Diversity and activity of soil bacterial communities under different management regimes

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Chapter I

General Introduction

1.1. Soil bacterial community structure and activity

Soil is a naturally occurring, structured, heterogeneous, and discontinuous system (Stotzky, 1997). The soil habitat is defined as the totality of living organisms inhabiting soil, which includes plants, animals, and microorganisms (Voroney, 2007). Soil harbors an enormous biomass of prokaryotic cells (Torsvik et al., 2002), with an estimate of 10^{10} cells per gram soil (Portillo et al., 2013; Sikorski, 2015). Dominant phyla such as *Proteobacteria*, *Acidobacteria*, *Actinobacteria*, *Chloroflexi*, and *Firmicutes* were previously described in many DNA-based studies investigating the soil habitat (Janssen, 2006; Will et al., 2010; Lauber et al., 2013; Pfeiffer et al., 2013; Rampelotto et al., 2013). In these studies, they accounted for up to 92% of all analyzed bacterial sequences and thus represent ubiquitous phylogenetic groups of the soil microbiome.

Less than 1% of microbial species are considered to grow under laboratory conditions (Torsvik et al., 2002). Therefore, the bacterial structure, ecology and their functioning in soil are of great interest. Next-generation sequencing (NGS) technologies (Mardis, 2008) are a promising approach to understand bacterial community composition and diversity. Pyrosequencing-based analysis of partial 16S rRNA genes has been successfully employed to gain insights into the microbial structure of various habitats such as water (e.g. Kirchman et al., 2010; Wemheuer et al., 2014), extreme habitats (e.g. Simon et al., 2009; Schneider et al., 2013; Röske et al., 2014), and soil (e.g. Uroz et al., 2010; Will et al., 2010; Nacke et al., 2011; Rampelotto et al., 2013).

Nonetheless, only a few studies investigated the active soil bacterial community using RNA-based approaches in combination with NGS (but see Urich et al., 2008; Baldrian et al., 2012; Pfeiffer et al., 2013). Baldrian et al. (2012) investigated the active microbial community in forest soils and found a stronger dominance of several phyla (e.g. *Acidobacteria* and *Firmicutes*) in the RNA dataset compared to the DNA-derived dataset. Taking into account that DNA-based approaches detect also dead cells, extracellular DNA, and dormant microorganisms (Lennon and Jones, 2011) RNA-based approaches provide a better overview of what is metabolic active at a given time. Thus, application of RNA-based studies results in deeper insights into the prokaryotic community response to changing of environmental conditions.

1.2. Interaction of soil rhizosphere bacteria and plant species

The rhizosphere is the soil part, which is most affected by the roots of growing plants (Pinton et al., 2007). It is considered to be a narrow zone of soil where root exudates stimulate or inhibit microbial populations and their activities. Rhizospheric soil includes a high density of prokaryotic cells, with estimates up to 10^{11} cells per gram soil (Torsvik et al., 1990; Sikorski, 2015). The bacterial community composition in the rhizosphere depend mainly on soil type and plant species (Berg and Smalla, 2009; Lundberg et al., 2012). The bacterial composition seems to be similar of the enclosing bulk soil (Bulgarelli et al., 2012; Lundberg et al., 2012) with *Proteobacteria* as the most abundant phylum (Hawkes et al., 2007). Bulgarelli et al. (2012) investigating the bacterial community in the rhizosphere of *Arabidopsis* plants by 454-pyrosequencing approaches and found that the core community of rhizospheric bacteria was recruited from the surrounding soil, which were able to colonize the plant-root surface.
Many members of the rhizospheric bacterial community have been reported to improve plant growth and health, e.g. by disease suppression (Sturz and Nowak, 2000; Bastida et al., 2009; Mendes et al., 2011; Koeberl et al., 2013). Mendes et al. (2011) indentified key bacterial taxonomic groups such as *Proteobacteria*, especially *Gammaproteobacteria*, *Firmicutes*, and *Actinobacteria* which are associated with disease suppression. A multitude of compounds are released into the rhizosphere of soil-grown plants, most of which are organic compounds (e.g. sugars, amino acids, organic acids, or fatty acids) and plant constituents derived from photosynthesis and other processes, which in turn stimulate and define the rhizospheric soil bacterial community and diversity (Rovira, 1969; Lynch and Whipps, 1991; Singh and Mukerji, 2006).

