed in CMAs with both strains. Ratios of iso/antiiso and trans/cis fatty acids, used as stress indicators for G+ and G- bacteria, respectively, were as expected initially higher in the CMAs with stressed cells, compared to unstressed ones. However, this difference was not observed already after 50 days in *B. subtilis* CMAs. In *P. fluorecnes* CMAs the trans/cis ratio was higher in the unstressed CMAs, which was accompanied with significantly higher cy/pre-ratio, indicating reduced metabolic activity.

5.4 Impact of soil water content on wettability

Soil samples, collected from two different locations, were incubated at three different incubation modes (details in section 4.3.1). The initial physical and chemical properties of both soils are presented in Table 4. Though both soils were similar in their physico-chemical properties analyzed, they had significantly different surface hydrophobicity. The LUE soil collected from Luneburg Heath in Lower Saxony exhibited moderate surface hydrophobicity ($\theta = 82 \pm 7°$), while the CAL soil collected from Altmark region in Saxony-Anhalt was initially extremely hydrophobic ($\theta = 128 \pm 6°$).

As previously mentioned in section 4.3.3, the soils were adjusted to different water contents, corresponding to pF2.5 (“wet” incubation mode, at field capacity), pF4.2 (“dry” mode, permanent wilting point) and cycling between pF2.5 and pF4.2 (“intermittent” mode) The measured WC at each sampling points are presented in Figure 11.

The water content of LUE soil was adjusted to 29 ± 0% for “wet” and “intermittent” modes and to 16 ± 1% for “dry” mode. The “intermittent” mode was adjusted to fluctuate between pF2.5 (29 ± 0%) and pF4.2 (16 ± 1% WC), however, lowest water content achieved was 22 ± 0%.

Water content of “wet” and “intermittent” modes in CAL soil was adjusted 22 ± 0%.

Water content of “dry” mode was initially adjusted to 16 ± 0%. The “intermittent” mode fluctuated between 17 ± 0%.and 22 ± 0%. About 5% water loss was observed in samples, due to evaporation from the system, therefore after the sampling soil water content was adjusted by addition of deionized water to pF2.5 and pF4.2.

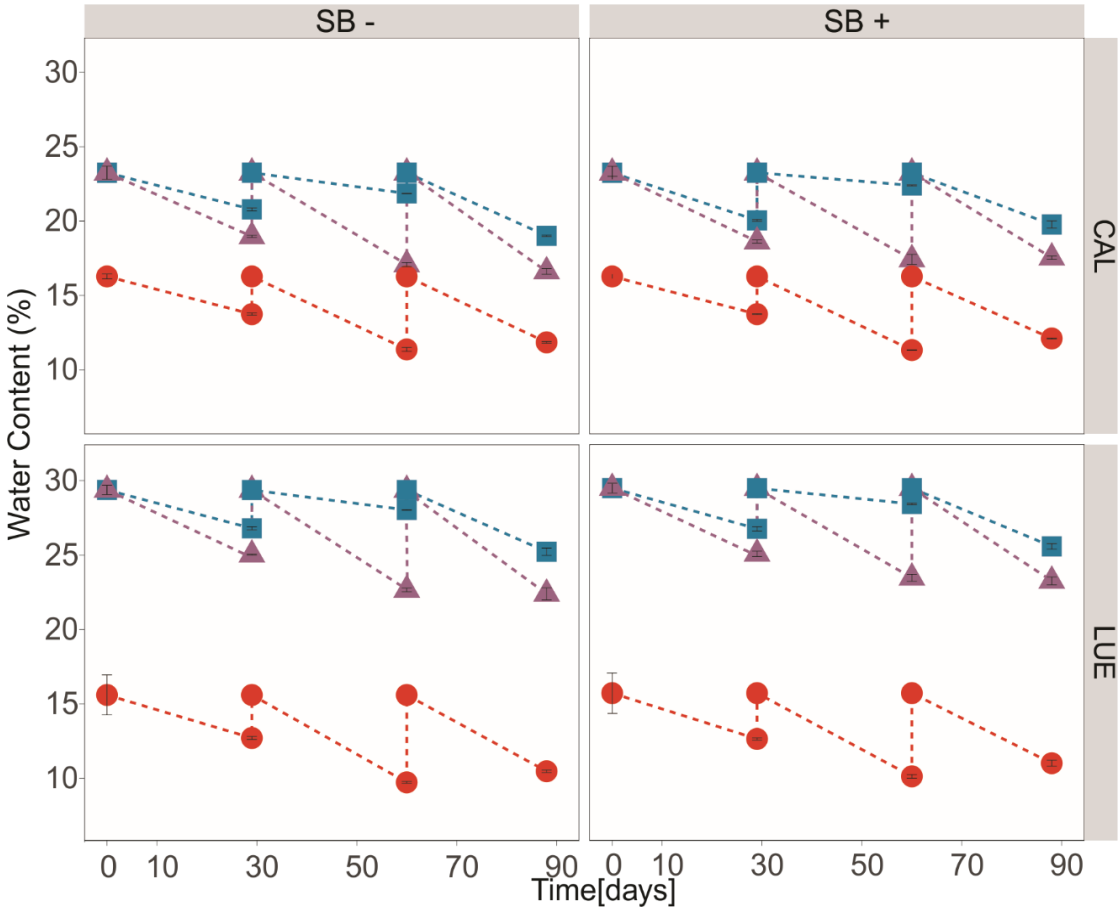

Figure 11 Measured WC of CAL and LUE soils during the incubation in “wet”–blue, “dry”–red and “intermittent” –purple incubation modes, without (SB-) and with substrate amendment (SB+). The values are arithmetic means of n = 3 measurements with the error bars indicating the standard error.

After adjustment of soil moisture to corresponding pF values the contact angle of CAL soil increased from 128° ± 6° to θ = 131 ± 2° but stayed unchanged throughout the incubation.

The adjustment of soil moisture to pF2.5 had no significant impact on the contact angle of LUE soil, while at pF4.2 it increased to 100 ± 1°. Further changes in soil surface hydrophobicity, driven by the different incubation modes, were assessed throughout the 88 days of incubation and are presented in Figure 12. No differences were observed between the incubation modes and substrate levels in CAL soil. In contrast, the “dry” incubation mode and substrate amendment significantly affected soil surface

hydrophobicity of LUE soil. The contact angle of non-amended soil at the “dry” (θ = 100 ± 2°) incubation mode was initially higher, compared to “wet” and “intermittent” (θ = 73 ± 5°) modes and slightly increased by the end of incubation period (θ = 114 ± 4°). Substrate amendment resulted in higher contact angle already at the beginning of the incubation in all incubation modes (θ = 88 ± 2° (“wet”) and θ = 124 ± 4° (“dry”)), compared to non-amended soils (θ = 73 ± 5° (“wet”) and θ = 100 ± 2° (“dry”)). With exception of “intermittent” mode the contact angle of the soils stayed unchanged. After 88 days sight reduction in surface hydrophobicity was observed in the soil at “intermittent” incubation mode, reaching θ = 76 ± 2° from initial θ = 88 ± 2°.

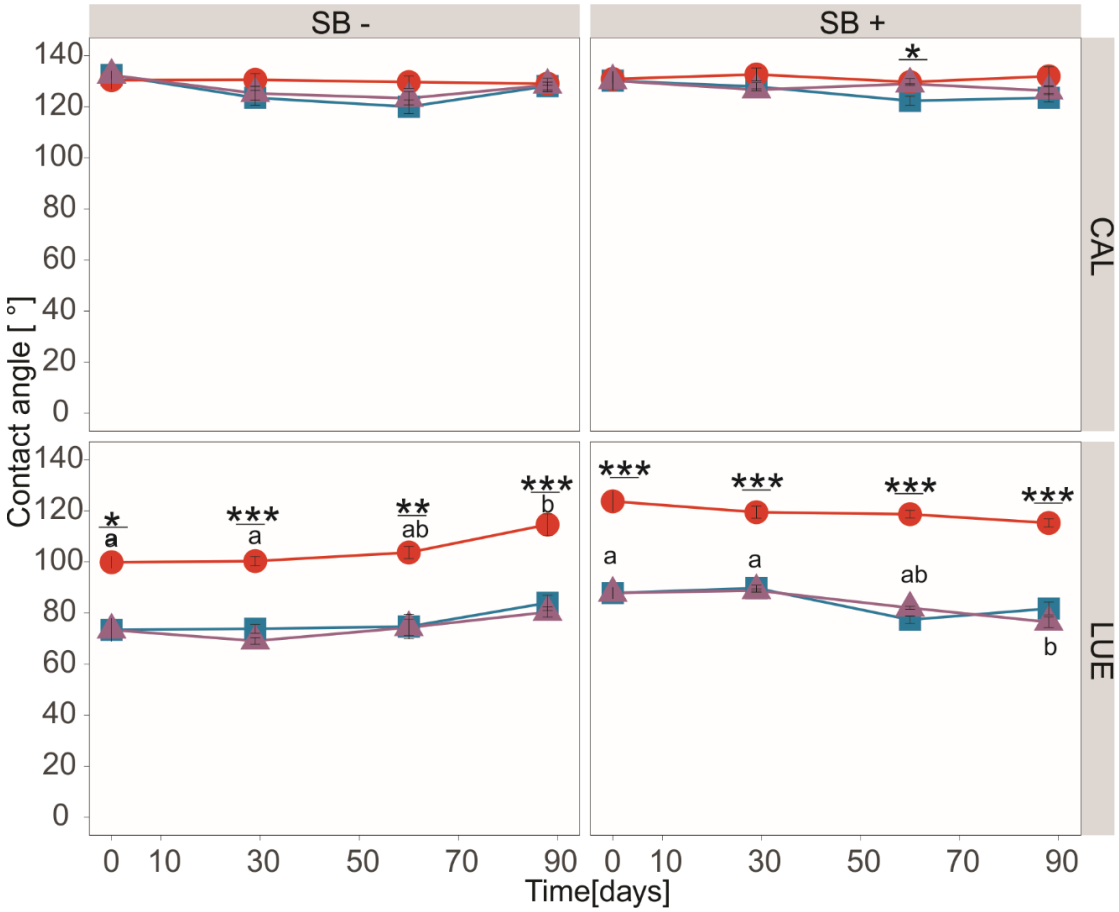

Figure 12 soil surface hydrophobicity of CAL and LUE soils, expressed as contact angle. Red represents “dry”, blue –“wet” and purple –“intermittent” incubation modes, without (SB-) and with substrate amendment (SB+). The values are arithmetic mean of n=10 measurements with the error bars indicating the standard error. Asterisks indicate the significant difference between the water regimes and letters the within-treatment differences over time.

In summary, neither the water regime, nor substrate amendment had an impact on the CA of the initially hydrophobic (CAL) soil. In contrast, the CA of moderately hydrophobic (LUE) soil was influenced by both, drying and substrate amendment. The

CA of the “dry” LUE soil was already significantly higher compared to “wet” soil at the beginning of the incubation and continued increasing at a slow rate overtime. Substrate amendment resulted in slightly higher initial CA for all incubation modes, compared to unamended ones. However, in the amended soil, while no changes occurred in “dry” mode, slight decrease was observed in the “intermittent” mode.

5.5 Soil microbial community structure under different water regimes

Impact of soil water content on the microbial community composition in 2 soils with different levels of water repellency and drought history was assessed via and incubation experiment. Changes in community composition were analyzed by means of PLFA and next generation sequencing of 16S rRNA genes. Preliminary analysis of the results indicated that in the range of the water contents analyzed, the impact of soil moisture level was less pronounced compared to the differences observed between soils and driven by addition of substrate. Therefore, the results will be presented separate for the two soils.

5.5.1 LUE soil microbial biomass and community composition

5.5.1.1 PLFA profile of LUE soil and changes over time

Total microbial biomass in all soils was analyzed by extraction of PLFA and is presented in (Figure 13; Table 10, Appendix). The initial amount of total PLFA was significantly higher in the “dry” mode, compared to “wet” soil. Bacterial biomass in “wet” and “intermittent” LUE soil at both substrate levels stayed relatively stable during the incubation. In the non-amended “dry” treatment initially significant higher amount of total PLFA was detected (8.9 ± 0.4 µmol·g^{-1}), however it reduced to 4.7 ± 0.4 µmol·g^{-1} after 29 days and stayed stable hereafter. The substrate amendment increased the total amount of PLFA in “dry” treatment after 88 days (7.1 ± 0.2 µmol·g^{-1}) compared to initial amount (5.3 ± 0.6 µmol·g^{-1}).

At the beginning of the experiment all the incubation modes were dominated by the abundance of monounsaturated fatty acids (Figure 13), indicating abundance of G-bacteria.

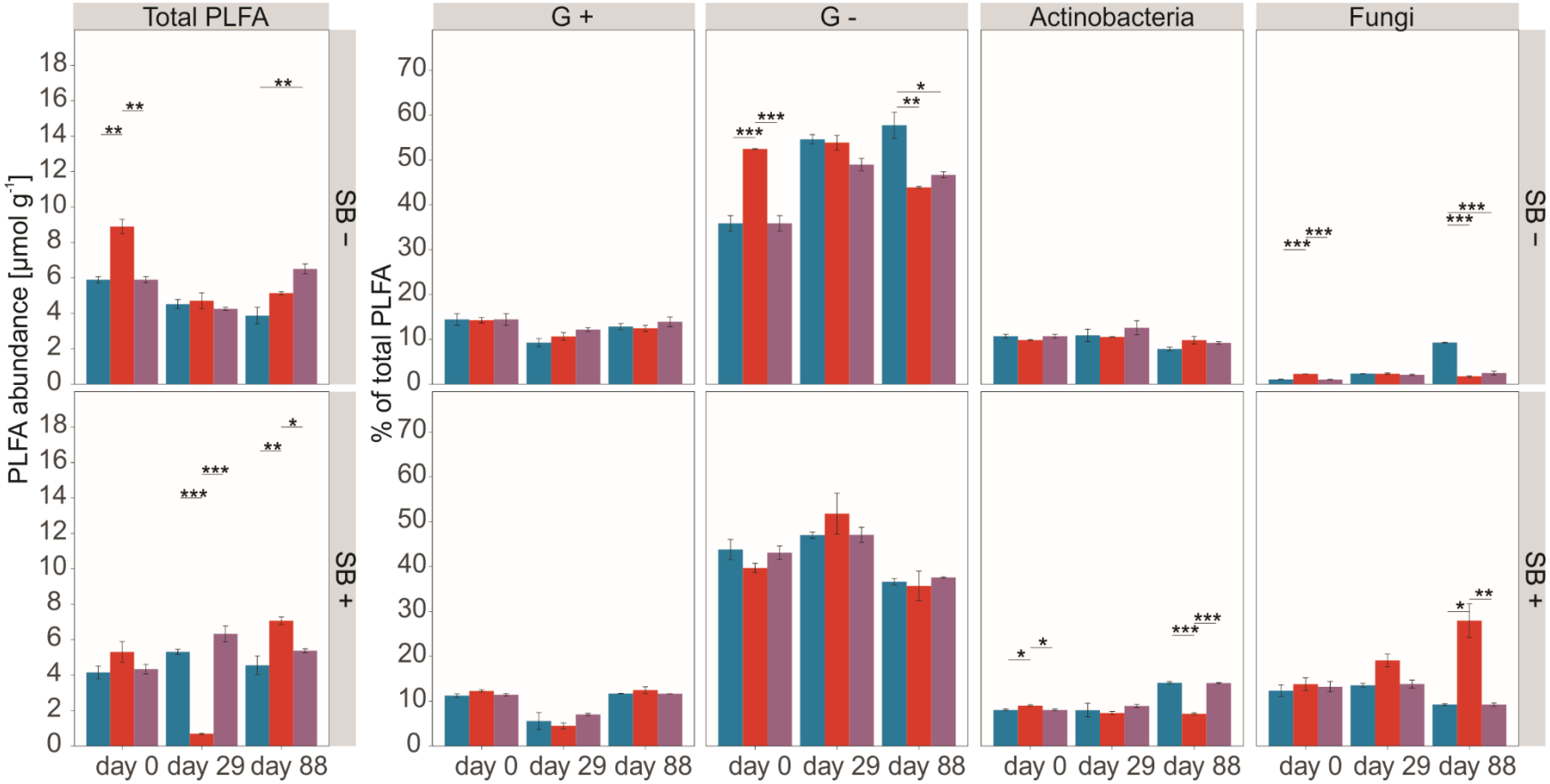

Figure 13. PLFA composition of LUE soil in non-amended (SB-) and substrate amended (SB+) treatments. Blue stands for “wet” incubation mode, purple –“intermittent” and red for “dry” mode. Values are arithmetic mean of n = 3 measurements with the error bars indicating the standard error.

In the “wet” and “intermittent” soils the relative abundance of G- bacteria increased from initial 36% ± 2 to 58% ± 3 (“wet”) and 47% ± 1 (“intermittent”) at the end of the incubation. In non-amended “dry” soil it decreased significantly from initial 52% ± 2 to 44% ± 4.

G-bacterial PLFA in substrate amended soil were more resistant to “dry” treatment. G+ bacterial PLFA were relatively stable throughout the incubation at all incubation modes and substrate levels.

Relative abundance of Actinobacteria was stable during the incubation at all incubation modes in the non-amended soil. Substrate amendment increased the relative abundance of Actinobacterial PLFA in “wet” and “intermittent” treatments from initial 8 ± 0% to 14 ± 0% after 88 days. No changes occurred in the “dry” treatment.

Fungal PLFA (18:2ω6, 9) stayed relatively stable during the incubation experiment in the soil at both substrate levels and all incubation modes, with exception of non-amended “wet” and substrate amended “dry” soil. In the non-amended “wet” treatment a significant increase was observed after 88 days, reaching 9± 0% relative abundance from initial 1%. Substrate amendment resulted in increase of fungal PLFA from 14 ± 1% to 28 ± 4% “dry” soil within 88 days.

In general, the amount of total PLFA slightly fluctuated in all incubation modes and substrate levels, but no significant changes were observed. The relative abundances of G+ and Actinobacterial PLFA were also relatively stable. The main changes were observed in the relative abundance of G- and fungal PLFA. Substrate amendment induced the growth of fungi in all modes, while the G- PLFA were more affected in non-amended soils. Additionally, in non-amended soil the relative abundance of fungi increased over time in the “wet” mode, while in substrate amendment promoted fungal growth in “dry” incubation mode.

5.5.1.2 Impact of soil water content on microbial community structure of LUE soil

The total amount of fatty acids detected in the non-amended LUE soil at the beginning of incubation of was significantly lower in “wet” and “intermittent” soils (6 ± 0 $\mu mol \cdot g^{-1}$) compared to “dry” soil (9 ± 0 $\mu mol \cdot g^{-1}$) (Figure 13), but no significant differences were observed at the end of the incubation. While water content had no significant impact on the total FAs in the substrate amended soil at the beginning of the incubation, after 88 days it was significantly higher in “dry” soil (7.0 ± 0.2 $\mu mol \cdot g^{-1}$), compared to “wet” (4.6 ± 0.5 $\mu mol \cdot g^{-1}$) and “intermittent” (5.4 ± 0.1 $\mu mol \cdot g^{-1}$) soils.

Relative abundance of G- bacterial PLFAs was higher in non-amended “dry” (52 ± 0%)

soil at the beginning of the incubation, compared to “wet” and “intermittent” soils (36 ± 3%) soils (Figure 13). After 88 days it decreased to 44 ± 0% in the “dry” soil, while increased to 58 ± 3% in the “wet” soil. The abundance of G- bacterial PLFA in the “intermittent” mode also increased at the end of the incubation but reached to 47 ± 1%. No differences between the incubation modes were observed in substrate amended soil. No differences in relative abundance of G+ bacterial PFLA was observed between the incubation modes at both substrate levels.

Initially, fungal PLFAs were more abundant in the non-amended “dry” (2.3 ± 0.0%) soil, compared to the “wet” (1.1 ± 0.0%) modes. However, due to an increase in “wet” treatment after 88 days it was significantly higher in “wet” soil (9.3 ± 0.1%). A slight increase in the “intermittent” treatment resulted in relative abundance similar to “dry” mode, 1.8 ± 0.1% and 2.5 ± 0.4% in “dry” and “intermittent” modes, respectively. In contrast, no differences in relative abundance of fungi were observed in the substrate amended soil between the incubation modes at the beginning of incubation (~13 ± 1%). After 88 days the fungal PLFA constituted only 9 ± 0% in “wet” and “intermittent” soils and 28 ± 6% in the “dry” soil.

No differences in relative abundance of Actinobacteria was observed between the incubation modes at the beginning of the incubation at both substrate levels. After 88 days in substrate amended soil, it was twice lower in the “dry” soil (7 ± 0%), compared to “wet” and “intermittent” modes (14 ± 0%).

Overall no major changes were observed in the total microbial biomass due to differences in water contents between incubation modes. No major differnces were observed besides significantly higher relative amount of fungal PLFA in the substrate amended “dry” soil, compared to the “wet” modes at the end of the incubation.

5.5.1.3 Microbial community structures of LUE soil assessed by 16S rRNA gene sequencing

To obtain more detailed information about changes in microbial community structure and possibly identify the drivers of those changes, the 16S rRNA genes extracted from the soils were amplified and sequenced. Alpha diversity indices were calculated from rarefied to samples (122020 ASV counts per sample). Observed alpha diversity (diversity richness), showing the number of ASVs present in the sample, indicated no significant difference between “wet”, “dry” and “intermittent” incubation modes at the beginning of the incubation. Reduction in species richness from initial 856 ± 23 to 650 ± 19 was observed in the “dry” mode at the end of the incubation. No significant

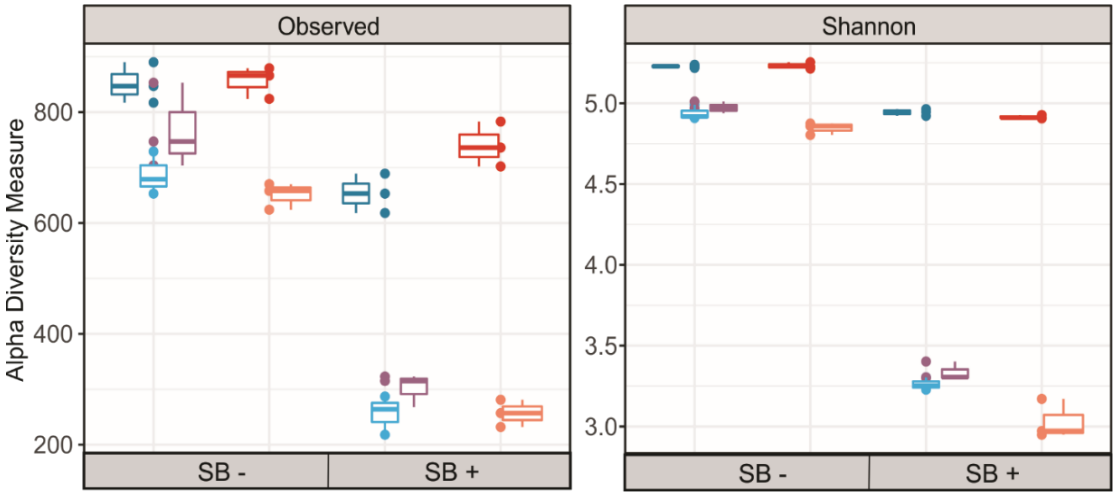

Figure 14 Species richness(observed) and Shannon diversity index of non-amended (SB-) and substrate amended (SB+) LUE soil. Red represents the “dry” mode at the beginning of incubation. Dark blue –“wet” and “intermittent” modes at the beginning, light blue –“wet” mode end, purple –“intermittent” end and orange –“dry” end.

changes happened in other incubation modes (Figure 14). In substrate amended soil the species richness reduced from initial 653 ± 29 in “wet” and 740 ± 33 in “dry” modes to 256 ± 25 in “dry” and “wet” modes. In the “intermittent” mode it was slightly higher (302 ± 24).

Shannon index was also calculated to characterize the species diversity of the samples (Figure 14). In non-amended soil no significant differences were observed between the incubation modes, both at the beginning (5.2 ± 0.0) and at the end of the incubation (5 ± 0) in “wet” and “intermittent” modes and 4.8 ± 0.0 in “dry” mode). As in case of species richness, no difference in species diversity was observed at the beginning of the incubation between incubation modes (4.9 ± 0.0) in substrate amended soils.

At the end of the incubation species diversity reduced in all mode (3.3 ± 0.0 in “wet”, 3.2 ± 0.0 in “intermittent” modes and 3 ± 0 in “dry” samples). Substrate amendment resulted in reduced species diversity in all incubation modes, compared to non-amended soils, following the same pattern as the observed diversity.

In summary, in LUE soil species richness and Shannon index reduced in all incubation modes, however, the reduction was more pronounced in the “dry” mode. Substrate amended soils were initially contained slightly a smaller number of species, compared to unamended ones. Furthermore, the number of species of the amended soil declined more significantly. While the incubation modes did not cause any differences in the species richness of amended soils, the species heterogeneity was significantly lower in “dry” soil.

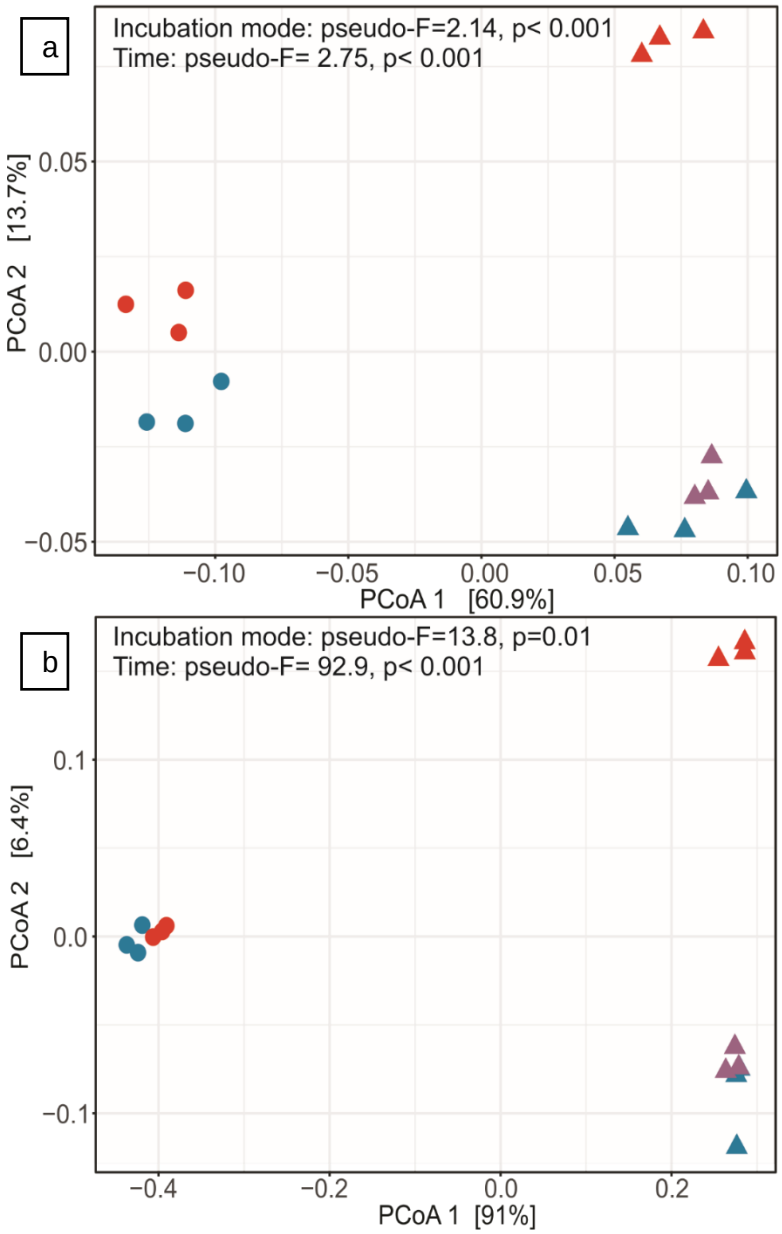

Figure 15 PCoA of beta diversity of LUE soil, based on Bray-Curtis dissimilarities for a) non-amended, b) substrate amended soil. Red circles represent “dry” mode at the beginning, blue circles –“wet” and “intermittent” beginning. Red, blue and purple triangles represent “dry”, “wet” and “intermittent” modes, respectively, at the end of incubation.

To obtain information on changes in the overall species diversity in the soils that occurred due to different incubation modes and substrate levels in the LUE soil, beta diversity was assessed and is presented in Figure 15. The overall variance of 74.6% and 97.4% was explained by the PCoA, respectively for non-amended and substrate amended soils.

In non-amended soil 60.9% of the variance was explained by the first principal component (PC), and 13.7% by the second one. In substrate amended soil 91% of variation was explained by the first PC, and 6.4% by the second one (Figure 15, a). The PCoA revealed no differences in composition of microbial communities between the incubation modes at the beginning of the incubation, neither in non-amended, nor in substrate amended (Figure 15, b) soils. At the end of the incubation, differentiation between “dry” and “wet” and “intermittent” incubation modes occurred at both substrate levels.

Further assessment of high-throughput sequencing data showed that both, non-amended “wet” and “dry” soils were initially dominated by 12 orders (> 1% relative abundance), belonging to phylum Proteobacteria (~39%), Acidobacteria (~29%), Actinobacteria (~27%), Firmicutes (~3%) and Planctomycetes (~1%; Figure 16).