1.3. Factors influencing the bacterial community

Bacterial community structure, function, and diversity are influenced by various factors; e.g., abiotic soil properties such as pH, C/N ratio, and water availability (Lauber et al., 2009; Nacke et al., 2011; Tripathi et al., 2012; Landesman et al., 2014). Analyzing the drivers changing the bacterial community structure and diversity, including evenness and richness, is very versatile and complex (Figure 1). The link between abiotic factors and bacterial communities are subject of several studies. Landesman et al. (2014) collected 700 soil samples across multiple spatial scales, tree species and forests in the eastern United

States. The authors generated 469,209 high quality partial 16S rRNA gene sequences with high-throughput sequencing and measured those soil properties thought to influence the bacterial community composition. They found that 81.7% of the explained deviance in overall bacterial composition was attributed to soil properties, especially soil pH. Tripathi et al. (2012) generated 74,802 16S rRNA gene sequences of 28 tropical soil samples and found that bacterial community composition and diversity was strongly correlated with soil properties such as pH, total carbon, and C/N ratio. They concluded that soil pH is the best predictor of bacterial community composition and diversity across various land use types, with the highest diversity close to neutral pH values.

Additionally to soil properties, land use type, management regimes, and plant species have an direct or indirect impact on soil bacterial community composition and diversity (e.g. Thoms et al., 2010; Fierer et al., 2012; Jorquera et al., 2013; Lauber et al., 2013; Pfeiffer et al., 2013; Rampelotto et al., 2013; Dean et al., 2014; Thomson et al., 2015). Rampelotto et al. (2013) investigated changes in diversity, abundance, and structure of bacterial communities under different land use systems. They observed a relevant impact on bacterial groups and differences in the abundance of bacterial phyla in soils with land use as the main driver. Pfeiffer et al. (2013) investigated the effect of different tree species on soil bacterial composition and diversity in a mesocosm experiment. They found that tree species such as beech and ash influenced bacterial community composition and diversity in different ways.

In addition to the impact of tree species, fertilizer application drives bacterial community composition and diversity. In a recent study by Jorquera et al. (2013), the relationship between nitrogen (N) and phosphorus (P) fertilization on bacterial community composition in rhizospheric soils of two Chilean Andisol pastures was investigated. They found that N fertilization without P amendment significantly affected the soil bacterial community, whereas the application of P and N did not significantly altered the bacterial community composition.

Moreover, the versatile effect of season and sampling time on the structure of soil and plant-associated bacterial communities is another subject of ongoing research (Smalla et al., 2001; Cruz-Martinez et al., 2009; Shade et al., 2013). Changes are caused by various factors including temporal differences in plant growth and substrate availability (Kennedy et al., 2005; Kuffner et al., 2012; Silva et al., 2012; McHugh and Schwartz, 2015) or by changes in soil environmental conditions (Barnard et al., 2015; Schostag et al., 2015). Cruz-Martinez et al. (2009) found only little differences in soil bacterial community

composition in a manipulated rainfall experiment over 5 years. Extreme weather conditions affected the microbial community, but repeated sampling over seasons and years revealed that these changes were short-term effects. Furthermore, Kuffner et al. (2012) analyzed 12 forest soil samples and generated 17,308 16S rRNA gene sequences per sample. They found that diversity indices did not differ between summer and winter, and seasonal shifts were coherent among related phylogenetic groups. In contrast, Rasche et al. (2011) monitored the seasonal impact on total bacterial community composition every two month over a 2-years period, employing T-RFLP analysis. The authors observed that seasonality had the greatest impact on the total bacterial community as well as on four selected taxa (*Alpha*- and *Betaproteobacteria*, *Acidobacteria*, and *Verrucomicrobia)*.

There is a great variety of factors influencing the soil bacterial community composition and diversity. In addition, the interaction between soil microbial communities, plant species, and abiotic factors is very complex and still not fully understood. Thus, this field of investigation is heterogeneous and intensive research is necessary to understand the ecological role of soil bacterial communities.