The most abundant phylum Proteobacteria consisted of Rhizobiales (~22%), followed by Elsterales (~10%), Acetobacteriales (~5%) and Micropepsales (~1.5%) orders (Figure16). Acidobacteriales (~22%) and Solibacteriales (~7%) constituted the second most abundant phylum Acidobacteria. Within Actinobacteria phylum Frankiales (~19%) was the most abundant order, followed by uncultured Acidimicrobiia (~5%) and IMCC26256 (~1%). Two uncultured orders from phylum Firmicutes (~3%) and Isospherales (~1%) from Planctomycetes phylum were also identified.

After 88 days of incubation the most affected phylum in all incubation modes was Acidobacteria. Relative abundance of Acidobacteriales order decreased from initial 23% to 20%, 17% and 14%, respectively in the “wet”, “intermittent” and “dry” soils. Solibacteriales decreased from initial 7% relative abundance to 3% in all incubation modes. In contrast, relative abundance of Frankiales order (Actinobacteria) increased from 19% to 28%, 29% and 30%, respectively in “wet”, “intermittent” and “dry” soils. Slight decrease in relative abundance of Rhizobiales order was also observed in “wet” soil in the end of the incubation (20%), compared to initial 23%.

Overall, the community of the unamended soil was relatively stable under all incubation modes. Slight shifts were observed due to increase in relative abundances of Actinobacteria and simultaneous decrease in Acidobacteria. The shifts in microbial community were more accentuated by addition of substrate. Representatives of several orders were very susceptible to substrate addition and were not detected in the amended soils (relative abundance >1%): Isospharales, Micropepsales, and 2 orders of Firmicutes phyla. Furthermore, significant differences were observed in relative abundances between the incubation modes in substrate-amended soils with the diversity declining in the following order “wet” >” intermittent” > “dry”.

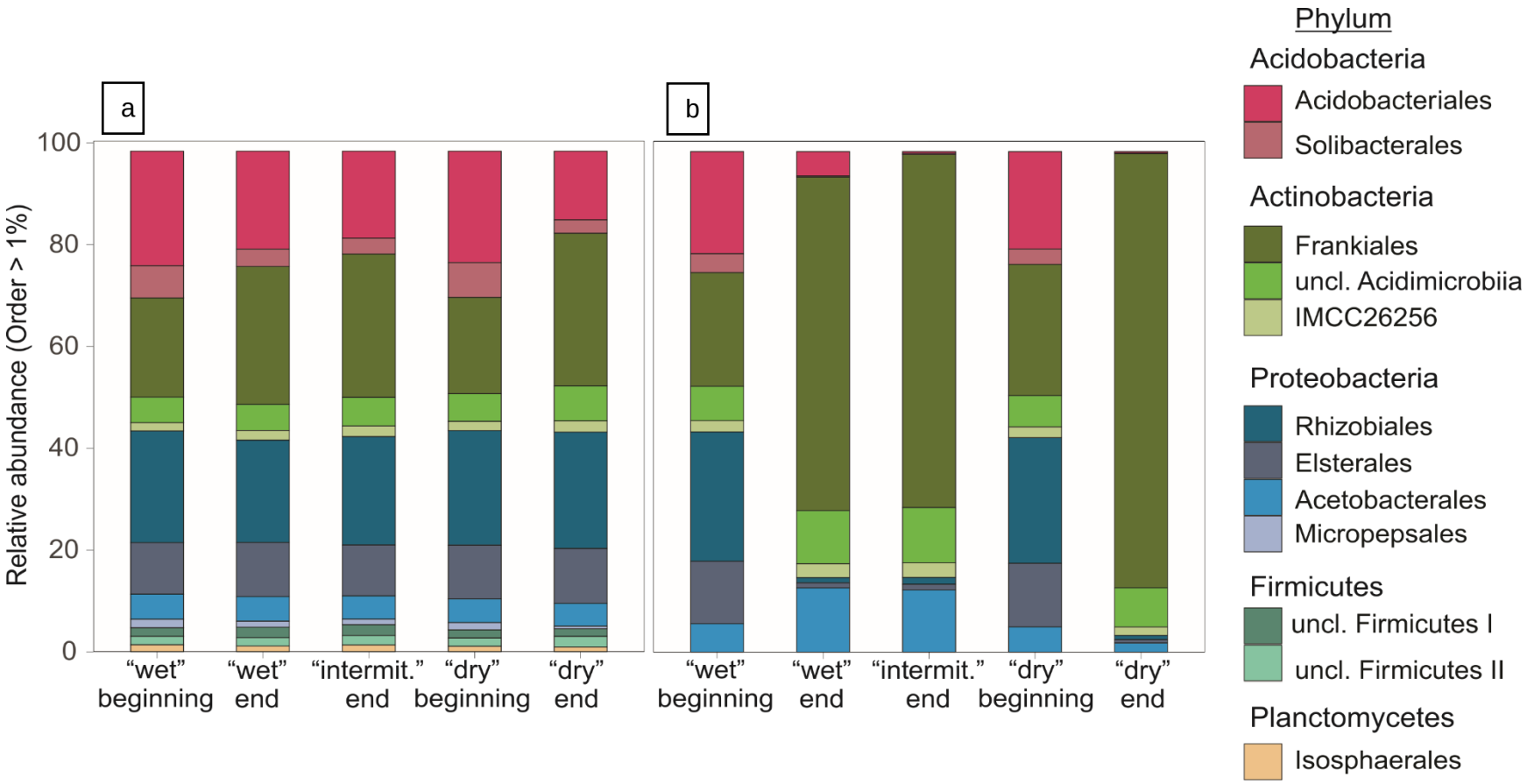

Figure 16 Dynamics of the 16S rRNA gene-based relative abundance of the main present bacterial groups at the phylum and order levels, a) in non-amended and b) substrate amended LUE soil

While the soil in all incubation modes predominantly contained Actinobacteria, in “wet” mode Acidobacteria were also detected. Furthermore, about 15% of identified ASV in “wet” and “intermittent” belonged to the Proteobacteria phylum, while in “dry” soil more than 90% of the identified ASVs belonged to Actinobacteria.In substrate amended soils were initially dominated with only 3 phyla, composed of 8 orders, compared to 5 phyla identified in non-amended soils. Proteobacteria were still the dominating phylum, making up 43% relative abundance, followed by 33% Actinobacteria and 23% Acidobacteria. After 88 days of incubation all the soils were dominated by Actinobacteria.

Order Frankilaes increased from initial 24% to 67%, 71% and 87%, respectively in “wet”, “intermittent” and “dry” soils. Rhizobiales and Elsterales, representatives of Proteobaceria phylum, decreased from 26% and 13%, respectively, to 1% in all incubation modes. Acetobacteriales increased from 5% relative abundance to 13% and 12% in “wet” and “intermittent” soils respectively, while reducing to 2% in “dry” soil. Acidobacteriales, from Acidobacteria phylum, reduced from 20% to 5% in the “wet” soil, but was not detected in “intermittent” and “dry” soils.

Fifteen ASVs significantly different ($p < 0.05$) between incubation modes were identified as bioindicators, possibly driving the changes in microbial community composition in the LUE soil (Figure 17). Though no order was dominating in any of the incubation modes, 6 out of 15 ASVs identified as bioindicators were classified to orders Solibacteriales and Acidobacteriales of Acidobacteria phylum. Five ASVs belonged to different orders of Proteobacteia phylum. Two ASVs belonging to Sacharimondales (Patescibacteria phyla) were also identified. While most of the ASVs were present in all incubation modes throughout the incubation, ASVs belonging to Solibacteriales were not identified at the beginning of the incubation but were detected in “wet” and “intermittent” soils at the end. The opposite was observed for a bacterium belonging to the orders Frankaels, Sacharimondales, uncl. Alphaproteobact and Acetobacteriales, which were initially identified, but were not present in “wet” and “intermittent” soils at the end. In substrate amended soils most of ASVs identified as bioindicators belonged to order Frankiaels of Actinobacteria phylum. Four ASVs belonging to Frankiales order were not detected at the beginning of incubation but were identified as bioindicators at the end. ASVs belonging to orders Sacharimondales, Alphaproteobacter, Acidobacteriaale were identified to be related to the differences between the “dry” and “wet” + “intermittent” soils at the end of the incubation.

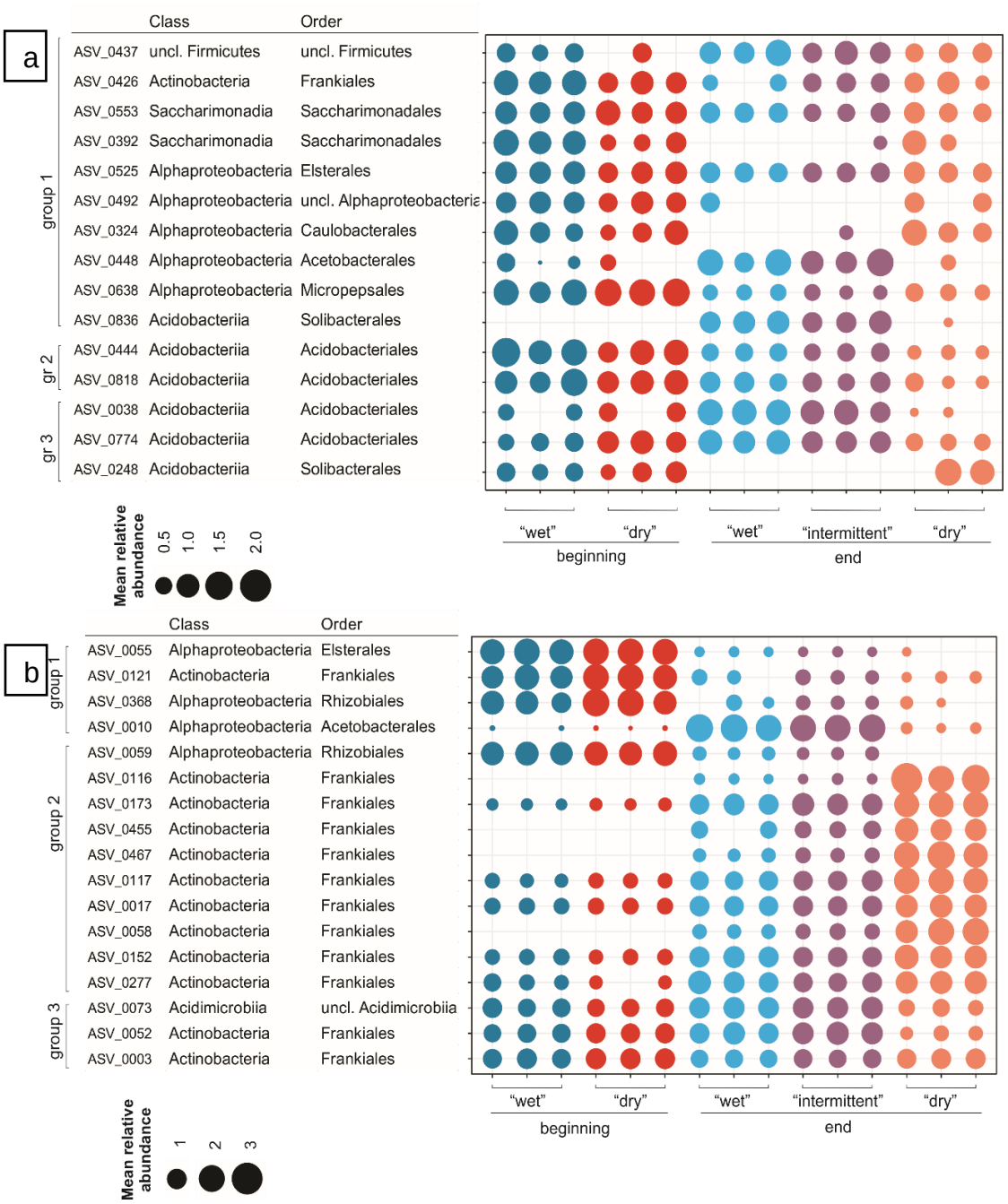

Figure 17 Mean relative abundance of ASVs identified as bioindicators in the a) non-amended and b) substrate amended LUE soil.

5.5.2 CAL microbial community composition and biomass

5.5.2.1 PLFA profile of CAL soil and changes over time

In non-amended “wet” and “intermittent” CAL soil total amount of PLFAs significantly decreased over time. After 88 days of incubation, it reduced from 11 ± 1 μmol·g^{-1} to 7 ± 0 μmol·g^{-1} and 5 ± 1 μmol·g^{-1} in “wet” and “intermittent” soils, respectively. In “dry” soil the initial amount of PLFA was 10 ± 0 μmol·g^{-1} and no significant changes over time have been observed. In contrast, the total amount of fatty acids stayed relatively constant in substrate amended “wet” and “intermittent” incubation modes through the incubation. The amount of total PLFA in “dry” soil increased from 6 ± 0 μmol·g^{-1} to 15 ± 1 μmol·g^{-1} after 29 days, followed by reduction (11 ± 1 μmol·g^{-1}) after 88 days of incubation (Figure 18; Table 11, Appendix).

While in non-amended soil the “wet” and “intermittent” incubation modes had no impact in the relative abundance of G+ bacterial PLFA, a slight increase was observed in the “dry” treatment (from initial 14 ± 0% to 17 ± 0%). In substrate amended soil the relative abundance of G+ PLFA significantly decreased in “intermittent” and “dry” soils after 29 days. After 88 days it increased to the initial level (11 ± 0%) in “intermittent” treatment. In substrate amended “dry” soil the relative abundance of G+ PLFA reduced to 10 ± 1%, compared to initial 14 ± 1% after 88 days.

G- bacterial PLFA were the most abundant group in non-amended soil and were not affected by the incubation modes (~28%). In substrate amended soil the relative abundance gradually decreased in all incubation modes during the incubation. In “wet” and “intermittent” modes it decreased from 31 ± 1% to 26 ± 0% and in “dry” mode from 36 ± 2% to 28 ± 1%. At the end of the incubation of non-amended “wet” and “dry” soils the relative abundance of Actinobacterial PLFA decreased by 4%, compared to initial 17% ± 1 and 20% ± 0, respectively. No changes have been observed in “intermittent” incubation mode. In substrate amended “wet” and “intermittent” soils the relative abundance of Actinobacterial PLFA increased from initial 11 ± 1% to 17 ± 0%.

However, in “wet” soil an increase was observed already after 29 days (14 ± 1μmol·g^{-1}), while in “intermittent” mode the increase was observed only after 88 days. In “dry” soil after a decrease to 7 ± 0% after 29 days, it increased to initial level (11 ± 1%) in the end of the incubation. In non-amended soil, relative abundance of fungal fatty acids was initially 2.3 ± 0.1% in all incubation modes. After 88 days it decreased to 1.3 ± 0.0% in “wet”, 1.5 ± 0.0% in “dry” and 1.8 ± 0.0% in “intermittent” soils. No changes in relative abundance of fungal PLFA occurred in substrate amended “wet” soil. In “dry”

mode it increased from 1 ± 0% to 28 ± 3% after 29 days, then decreased to19 ± 1% after 88 days.Same was observed in the “intermittent” mode: an increase after 29 days, followed by reduction to the initial amount of 13 ± 1%. In general, the living microbial biomass and the relative abundance of bacterial and fungal were not significantly affected by the incubation modes. The amount of total PLFA declined in non-amended soils at all incubation modes, however, the reduction was less pronounced in “dry” mode. Despite decreased total PLFA, the relative abundances bacterial and fungal PLFA were relatively stable. G+ and Actinobacterial PLFA were also relatively stable. Substrate amendment of the soil resulted in decrease in the relative abundance of G- PLFA, accompanied with the growth of more drought tolerant fungi in “dry” and Actinobacteria in “wet” and “intermittent” modes.

5.5.2.2 Impact of soil water content on microbial community structure of CAL soil

At the beginning of the incubation no difference in the amount of total fatty acids between different incubation modes was observed in non-amended soil (Figure 18). After 88 days the total fatty acids were significantly lower in the “intermiitent” soil (5 ± 1 μmol·g^{-1}), compared to “wet” (7 ± 0 μmol·g^{-1}) and “dry” soils (8 ± 0 μmol·g^{-1}). In substrate amended “wet” and “intermittent” soils (9±1 μmol·g^{-1}) initially had significantly higher amount of total PLFA compared to “dry” soil (6±0 μmol·g^{-1}). However, after 88 days difference was observed only between “wet” (7 ± 0 μmol·g-1) and “dry” (11 ± 1 μmol·g^{-1}) soils. No significant differences in the relative abundance of G+ bacteria PLFA have been observed between the incubation modes in non-amended soil throughout the incubation period. On the other hand, in substrate amended soil already after 29 days it was significantly higher in “wet” (11 ± 0%) soil, compared to “dry” (6 ± 0%) and “intermittent” (7 ± 0%) ones. After 88 days the relative abundance of G+ PLFA was only slightly higher in “wet” (13± 0%) soil, compared to “dry” (10 ± 0%) soil. At the beginning of the incubation no significant differences in G- bacteria PLFA were observed between different incubation modes at any substrate level. After 88 days of incubation G- bacterial PLFA in non-amended “wet” (28 ± 0%) soil was slightly lower, compared to “dry” (30 ± 0%) and “intermittent” (31 ± 0%) soils. In substrate amended soil differences have been observed only after 29 days of incubation between “wet” (32 ± 0%) soil, compared to “dry” (31 ± 1%) and “intermittent” (27 ± 0%) soils. In non-amended “wet” soil (13 ± 1%) the relative abundance of Actinobacterial PLFA detected was lower at the end of the inbcubation, compared to “dry” (16 ± 0%) and “intermittent” (15 ± 1%) soils.

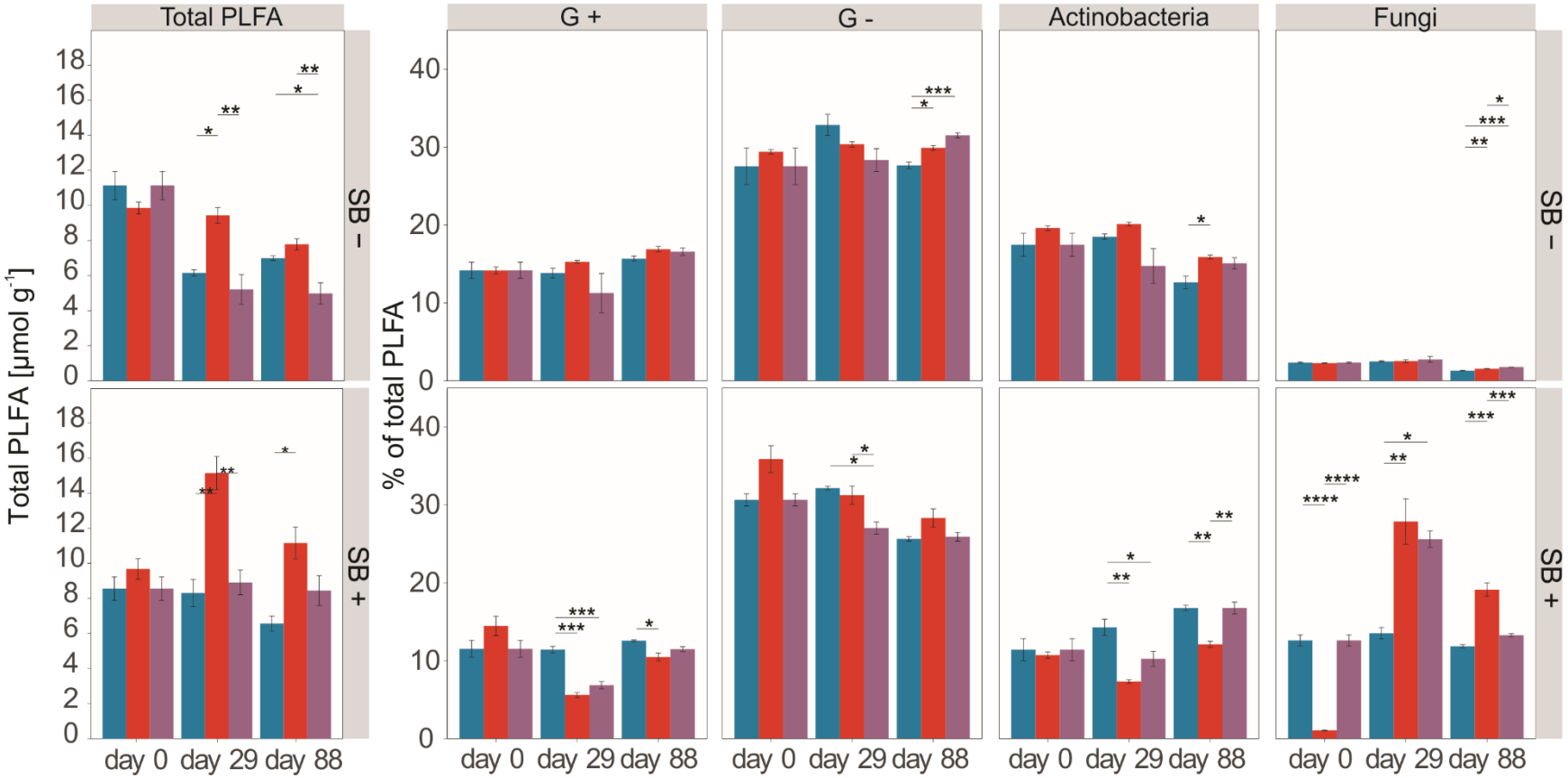

Figure 18 PLFA composition of CAL soil in non-amended (SB-) and substrate amended (SB+) treatments. Blue stands for “wet” incubation mode, purple –“intermittent” and red for “dry” mode. Values are arithmetic mean of n = 3 measurements with the error bars indicating the standard error.

In susbtrate amended soil already after 29 days the relative abundace was siginificantly higher in “wet” (14 ± 1%) soil, compared to “dry” (7 ± 0%) and “intermittent” (10 ± 1%) soils. However, after 88 days difference was observed between “dry” (17 ± 0%) soil, compared to “wet” (12% ± 0) and “intermittent” (17 ± 1%) soils. The relative abundance of fungal PLFA was not affected by the incubation modes in non-amended soil. In contrast, in substrate amended soils differences between incubation modes were already observed at the beginning of the incubation. Relative abundace of fungal PLFA was initially lower in “dry” (6 ± 0%) soil, compared to “wet” and “intermittent” soils (13 ± 1%). Howbeit, after 88 days of incubation, the relative abundance of fungal PLFA was significantly higher in “dry” soil (19 ± 1%), compated to “wet” (11 ± 0%) and “intermittent” (13 ± 0%) soils.

Overall, the total bacterial biomass and PLFA profile were less susceptible to the differences in water content, compared to LUE soil, in different incubation modes. No major differences were observed, besides significantly higher relative amount of fungal PLFA and low Actinobacterial PLFA in the substrate amended “dry” soil, compared to the “wet” modes at the end of the incubation.

5.5.2.3 Microbial community structures of CAL soil assessed by 16S rRNA gene sequencing

No significant differences have been detected in both, observed and Shannon diversity indices, at the beginning of the incubation between the modes at either substrate levels (Figure 19). After 88 days observed diversity index increased from initial 504 ± 18 to 611 ± 21 in non-amended “intermittent” soil, while the Shannon index stayed unchanged. No changes in observed and Shannon indices occurred in “wet” and “dry” soils as result of incubation. In substrate amended soil, however, both indices reduced after 88 days of incubation. Observed alpha diversity reduced from 484 ± 21 to 175 ± 16 in “wet”, 177 ± 10 in “dry” and 165 ± 10 in “intermittent” soils. Shannon index reduced from 4 ± 0 to 2.2 ± 0.0, and 2.3 ± 0.0 in “wet” and “intermittent” soils, respectively, and to 2 ± 0 in “dry” soil. The results show that the species richness and the Shannon index were not strongly affected in non-amended CAL soil at all incubation modes. An exception was the “intermittent” soil, in which the species richness slightly increased over time accompanied with increased diversity.

Substrate amendment caused a reduction in diversity and increased the homogeneity. However, while no differences were detected in the number different observed species between incubation modes in substrate amended soil, the Shannon index was lower in the “dry” mode, due to adaptation of fewer species in the sample and simultaneous

death of others.

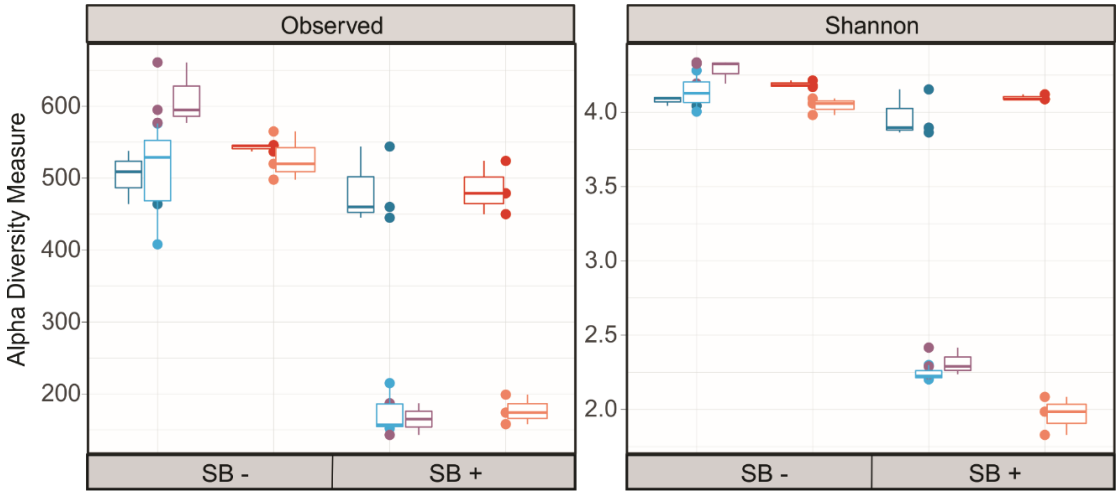

Figure 19 Species richness(observed) and Shannon diversity index of non-amended (SB-) and substrate amended (SB+) CAL soil. Red represents the “dry” mode at the beginning of incubation. dark blue –“wet” and “intermittent” modes at the beginning, light blue –“wet” mode end, purple –“intermittent” end and orange –“dry” end.

As in LUE soil, in CAL soil the PCoA did not reveal differences in composition of microbial communities between the incubation modes at the beginning of the incubation, neither in non-amended (Figure 20, a), nor in substrate amended (Figure 20, b) soils. At the end of the incubation, differentiation between “dry” and “wet” and “intermittent” incubation modes occurred at both substrate levels. In non-amended CAL soil 32.1% of variance was explained by first PC and 20.3% by the second.

At the beginning of incubation ASVs belonging to 4 phyla were detected in the CAL soil at both substrate levels (Figure 21). In non-amended soil, the most abundant phyla, In substrate amended soil 97.7% of variance was explained, with 90.7% accounted in PC1 and 7% in PC2. Acidobacteria phylum, uncultured Acidimicrobiia (4%), Rhizobiales (12%), Elserales (13%) and Acetobacteriales (5%) of Proteobacteria phyum and uncultured Firimicutes (3%) from Firmicutes phylum were also detected. After 88 days of incubation community composition of non-amended soil on order level remained relatively stable, with Acidobacteriales reducing from 15% to 13% and instead relative abundance of Frankiales increased from 44% to 47%. The initial community composition of substrate amended soil was similar to non-amended soil, with the exception of order Solibacteriales not being detected in the amended one. At the end of the incubation ASVs from only 3 orders were detected in “wet” and “intermittent” soils and 4 in “dry” soil.

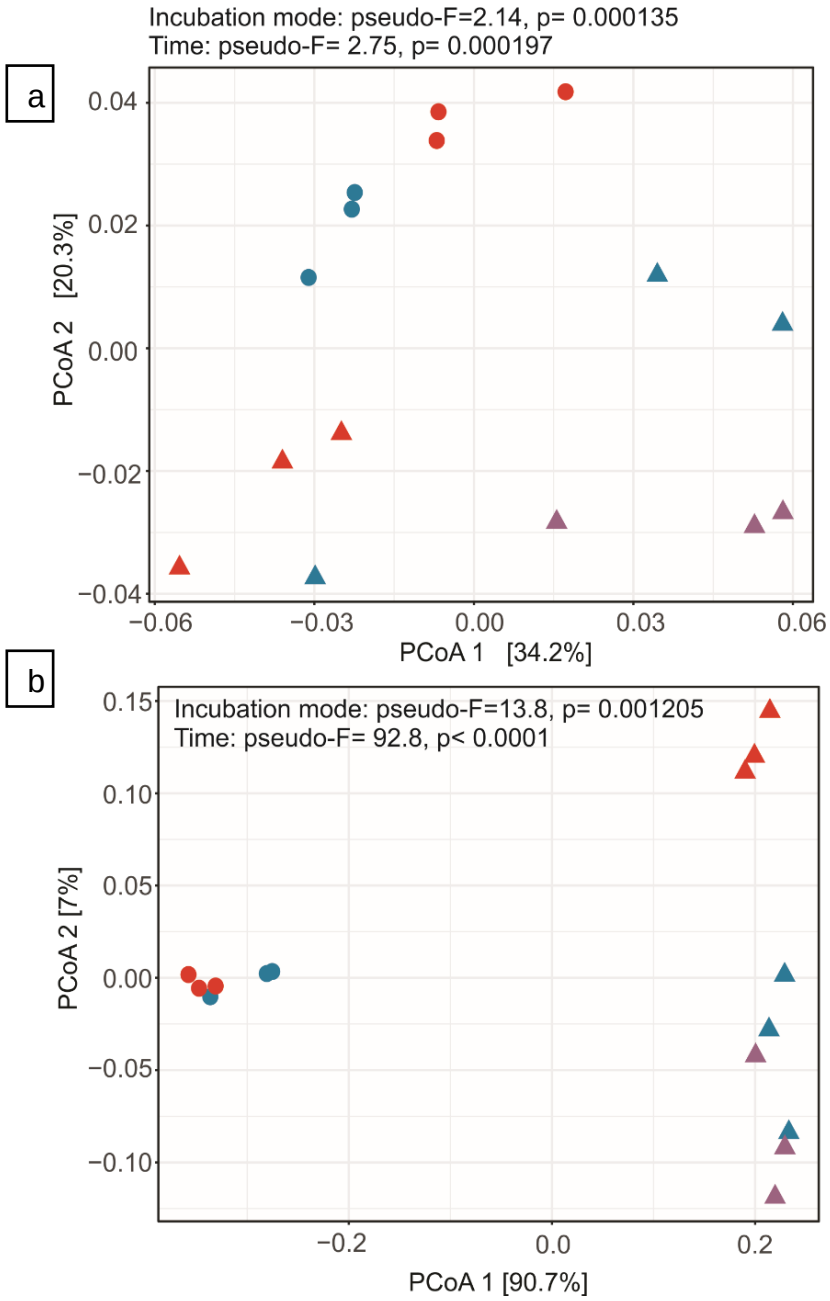

Figure 20 PCoA of beta diversity of CAL soil, based on Bray-Curtis dissimilarities for a) non-amended, b) substrate amended soil. Red circles represent "dry" mode at the beginning, blue circles –"wet" and "intermittent" beginning. Red, blue and purple triangles represent "dry", "wet" and "intermittent" modes, respectively, at the end of incubation.

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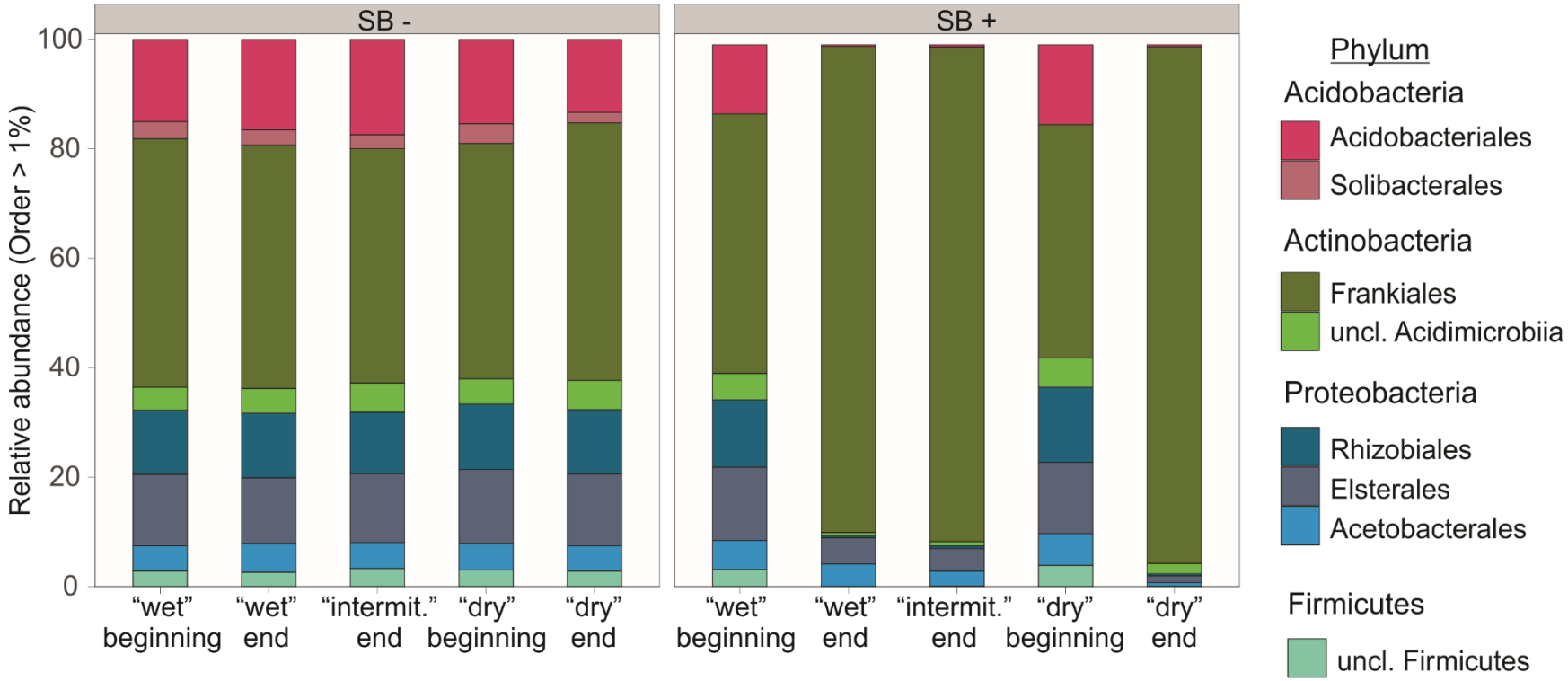

Figure 21 Dynamics of the 16S rRNA gene-based relative abundance of the main present bacterial groups at the phylum and order levels, in non-amended and substrate amended CAL soil.

Order Frankeales dominated in all incubation modes, constituting 90% and 92% in “wet” and “intermittent” and 96% in “dry” soils. Relative abundance of order Elsterales was 5% in “wet”, 4% in “intermittent” and 1% in “dry” soil.
Acetobacterales was also detected with relative abundance of 4%, 3% and 1% in “wet”, “intermittent” and “dry” soils, respectively. In contrast to “wet” and “intermittent” soils, in “dry” soil ASV belonging to an uncultured Acidimicrobiia with relative abundance of 1% was also detected. No Firmicutes were detected in substrate amended soil after 88 days.

The changes in the community composition of CAL soil followed similar pattern as in the LUE soil, with the community of the unamended soil being relatively stable under all incubation modes. Slight shifts were observed due to increase in relative abundances of Actinobacteria and simultaneous decrease in Acidobacteria. The shifts in microbial community were more accentuated by addition of substrate. Furthermore, significant differences were observed in relative abundances of members of particular orders between the incubation modes in substrate amended soils. While the soil in all incubation modes predominantly contained Actinobacteria, in “wet” and “intermittent” modes 2 different orders of Proteobacteria phylum were detected, while in “dry” soil more than 95% of the identified ASVs belonged to Actinobacteria.
RF analysis of CAL soil revealed 13 ASVs significantly different ($p < 0.05$) between incubation modes, driving the possible differences in microbial community composition (Figure 22). In non-amended CAL soil, majority of ASVs identified belonged to Acidobacteriales order of Acidobacteria phylum., followed by Proteobacteria. Two orders belonging to Planctomycetes phylum were also present (Tepidispherales and Gemmatales). The ASVs, that were unique to a particular treatment were identified to belong to orders Micropepsales (Proteobacteria) and Gemmatales (Planctomycetes) and a representative of Armatimondates phylum, Fimbrimondales order. The first two orders were not identified at the beginning of the incubation but were present in “wet” and “intermittent” soils after 88 days, and the latter was no longer present in “wet” and “intermittent” mode after 88 days. Substrate amendment resulted in changes in microbial community composition, and thus the “bioindicators”. ASVs of Acinobacteria phylum (Frankiales and Acidimicrobiales) were dominating in substrate amended CAL soil. Though Frankiales order was not detected in the non-amended soil, it was the most abundant ASV identified in substrate amended soil. Other unique ASVs were identified as well, such as uncultured Acetobacteriales order (Proteobacteria phylum).

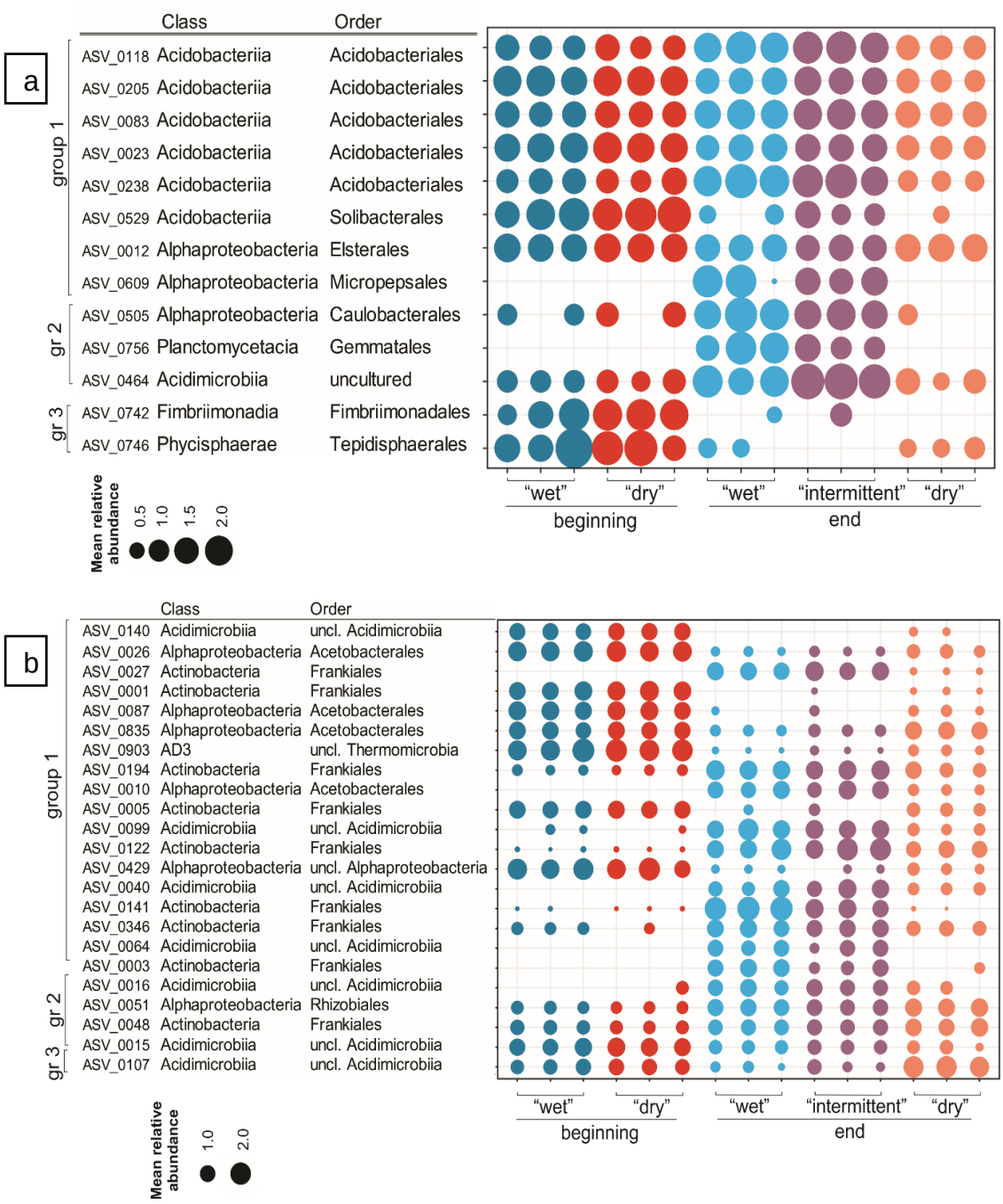

Figure 22 Mean relative abundance of ASVs identified as bioindicators in the a) non-amended and b) substrate amended CAL soil.

6 DISCUSSION

6.1 Impact of water stress on bacterial growth and cell surface hydrophobicity

In soil one of the major factors affecting the microbial activity is the soil water potential. Changes in cell surface hydrophobicity due to exposure to osmotic stress have already been reported (Baumgarten et al.,2012; Hachicho et al.,2017). Although in wet non-saline soils, the water potential is mostly determined by osmotic potential, as the soil dries out, matric potential becomes the dominant factor. By performing pure culture experiments, using NaCl to induce osmotic stress and PEG 8000 for matric stress, we aim is to independently assess the impact of osmotic and matric stresses on bacterial growth and surface properties. Moreover, bacteria hardly live as planktonic cells in the soil system and are mostly present in association with soil particles, thus being exposed to air/liquid/solid interface. Bacterial cells exhibit significant differences in growth behavior and stress tolerance in submersed growth, compared to surface growth. Thus, pure culture experiments were conducted to examine the different response to osmotic and matric stress in different growth conditions. The results showed that exposure of all 6 strains to water stress results in reduced growth rates. However, the effect water stress has on growth rates of different bacterial strains, depends on the type of water stress (osmotic or matric) and growth condition (submersed or surface). The impact of water stress on the surface hydrophobicity of the studied strains was also culture, stress type and growth condition dependent.

6.1.1 Impact of osmotic and matric stress on bacterial growth

Exposure to osmotic stress results in increased energy requirements for cell maintenance, thus reducing the available energy for new biomass production (Oren,1999).Growth inhibition was generally observed in this study as a result of exposure to osmotic and matric stress. However, as stated before, the degree of response to osmotic and matric stress varied between the strains. Numerous studies showed reduction in bacterial growth rate due to exposure to osmotic stress, both in pure culture (Hachicho et al.,2017; Holden et al.,1997; Worrich et al.,2016) experiments and in soil (Chowdhury et al., 2011; Kakumanu and Williams, 2014; Polonenko et al.,1986) showed that when exposed to osmotic stress growth *of P.*

putida mt-2 was fully inhibited when the water potential of the media was reduced by -3.5 MPa via addition of sodium chloride. In the study conducted by Worrich et al. (2016) the growth of *P. putida* KT2440 was inhibited when exposed to both osmotic and matric stress, however lower matric stress resulted in full growth inhibition at already -Δ 0.25 MPa matric potential, while osmotic stress caused full inhibition at -Δ 1.5 MPa. Contrary to these results, *P. putida* mt-2 has been shown to be less susceptible to matric stress (PEG8000) then to osmotic stress (NaCl) (Holden et al.,1997). When exposed to osmotic stress, growth inhibition was already observed at water potential of -0.25 MPa, but only started from -0.5 ± 0.1 MPa when exposed to matric stress. However, it should be taken into consideration that the growth media in these studies was different, which is known to impact the bacterial growth kinetics (Loutfi et al.,2020; Sánchez-Clemente et al.,2020). The impact of reduced water potential on the growth inhibition of another G- bacterium, *E. coli*, was also reported (McAneney et al.,1982). The growth of this strain was inhibited in the presence of both, PEG4000 and NaCl, at water potentials of -0.8 MPa and -4 MPa, respectively.

Not only the response to water stress varies between different spices and even strains, but also different bacteria have different levels of minimal water activity tolerance. In the experiment of Marshall et al. (1971) 16 different bacterial strains were grown at 30 °C for 28 days in media amended with NaCl. Results showed growth limiting A_w levels ranging from 0.97 to 0.83 (corresponding to -4.1 MPa to -25 MPa, respectively). Five different Bacillus spices exhibited high level of tolerance to osmotic stress, with *B. subtilis* being tolerant to up to-11MPa. In this study it was also concluded that G- rods exhibited higher susceptibility to low A_w, compared to G+ rods. In our study as well, G+ bacteria were slightly less susceptible to stress with exception of *M. pallens*. Though considered G+, Mycobacteria have rather different cell wall structure, cell cycle and asymmetric cell division (Kieser and Rubin, 2014; Thanky et al., 2007). Due to the presence of mycolic acids (C_{60}-C_{90}) *Mycobacterium* have a rigid cell wall (Brennan and Nikaido,1995), which affects the permeability towards nutrients and other hydrophilic substances (Jarlier and Nikaido,1990; Liu et al.,1996; Wick et al.,2002).

It is known that G+ bacteria are more adapted to survive in dry soils. Praveen Kumar et al. (2014) researched the impact of drought (-1.2MPa water potential caused by PEG 6000) and salinity (-6MPa water potential due to addition of 1.2 M NaCl) water stress on the growth of 120 *Bacillus* and 75 *Pseudomonas* isolates obtained from 31 locations in India. The isolates were considered tolerant, if the optical density (600nm) of 0.1 was reached after 6 days of incubation at 28 °C. Of these 120 *Bacillus* isolates 72 were able to tolerate osmotic pressure of -6MPa and 23 -1.2MPa matric potential.

In case of *Pseudomonas* isolates only 7 could tolerate the exposure to -6MPa osmotic pressure and 14 to matric pressure. Also, while 60 percent of *Bacillus* isolates were tolerant to low osmotic pressure, compared to only 10% of *Pseudomonas* isolates, only around 20% isolates from both genera exhibited tolerance to matric stress. Similarly, McAneney et al.(1982) reported that with reduction of water potential, with addition of both, NaCl and PEG4000 inhibited the growth of *E. coli* at -0.8MPa matric potential and -4 MPa osmotic potential.

In this study, though the most susceptible strain was G- *P. fluorescens*, growth of some of the G+ strains was also inhibited by reduced water potential. In general, however, this study also shows that different strains react differently to reduced water potential and the stress type.

The impact of water stress on bacterial growth in various growth conditions has also been reported. Hachicho et al. (2017) showed different response to osmotic stress of *P. putida* in planktonic (submersed) and agar plates (surface) growth. The 6 strains used in this study exhibited different levels of growth inhibition depending on the growth conditions as well. Higher tolerance of most bacterial strains to reduced water potentials was observed when grown as surface culture. In soil most bacteria live in biofilms and microcolonies on the surface of soil minerals. In these conditions, bacteria have been shown to exhibit higher tolerance to environmental stress, compared to submersed growth (Baumgarten et al., 2012; Keweloh et al.,1989). In surface growth the substrate diffusion and the removal of metabolites is limited to cell-agar interface, therefore, the colony growth rate on the surface is suppressed, compared to submersed growth (Kabanova et al., 2012; Skandamis and Jeanson, 2015). Thus, during surface growth with limited cell dispersal, the impact of substrate diffusion limitation on microbial growth conceal the impact of osmotic stress (Tronnolone et al., 2018; Worrich et al., 2016).

Studies available on the bacterial response to matric and osmotic stress vary in growth conditions and factors that affect stress response significantly (growth media, degree of stress, stress type). Therefore, the comparison between studies should be done very cautiously. In this study we provide an overview of the impact of both matric and osmotic stress on the growth inhibition and changes in surface hydrophobicity of a wide range of typical soil microorganisms exposed to same conditions, hence allowing a direct comparison between the strains.

6.1.2 Bacterial cell surface hydrophobicity

Impacts of matric and osmotic stress on the surface hydrophobicity of different bacterial strains have been studied under submersed and surface growth conditions. Increase in surface hydrophobicity in response to water stress have been observed for most of the strains used in this study. However, only *P. fluorescens* surface hydrophobicity increased in all the investigated growth conditions and the surface hydrophobicity of *R. erythropolis* was not affected at all. In other words, our results show that the response on the water stress is dependent on stress level, type, and strain and growth conditions.

P. fluorescens was the most susceptible strain and showed increased hydrophobicity in all growth conditions. In the study of Hachicho et al. (2017) increase in surface hydrophobicity of *P. putida* mt-2 compared to unstressed control was observed during submersed growth, similar to increase in CSH of *P. fluorescens* in this study. However, Hachicho et al. (2017) reported 90° contact angle for *P. fluorescens* during surface growth, compared to 40° obtained in this study. Exposure to osmotic stress resulted in decrease in contact angle, while *P. fluorescens* contact angle increased. Increase in contact angle due to exposure to osmotic stress of 2 *P. putida* strains were also reported by Achtenhagen et al. (2015) and Baumgarten et al. (2012). But the water potential values used in the studies were significantly lower than the ones used in this study (-9.8 MPa (Achtenhagen et al.,2015) and -7 MPa (Baumgarten et al., 2012)). During the surface growth *A. chlorophenolicus* strains reduced the surface hydrophobicity compared to the unstressed control. (Hachicho et al., 2017) observed this in her study with *P. putida*, where the surface hydrophobicity of the strain reduced with lower water potentials. *R. erythropolis* and *M. pallens* exhibited very high surface hydrophobicity of unstressed culture but did not get affected by water stress. The high level of initial surface hydrophobicity can be explained by the presence of mycolic acid in the cell wall of these bacteria (Stratton et al., 2002). Our results also indicate an upper limit for increase in surface hydrophobicity (~110°), which could be associated with maintaining the essential cell functions. High levels of surface hydrophobicity can affect the permeability, thus limiting the water and nutrient diffusion to the bacterial cell (Liu et al.,1996). Additionally, it is known, that exposure to osmotic stress results in reduced cell size, due passive loss of cytoplasmatic water, which in turn results in protein crowding (Zhou et al., 2008). Protein crowding has been shown to be associated with reduced diffusion coefficient (Mika et al., 2010) and can affect

structural and functional properties of membrane proteins (Miller et al., 2016).
In summary, the results of the stress exposure experiments in pure culture confirmed the hypothesis, that growth of microorganisms under water stress is accompanied with growth inhibition and changes in cell surface hydrophobicity. Furthermore, we confirmed that the degree of stress response was different depending on the stress type, with matric stress having stronger impact on the growth but not the surface hydrophobicity at lower water potentials. Additionally, the assumption is that bacterial response to osmotic stress will be different in growth conditions similar to one in soil, compared to liquid cultures. During surface growth bacteria were less susceptible to osmotic stress.

6.2 Cell mineral associations: surface wettability development and persistence

It is known that microbial living biomass and necromass contribute to SOM formation (Miltner et al., 2012), thus can influence surface properties of soil minerals, such as wettability. As already shown before (Figure 5), increase in bacterial cell surface CA occurs as an adaptation mechanism to water stress. To test our hypothesis, that the direct attachment of bacterial cells to soil particles will impact the wettability of the minerals and that osmotic stress-induced changes in bacterial cell surface hydrophobicity will exacerbate the impact, incubation experiment with bacterial cell-mineral associations was conducted.
The results showed that the attachment of bacterial cells renders originally wettable minerals hydrophobic. The attachment of stressed bacterial cells of both strains hindered quartz particles initially more hydrophobic. However, *B. subtilis* had stronger impact, compared to *P. fluorecens*. CMAs with *B. subtilis* had significantly higher CA, compared to the CA of the pure culture, in both, stressed and unstressed CMAs, while the initial CA of CMAs with *P. fluorescens* cells were similar to the CA of the unstressed and stressed pure culture. Nevertheless, within the first 7 days of incubation the CA of *B. subtilis* CMAs decreased, while increase was observed in *P. fluorescens* CMAs, followed by reduction in the CMA with stressed *P. fluorescens* cells. These changes in surface wettability of CMAs were accompanied by reducing respiration rates, which completely ceased after 7 days in CMAs with both strains. Achtenhagen et al. (2015) reported similar results, when studying the attachment of *P. putida* ($\theta_{unstressed} \approx 42°$, $\theta_{stressed} \approx 65°$) cells to mineral particles. The surface hydrophobicity of the minerals

significantly increased, and stress exposed cells rendered the minerals more hydrophobic, than the unstressed control ($\theta_{unstressed}$ = 61° and $\theta_{stressed}$ = 86°). Additionally, the degree of hydrophobicity of minerals with P. putida were higher, compared to the pure culture CA. However, in this study, this was observed for *B. subtilis*, but not *P. fluorescens*.

The results of Achtenhagen et al. (2015) also showed, that with increasing surface coverage of minerals by bacterial cells, contact angle of cell-mineral associations increased, reaching the maximal contact angle already at 20% and 10% surface coverage, for unstressed and stressed *P. putida* cells, respectively. Further increase in surface coverage had no additional impact on contact angle. In our study, the surface coverage was estimated to be between 10- 20% (based on bacterial cell size and SSA of medium sized quartz (Achtenhagen et al., 2015)).

The decrease in the total amount of PLFA identified and reduced microbial respiration in the CMA microcosms with both strains, indicate decrease in surface coverage of CMA, thus resulting in reduced contact angle, in all treatments except CMAs with unstressed *P. fluorecense*. The contact angle of CMAs with addition of unstressed *P. fluorescens* increased over the first 8 days of incubation but stayed relatively constant afterwards. Bacterial survival under osmotic stress is an extremely energy requiring process (Oren,1999), in addition, after removal of osmotic stress, bacteria are transferred to an environment with low energy and nutrient availability. In this environment unstressed cells have bigger chance of survival, using the nutrients derived from cell death, they maintain their population. In low energy conditions bacteria are prone to reduce their size, either by shrinking (Kieft et al.,1997) or by cell fragmentation (division without growth) (Amy et al.,1993), which would result in reduced surface coverage and consequently reduction in surface hydrophobicity of CMAs with stressed *B. subtilis* and *P. fluorescens*. Furthermore, G- bacteria (*E. coli*) exhibited increase in surface hydrophobicity when exposed to starvation. This improves their adhesion to particles, which has been indicated to be a survival strategy of copiotroph bacteria in oligotrophic growth conditions (Saini et al., 2011).

The incubation experiment with CMAs confirmed the hypothesis, that bacterial cell surface hydrophobicity impacts the wettability of soil minerals. Moreover, the extent of the induced water repellency depends on strain. The results indicate that the stress-induced increase in hydrophobicity do not persist for long, however full wettability is not recovered, even under extreme nutrient limitation.

6.3 Impact of soil water content on soil wettability and microbial community composition

6.3.1 Changes in soil wettability

The two soils exhibited different response to incubation modes in terms of the surface hydrophobicity. The CAL soil, collected from the location with lower MAP was initially more hydrophobic (θ=128 ± 6°) and the CA was not affected by the incubation modes. In contrast, LUE soil, the “dry” incubation mode rendered the LUE soil significantly more hydrophobic, while no major changes were observed in “wet” and “intermittent” modes. Soil water content is shown to be one of the major factors controlling the SWR and it is negatively correlating to SWR (de Jonge et al.,1999; Goebel et al., 2011; Hermansen et al., 2019; Karunarathna et al., 2010; Wijewardana et al., 2016). Thus, as expected the contact angle of Luss soil increased when dried. In the initially hydrophobic CAL soil changes in soil contact angle were not observed. Various models have been proposed for predicting the relationship between soil water content to soil hydrophobicity and its persistence (Bachmann et al., 2007; Karunarathna et al., 2010; Kawamoto et al., 2007; Vogelmann et al., 2013). Irrelevant of the method used in these studied to determine soil surface hydrophobicity, the common conclusion was, that there is a critical soil volumetric water content, below which the surface hydrophobicity of the soil increases, until it reaches a maximal value at the critical WC. Increase in soil WC above this point will render the soil hydrophilic. The threshold water content at which hydrophobic soil will become hydrophilic is dependent on SOC and texture. For hydrophobic soils with similar texture and C content as the CAL soil, this threshold would be above 45% volumetric water content (Karunarathna et al., 2010; Wijewardana et al., 2016). However, in our experiment volumetric water content of the “wet” treatment was 19%, which is far below the threshold. With increasing soil water content hydrophobicity remains high until it reaches water content close to field capacity. If the soil water content remains at field capacity or continues increasing for prolonged period of time the soil eventually will become hydrophilic (Vogelmann et al., 2013), due to the re-establishment of sorptivity. Furthermore, wetting of a dry soil can lead to the rearrangement of amphiphilic compounds, coating the surface of soil aggregates. Goebel et al. (2011) suggested, that SWR reduced infiltration, thus protecting the soil aggregates from fast breakdown. Prolonged exposure to water will allow the reorientation of amphiphilic compounds on the surface of the aggregates,

allowing the infiltration and consequent release of the SOM entrapped within the aggregates. This possibly could lead to an extended release of hydrophobic compounds, increasing the time required for the soil to become hydrophilic.

6.3.2 Changes in soil microbial community composition

Exposure to environmental stressors, such as drought, are known to impact soil microbial activity and community composition. The two soils were chosen for this experiment because of their exposure to different levels of mean annual precipitation and differences in their surface hydrophobicity. However, prior to the start of the experiment both soils were wetted to approximately 45% gravimetric water content and then dried to reach water contents corresponding to pF2.5 and pF4.2.

6.3.2.1 Water regime related changes in living microbial biomass

Total PLFA amount was initially significantly higher in CAL soil, compared to LUE soil. (Lozano et al., 2014) analyzed microbial community structure and composition in soils with different levels of water repellency and found that the soils with strong level of water repellency ((log (WDPT)>1) had the highest amount of total PLFA. Also increase in total PLFA in forest soil during the dry season was also reported by (Bhardwaj et al., 2020). The presence of hydrophobic compounds in water repellent soil may cause a shift in the community structure, selecting microorganisms capable to degrade these compounds (Roper,2004). The major proportion of identified microorganisms, capable to degrade hydrophobic compounds belongs to Actinobacteria (Roper,2004). In the CAL soil Actinobacteria were relatively more abundant than in LUE soil, indicating a possible adaptation to the high levels of surface hydrophobicity. The high relative abundance of Actinobacteria, could also be responsible for the significantly higher CA of CAL soil, compared to LUE soil (Figure 12). Most strains isolated in the study of (Roper, 2004; Roper, 2006) belonged to *Rhodococcus* and *Mycobacterium* genera. Both, *Rhodococcus* and *Mycobacterium* strains used in this study, exhibited extremely high levels of CSH, indicating a possible involvement of bacterial in the development of SWR.

Addition of substrate resulted in decrease in total PLFA amount in both soils. However, in LUE soil, the reduction was more prominent, compared to CAL soil. It is known that exposure to salt stress can result in rapid decline in number of microorganisms and

species diversity in soil (Polonenko et al., 1986; Rath and Rousk, 2015). However, large number of the soil microorganisms are able to rapidly adapt salt stress, (Bremer and Krämer, 2019). In this study, addition of substrate resulted in rapid and significant reduction of osmotic potential in the soil solution (approx.-2.5 MP for CAL and -1.6MPa for LUE soil). (Kakumanu and Williams, 2014) compared the impact of osmotic stress on microbial biomass in two soils. Results showed that the more drought-prone soil was less susceptible to osmotic stress, than the frequently exposed to floods soil. Differences between adaptation levels to water stress of microbial communities can explain different responses of LUE and CAL soils to increased osmotic potential.

In “dry” LUE soil an initial increase in total microbial biomass was observed, which contradicted to our expectations (Figure 13). The adjustment of water content to pF4.2 took approx. 2 months, compared to 11 days to achieve pF2.5. Increase in microbial biomass in a California grassland over the dry summer was reported by (Schimel et al., 2011).

Production of cyclopropane fatty acids is a response to various environmental stressors, including starvation in G- bacteria (Murínová and Dercová, 2014; Ramos et al., 2001). In our experiment the ratio of cyclopropane fatty acids to their precursor was initially significantly higher in “dry” soil, indicating growth, followed by stress adaptation to reduced substrate availability of G- bacteria during the 2-month pre-incubation.

Overall, the relative abundance of PLFA biomarkers (G+, G-, Fungi and Actinobacteria) bacterial biomass were more strongly influenced by substrate amendment and incubation time, rather than the incubation modes. Similar results were reported by (Bastida et al., 2017), showing that substrate amendment mitigates the response to water stress.

In our experiment the relative abundance of different PLFA groups were relatively stable throughout the incubation period. In an experiment of (Ouyang and Li, 2020) forest soil from a northwestern China, with mean annual precipitation of 600 mm, was exposed to drying-rewetting cycles with different frequencies: 2-14 cycles during 80-day period. Results indicated no significant differences between total, bacterial and fungal PLFAs in any of the treatments. SOM is one of the major factors influencing microbial resistance to repeated drying-rewetting cycles and soils with high C level are more resistant to drought (Hueso et al., 2012; Kaiser et al., 2015; Zhao et al., 2010). Both of the soils used in our experiment have relatively high organic C which could explain the resistance to water stress.

Nevertheless, it is important to keep in mind that the taxonomic resolution of PLFA

biomarkers is rather coarse, thus cannot provide accurate picture of water stress driven changes in community composition.

6.3.2.2 The effect of water regimes on diversity and structure of bacterial communities

Exposure to drying-rewetting cycles and drought have little impact on the diversity of soil communities (Fierer et al., 2003; Kundel et al., 2020; Meisner et al., 2018) and pre-exposure to drought can mitigate the response (Bouskill et al., 2013). This is in line with our finding that no major changes in species richness and diversity in CAL soil was overserved, while LUE was more susceptible to drought. The spices richness and diversity were decreased more by incubation time and substrate amendment, rather than water stress. This finding is in line with the results reported by Bastida et al. (2017). However, substrate addition resulted in reduced species diversity and richness, which is contradicting to their results.

In this study bacterial communities in both soils were dominated by Proteobacteria, Acidobacteria, Actinobacteria. Members of Firmicutes and Planctomycetes were also identified but were less abundant. Proteobacteria, Acidobacteria and Actinobacteria are reported to be the most abundant bacterial phyla found in forest soils (Hartmann et al., 2017; Wei et al., 2018).

While in LUE soil most abundant phylum was Proteobacteria, CAL soil was dominated by Actinobacteria. Prevalence of Actinobacteria over other phyla, including Proteobacteria has been observed in dry soils, while Proteobacteria are more dominant in soils with high moisture level (Chodak et al., 2015; Evans et al., 2014). Next most abundant phyla was Acidobacteria,in particular, members of order Acidobacteriales and Solibacteriales.

Both orders belonging to subdivion 1 (Kalam et al., 2020) are reported to be abundant in forest soils with different plant covers (Foesel et al., 2014; Männistö et al., 2018; Navarrete et al., 2015; Zhang et al., 2020). Members of the subdivision 1 have been reported to promote plant growth (Kielak et al., 2016) and produce EPS with high industrial and ecological value (Kielak et al., 2017). Relative abundance of Acidobacteria was reported to be negatively correlated with soil pH, with significant increase below pH 5.5 (Griffiths et al., 2011) and N amendment (Fierer et al., 2012). Decrease in relative abundance of Acidobacteria, with increasing amount of N has been reported (Ramirez et al., 2010). Furthermore, Acidobacteria are reported to be

mainly oligotrophs and play an important ecological role in degradation of polysaccharides in forest soils (Lladó et al., 2016).

In both non-amended soils, the changes in community composition, driven by “dry” and “intermittent” modes were extremely moderate. Most studies reporting more drastic changes in community composition due to drought or drying rewetting use a wide range of water contents, with more extreme differences (Evans and Wallenstein, 2012; Iovieno and Bååth, 2008; Veach and Zeglin, 2020; Yemadje et al., 2017). The soil water content of the “dry” incubation mode was adjusted to water potential, corresponding to the permanent wilting point. At this water potential, however, the water content of both soils was approximately 50-60% of WHC, which is an optimal condition for microbial activity (Franzluebbers, 2020; Uhlířová et al., 2005), thus the microbial growth condition did not drive major changes in community composition. However, substrate amendment accentuated the differences between incubation modes.

In LUE soil at both substrate levels the relative abundance of Acidobacteria slightly reduced in “dry” and “intermittent” incubation modes, while Actinobacteria increased (“wet” < “intermittent” < “dry”). This response was more prominent in substrate amended soils. A decrease in relative abundance of Acidobacteria, accompanied with increase in Actinobacteria during dry-down were also reported in a study of (Barnard et al., 2013). Furthermore, Actinobacteria play an essential role in decomposition of recalcitrant carbon abundant in drought-prone, oligotrophic soils (Bao et al., 2021; Hartmann et al., 2017). Despite that, Fierer et al. (2007) reported that some orders of Actinobacteria could not be assigned into copiotrophic or oligotrophic category and certain representatives of Actinobacteria phyla have been shown to increase in relative abundance when sucrose was added to soil (Chodak et al., 2015; Goldfarb et al., 2011). Therefore, though the increase in Actinobacteria was observed in non-amended soil, substrate amendment provided perfect growth conditions for the growth of Actinobacteria, particularly order Frankiales, compared to oligotrophic Acidobacteria. Members of order Frankiales are capable of forming symbiont root nodules in dicotyledonous plants, such as Beech, thus fixing nitrogen for their growth and nutrition (Ghodhbane-Gtari et al.,2019). Furthermore,

Frankiales members are drought tolerant, due to hyphal growth and the ability to produce spores (Burleigh and Dawson,1994). Frankiales have been shown to improve plant growth under drought (Kucho et al., 2019; Srivastava et al., 2013) and can be used as biofertilizers in drought affected regions (Sayed, 2011) to improve the crop production.

Decrease in Proteobacteria was observed as well. G- Proteobacteria are known to be sensitive to changes in environmental conditions and are strongly affected by drought (Barnard et al., 2013; Uhlířová et al., 2005).

CAL soil, characterized with lower mean annual precipitation, compared to LUE soil, was less affected by different water regimes. It is known, that soils with drought history, can exhibit more tolerance to dry conditions (Bouskill et al., 2013; Leizeaga et al., 2020). In non-amended CAL soil almost no changes in relative abundances were observed. Only slight increase in Actinobacteria and decrease in Acidobacteria were observed in the “dry” incubation mode. The changes in community composition were accentuated by addition of substrate. As in case of LUE soil, relative abundance of Actinobacteria increased in all substrate amended soil, however, was the highest in “dry” mode, followed by “intermittent” and “wet” modes. As in LUE, significant reduction in the relative abundance of Proteobacteria, was observed as well.

6.4 Microbial drivers of SWR

Results (Figure 5) indicated that water stress results in increased bacterial cell surface hydrophobicity, consequently rendering soil minerals more hydrophobic, compared to unstressed cells (Figure 7). Bacterial living biomass and necromass impact the quality of SOM, a determining factor for occurrence of SWR, indicating the involvement of soil microbes in the development of soil hydrophobicity. There are several challenges in identifying specific bacterial drivers of SWR. Culturable bacteria are considered to be a useful tool to assess changes driven by various environmental factors (Bakken, 1997). However, most bacteria remain uncultured (Steen et al., 2019). Furthermore, it has been shown that the culturability of bacteria is significantly lower in hydrophobic soils, compared to hydrophilic ones (Braun et al., 2010).

Soil microorganisms can both be the source of SWR (Achtenhagen et al., 2015) and some (e.g. Actinobacteria, (Roper, 2004)) have been shown to mediate the decomposition of hydrophobic compounds (Doerr et al.,2000). However, we observed stress driven increased CA in all the strains tested, irrelevant of their metabolic versatility. Therefore, deciphering relationship between specific microbes and SWR based on culture-dependent or conventional culture-independent methods is challenging.

Several studies successfully used RF analysis of 16S rRNA sequences to identify “bioindicators” for litter decomposition (Thompson et al., 2019), microbial response to

physical disturbances in soil (Wang et al., 2020a), predict soil physico-chemical properties based on microbial community (Hermans et al., 2020) and drivers of community resistance to global changes in drylands (Delgado-Baquerizo et al., 2017). In this study random forest analysis was implemented to identify bioindicators responsible for SWR. A low taxonomic resolution was used, as information on low taxonomic levels is becoming increasingly available (Fierer et al., 2007), and are present in different soils across the globe, allowing comparison between different ecosystems. This analysis allowed the identification of particular microbial taxa as drivers of changes in microbial community composition due to changes in soil water availability and possible determinants of SWR. A relatively small number of ASVs were selected by RF as “bioindicators” for different treatments. Only a few ASVs were unique to a treatment; most ASVs were common between treatments but exhibited different abundances. This result indicates that the relative abundances of certain microbial taxonomic groups rather than their presence or absence, could be more important for differentiation between different treatments. Once the samples were classified with low out-of-bag error rate, RF was effective in identifying “bioindicator” ASVs. In our study we observed a high out-of-bag error rates, probably due to heterogeneity in soil samples and similarity in moisture conditions between incubation modes. The differences between the incubation mode were driven mostly by changes in the relative abundance of representatives of different phyla.

In both non-amended soils, most of ASVs identified as bioindicators belong to Proteobacteria and Acidobacteria phyla. All the ASVs from Proteobacteria phylum belonged to Alphaproteobacteria class, which is characteristic for forest soil microbial communities (Nacke et al., 2011). In substrate amended soils however, majority of ASVs belonged to Actinobacteria and Proteobacteria. As previously mentioned, addition of substrate resulted in reduced relative abundance of oligotrophic Acidobacteria. In LUE soil an ASV representative of order Saccharimondales of Pateschibacteria phylum were identified at the beginning of the incubation but were absent after the incubation. Saccharimondales are associated with soils from high moisture climates (Chen et al., 2020). Three ASVs belonging to two different orders of Planctomycetes and 1 Armatimonadetes phylum were detected in CAL soil. Increase in relative abundance of Planctomycetes was observed in the study of (Dai et al., 2019) in drought affected soils. Armatimonadetes are found to be abundant in paddy soils, and decrease in relative abundance, when exposed to drought (Jang et al., 2020). The presence of ASV corresponding to this phylum at the beginning of the incubation can be explained by the wetting of the soil to 45% WC for the pre-incubation.

The proposed hypothesis, that dry conditions increase SWR levels in soil and that the changes differ depending on soil moisture history, was confirmed. The results showed that the drying and drying-rewetting event increase the SWR, however the extent of these changes is mitigated by the soil drought history. Furthermore, as expected, in the soil with high level of SWR the microbial community was initially more adapted to dry conditions and was more resistant to reduced water availability, compared to the moderately repellent soil. Additionally, the results showed that only a small fraction of total community is involved in driving the changes in its structure, including members of several less abundant orders.

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7 CONCLUSIONS

Hydrophobic compounds derived from plants and microorganisms, coating soil particles and soil aggregates, cause SWR. However, due to their significant contribution to SOM (Miltner et al., 2012), understanding the direct involvement of bacteria in the development of SWR would lead to better understanding of the occurrence and persistence of SWR in soil. As global climate change becomes more severe the frequency and intensity of droughts increases, lowering availability of water from soil microorganisms. Our results showed that reduced water potential in most cases leads to increase in surface hydrophobicity of bacterial cells. However, the response to water stress can vary depending on the strain, origin of the stress and the growth conditions. Growing in biofilms or microcolonies as they occur in soil show advantage over submersed growth regarding tolerance of environmental stress. The CSH of G+ bacteria were not affected during surface growth; however, most were initially hydrophobic. As an adaptive response to water stress G- bacterial CSH increased with increasing water stress. This means that when exposed to water stress (pF value >4.2) in soil G- bacteria increase CSH hydrophobicity to survive the drought. In addition, results obtained in the CMA incubation experiment confirmed that bacterial adhesion to mineral particles renders them less hydrophilic, with G+ bacteria having initially stronger short-term impact, significantly declining with improvement of environmental conditions. In contrast, surface hydrophobicity induced by G- bacteria can increase even after the removal of the stressor, and it can persist for longer periods. All the hypotheses in this study were based on the assumption that soil water repellency is caused by the cell surface hydrophobicity of soil bacteria. The investigation of microbial community and SWR response to reduced water availability showed, that the community composition reflects our assumptions, with the more hydrophobic soil being dominated with G+ bacteria, while G- bacteria were relatively more abundant in the moderately repellent soil. Furthermore, the increased SWR in the moderately hydrophobic soil was accompanied with increase of G+ bacteria, in particular Actinobacteria, representatives of which exhibited higher level of water repellency. Considering the similarity of physico-chemical properties of the two soils, it is safe to assume a correlation between the SWR and microbial community.

In conclusion, considering increase in cell surface hydrophobicity as an adaptive stress response, the impact of bacterial cells on wettability of minerals and the changes in SWR and microbial community in response to variations in soil WC, it is inferred, that soil bacteria play a significant role in development of SWR, not only by production or

degradation of hydrophobic compounds found in soil, but also by directly impacting the surface properties of soil particles.

The fast response of microbial communities to environmental stress indicates, that short term SWR is induced by microbes. However, prolonged dry conditions will eventually lead to a shift towards more adapted microbial community with more hydrophobic cells, thus contributing to the persistent SWR. We conjecture that SWR is caused by changes in the microbial community, rather than the opposite and there is positive feedback between bacterial CSH and SWR, which drives the formation of hydrophobic domains in soil.

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10 APPENDIX

Table 7 Incubation length of different bacterial strains in submerged and surface growth

	Growth condition	
Microorganism	Submersed growth	Surface growth
Bacillus subtilis	6 h	30 h
Arthrobacter chlorophenolicus	6 h	30 h
Pseudomonas fluorescens	6 h	30 h
Novosphingobium aromaticivorans	16 h	42 h
Rhodococcus erythropolis	8.5 h	30 h
Mycobacterium pallens	22 h	65 h

Table 8. Abundance of PLFA present in CMAs amended with *B. subtilis* cells, presented as % of total PLFA

	Incubtion time	Total PLFA	iso: antiiso	i15:0	a15:0	i16:0	i17:0	a17:0	10me17:0	18:2ω6,9	18:1ω9
	[days]					% of total PLFA					
unstressed *B. subtilis*	0	647 (± 27)	0.5 (± 0.0)	4.2 (± 0.7)	20.0(± 2.3)	5.4 (± 0.8)	5.3 (± 1.4)	12.1 (± 3.1)	0.2 (± 0.3)	0.4 (± 0.5)	0.2 (± 0.3)
	30	185 (± 10)	0.5 (± 0.1)	0.6 (± 0.3)	1.9 (± 0.7)	0.5 (± 0.2)	0.6 (± 0.1)	1.4 (± 0.3)	0.3 (± 0.2)	0.3 (± 0.2)	0.6 (± 0.5)
	50	226 (± 28)	0.7 (± 0.2)	0.3 (± 0.0)	1.0 (± 0.3)	3.0 (± 3.8)	0.5 (± 0.1)	0.7 (± 0.4)	0.4 (± 0.3)	0.6 (± 0.5)	8.0 (± 1.2)
	80	88 (± 7)	8.1 (± 2.7)	0.2 (± 0.1)	0.5 (± 0.4)	3.8 (± 1.4)	0.4 (± 0.2)	0.1 (± 0.1)	n.d.	0.4 (± 0.0)	0.4 (± 0.0)
stressed *B. subtilis*	0	562 (± 26)	2.8 (± 0.1)	6.7 (± 0.1)	4.7 (± 0.0)	6.6 (± 0.6)	n.d.	n.d.	1.3 (± 0.1)	3.4 (± 0.1)	19.0 (± 5.3)
	30	178 (± 18)	3.6 (± 2.4)	0.4 (± 0.1)	1.7 (± 0.5)	0.5 (± 0.1)	7.2 (± 4.0)	0.6 (± 0.1)	0.3 (± 0.0)	0.6 (± 0.1)	0.7 (± 0.0)
	50	202 (± 14)	0.5 (± 0.1)	0.2 (± 0.1)	0.8 (± 0.4)	0.2 (± 0.1)	0.3 (± 0.1)	0.8 (± 0.2)	n.d.	0.7 (± 0.5)	0.5 (± 0.1)
	80	267 (± 15)	10.3 (± 2.0)	0.3 (± 0.1)	0.7 (± 0.3)	0.3 (± 0.1)	13.8 (± 2.3)	0.4 (± 0.1)	0.4 (± 0.1)	0.8 (± 0.4)	1.2 (± 1.2)
Quartz	0	143 (± 6)	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	2.4 (± 0.2)
	30	39 (± 9)	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	50	24 (± 8)	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	80	33 (± 4)	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.

Table 9 Abundance of PLFA present in CMAs amended with *P. fluorescens* cells, presented as % of total PLFA

	Incubtion time	Total PLFA	16:1ω7t	16:1ω7c	16:0	17:0cyclo	18:1ω7t	18:1ω7c	18:0	19:0cyclo
	[days]	nmol PLFA g^{-1} soil	% of total PLFA							
unstressed *P.fluorescens*	0	262 (± 38)	5 (± 2)	3 (± 1)	43 (± 4)	9 (± 1)	1 (± 0)	19 (± 1)	18 (± 0)	1 (± 0)
	30	45 (± 12)	n.d.	n.d.	24 (± 8)	5 (± 1)	2 (± 2)	8 (± 1)	55 (± 11)	5 (± 1)
	50	52 (± 17)	n.d.	n.d.	34 (± 4)	5 (± 1)	5 (± 1)	8 (± 2)	43 (± 3)	4 (± 1)
stressed *P.fluorescens*	0	59 (± 59)	5 (± 5)	2 (± 2)	4 (± 4)	2 (± 2)	0 (± 0)	4 (± 4)	3 (± 3)	0 (± 27)
	30	36 (± 36)	n.d.	n.d.	27(± 27)	2 (± 1)	2 (± 2)	8 (± 8)	3 (± 26)	6 (± 7)
	50	11 (± 11)	1 (± 1)	n.d.	5 (± 5)	1 (± 1)	1 (± 1)	3 (± 3)	5 (± 5)	1 (± 1)
Quartz	0	53 (± 9)	n.d.	n.d.	49 (± 6)	n.d.	n.d.	n.d.	49 (± 6)	n.d.
	30	32 (± 1)	n.d.	n.d.	19 (± 6)	n.d.	n.d.	n.d.	78 (± 6)	n.d.
	50	148 (± 9)	n.d.	n.d.	39 (± 2)	n.d.	3 (±0)	2 (±0)	56 (±1)	n.d.

Table 10 Relative abundance of PLFA groups present in LUE soil, presented at % of total PLFA

	Incubation time	Total PLFA	Terminally branched fatty acids	Methyl brenched fatty acids	Mono unsat. fatty acids	Cyclopro. fatty acids	Poly unsat. fatty acids	Poly: mono+ branched	Cyclo: precursor	Mono: sat
	[days]	nmol PLFA g-1 soil	% of total PLFA							
wet	0	306 (± 7)	14 (± 1)	11 (± 1)	34 (± 0)	4 (± 2)	1 (± 0)	0.02	0.3	1
	29	**215 (± 46)**↓	10 (± 1)	11 (± 1)	**40 (± 0)**↑	16 (± 1)	2 (± 0)	0.02	0.9	1.5
	88	226 (± 41)	13 (± 1)	8 (± 5)	42 (± 1)	**21 (± 2)**↑	**6 (± 0)**↑	**0.08**↑	3.6↑	**2.8**↑
dry	0	469 (± 35)	14 (± 0)	10 (± 0)	35 (± 0)	20 (± 0)	2 (± 0)	0.03	1.3	2.3
	29	**255 (± 31)**↑	11 (± 0)	11 (± 0)	37 (± 0)	19 (± 0)	2 (± 0)	0.03	1.2	1.6
	88	266 (± 12)	12 (± 1)	**7 (± 1)**↓	34 (± 0)	9 (± 1)	**28 (± 1)**↑	0.44	5↑	2.4
intermit.	0	306 (± 7)	14 (± 1)	11 (± 1)	34 (± 0)	4 (± 2)	1 (± 0)	0.02	0.3	1
	29	226 (± 11)	12 (± 3)	13 (± 5)	36 (± 1)	16 (± 1)	2 (± 1)	0.03	1.1	2
	88	299 (± 58)	14 (± 1)	9 (± 1)	31 (± 2)	**18 (± 2)**↑	2 (± 0)	0.03	1.4↑	1.3
wet	0	225 (± 17)	11 (± 1)	8 (± 1)	30 (± 0)	16 (± 1)	13 (± 1)	0.2	2	1.7
	29	210 (± 101)	7 (± 1)	9 (± 1)	37 (± 0)	**8 (± 1)**↓	18 (± 1)	0.29	0.9	1.4
	88	260 (± 40)	12 (± 0)	**14 (± 0)**↑	29 (± 0)	10 (± 0)	**9 (± 0)**↓	0.14↓	1.2	1
dry	0	277 (± 40)	12 (± 0)	9 (± 0)	30 (± 0)	14 (± 1)	14 (± 1)	0.21	1.8	1.7
	29	37 (± 2)	4 (± 0)	7 (± 0)	45 (± 0)	**9 (± 0)**↓	19 (± 0)	0.29	1.3	0.7
	88	**372 (± 5)**↑	12 (± 1)	7 (± 1)	34 (± 0)	9 (± 1)	**28 (± 1)**↑	**0.44**↑	5	2.4
intermit.	0	225 (± 17)	11 (± 1)	8 (± 1)	30 (± 0)	16 (± 1)	13 (± 1)	0.2	2	1.7
	29	350 (± 26)	7 (± 0)	9 (± 1)	41 (± 0)	7 (± 0)	14 (± 1)	0.22	0.9	2.1
	88	283 (± 9)	12 (± 0)	**14 (± 1)**↑	29 (± 0)	11 (± 0)	**9 (± 0)**↓	0.14	1.3	1.2

Table 11 Relative abundance of PLFA groups present in CAL soil, presented at % of total PLFA

	Incubation Time	Total PLFA	Terminally branched fatty acids	Methyl brenched fatty acids	Mono unsat. fatty acids	Cyclopro. fatty acids	Poly unsat. fatty acids	Poly: mono+ branched	Cyclo: precursor	Mono: sat
	[days]	nmol g^{-1} soil	% of total PLFA							
wet	0	572 (± 58)	9 (± 1)	17 (± 1)	14 (± 0)	15 (± 2)	2 (± 0)	0.05 (± 0.0)	5.43 (± 0.16)	0.61 (± 0.01)
	29	**314 (± 37)↓**	**14 (± 1)↑**	18 (± 0)	**17 (± 0)↑**	18 (± 0)	2 (± 0)	0.05 (± 0.0)	5.96 (± 0.15)	0.61 (± 0.01)
	88	368 (± 5)	16 (± 1)	13 (± 5)	**25 (± 1)↑**	17 (± 2)	1 (± 0)	**0.02 (± 0.0)↓**	**9.01 (± 0.55)↑**	**0.74 (± 0.03)↑**
dry	0	510 (± 37)	8 (± 1)	20 (± 0)	16 (± 0)	16 (± 0)	2 (± 0)	0.04 (± 0.0)	5.76 (± 0.15)	0.62 (± 0.01)
	29	513 (± 35)	**15 (± 0)↑**	20 (± 0)	17 (± 0)	16 (± 0)	3 (± 0)	0.05 (± 0.0)	5.76 (± 0.15)	0.68 (± 0.01)
	88	**410 (± 11)↓**	17 (± 0)	**16 (± 0)↓**	14 (± 0)↓	18 (± 0)	2 (± 0)	0.03 (± 0.0)	**8.84 (± 0.28)↑**	**0.48 (± 0.01)↓**
intermit.	0	572 (± 58)	9 (± 1)	17 (± 1)	14 (± 0)	15 (± 2)	2 (± 0)	0.05 (± 0.0)	5.43 (± 0.16)	0.6 (± 0.01)
	29	**283 (± 40)↓**	11 (± 0)	15 (± 1)	**18 (± 0)↑**	13 (± 0)	3 (± 1)	0.06 (± 0.03)	3.65 (± 0.18)	0.57 (± 0.03)
	88	256 (± 43)	**17 (± 1)↑**	15 (± 1)	16 (± 2)	17 (± 2)	2 (± 0)	0.04 (± 0.0)	7.33 (± 1.03)	0.54 (± 0.02)
					substrate amended					
wet	0	463 (± 58)	7 (± 1)	11 (± 1)	18 (± 0)	14 (± 1)	13 (± 1)	0.31 (± 0.02)	5.68 (± 0.18)	0.66 (± 0.02)
	29	449 (± 51)	**11 (± 1)↑**	14 (± 2)	19 (± 1)	15 (± 1)	14 (± 1)	0.31 (± 0.03)	6.17 (± 0.62)	0.77 (± 0.05)
	88	352 (± 36)	13 (± 0)	17 (± 0)	20(± 0)	**7 (± 0)↓**	12 (± 0)	**0.24 (± 0.0)↓**	4.42 (± 0.13)	0.69 (± 0.0)
dry	0	509 (± 36)	8 (± 0)	17 (± 0)	16 (± 0)	14 (± 1)	6 (± 1)	0.13 (± 0.01)	5.54 (± 0.12)	0.61 (± 0.01)
	29	832 (± 79)	6 (± 0)	**7 (± 0)↓**	**26 (± 0)↑**	**6 (± 0)↓**	**28 (± 0)↑**	**0.71 (± 0.08)↑**	4.02 (± 0.21)	**0.84 (± 0.02)↑**
	88	574 (± 57)	**10 (± 1)↑**	**12 (± 1)↑**	21 (± 0)↓	8 (± 1)	**19 (± 1)↓**	**0.44 (± 0.03)↓**	5.17 (± 0.25)	**0.65 (± 0.07)↓**
intermit.	0	463 (± 37)	7 (± 1)	11 (± 0)	18 (± 0)	14 (± 0)	13 (± 0)	0.31 (± 0.02)	5.68 (± 0.18)	0.66 (± 0.02)
	29	488 (± 40)	7 (± 0)	10 (± 1)	**22 (± 0)↑**	**6 (± 0)↓**	**26 (± 1)↑**	**0.65 (± 0.03)↑**	**3.63 (± 0.18)↓**	0.76 (± 0.03)
	88	435 (± 67)	**11 (± 0)↑**	**17 (± 1)↑**	20 (± 0)	7 (± 0)	**13 (± 0)↓**	**0.27 (± 0.0)↓**	4.44 (± 0.33)	0.65 (± 0.02)

Curriculum vitae

PERSONAL DATA

Family name: Karagulyan

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EDUCATION

Since Oct 2017 — External PhD student in the Department of Soil Physics at Leibniz Universität Hannover, Hannover, Germany

Practical work performed in the Department of Environmental Biotechnology at Helmholtz Center for Environmental Research - UFZ, Leipzig, Germany

Thesis: “Impact of Bacterial Biomass on Soil Particle Wettability under Various Moisture Conditions”

2014 - 2016 — MSc in Agricultural Engineering at Szent Istvan University, Gödöllő, Hungary
FAO-Hungarian Government Scholarship

Thesis: "Analyzing Land Cover Change in Hungary using CORINE Land Cover Maps"

2011-2013 — MSc in Desert Studies at Ben-Gurion University of Negev, Sde-Boker, Israel

Thesis: "Effect of Nitrification Inhibitors on Activity, Diversity and Abundance of Ammonia Oxidizers"

2007-2011	BSc in Biology and Qualification of Biology teacher at Yerevan State University, Yerevan, Armenia **Thesis**: “Identification of Industrial Strains of Lactic Acid Bacteria by Means of Molecular Genetic Methods”