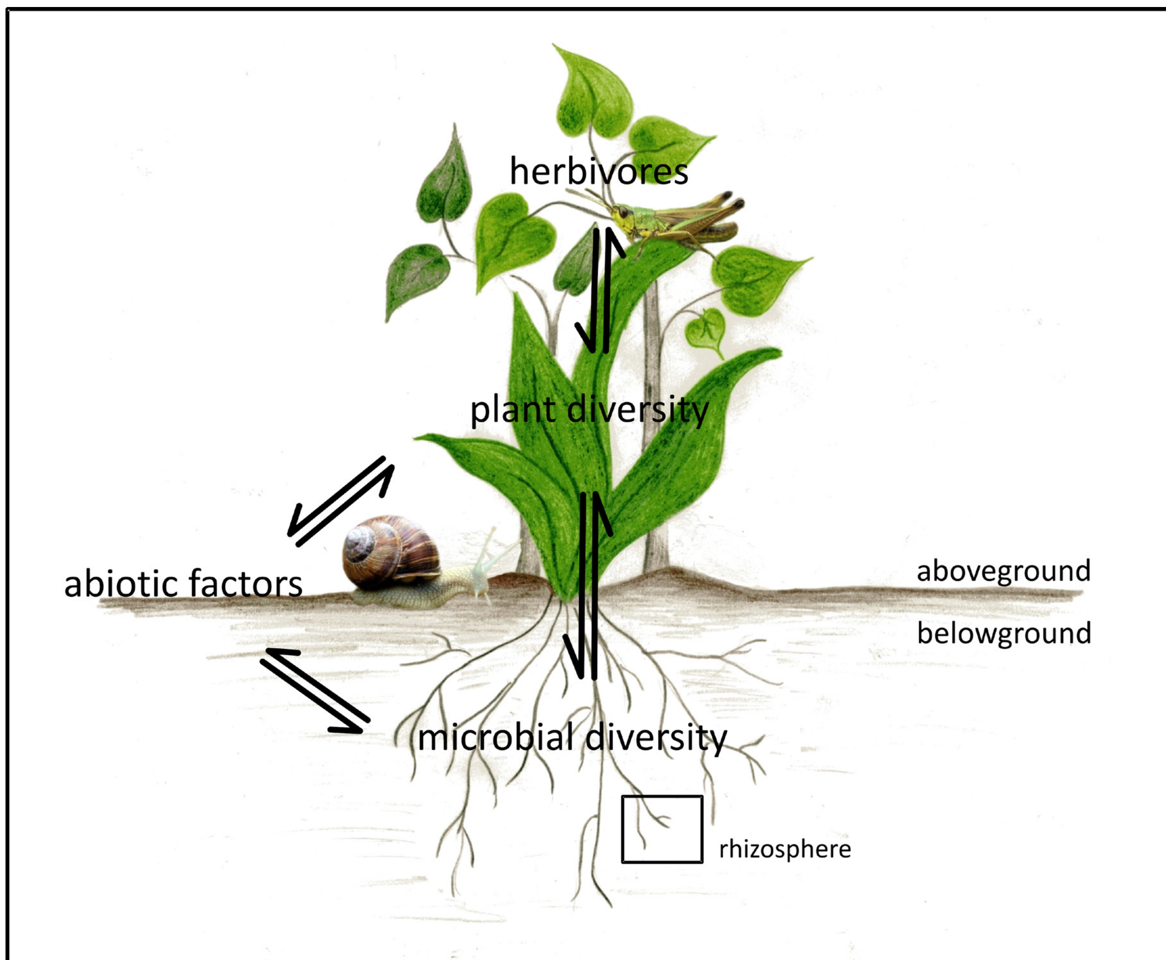

Figure 1. Interactions between microbial diversity, plant diversity, herbivores, and abiotic factors. Depicted is the rhizosphere (soil-root interface) where bacteria can colonize and play important roles.

1.4. The Poplar Diversity Experiment

The Poplar Diversity Experiment (PopDiv) was established within the framework of the Göttingen cluster of excellence "Functional Biodiversity Research". The general goal was to investigate inter- and intraspecific diversity of different poplar demes with respect to ecosystem functioning and biodiversity. The experiment was established in October 2008 on a former historically documented permanent grassland (Thurengia, 1910). The study site is located in the Solling mountains (51°44´56´´ N, 9°32´28´´ E), approximately 60km west of Göttingen (Lower Saxony, Germany). The moderately nutrient poor and acidic soil type is a Haplic Cambisol with a loamy silt texture (Hoeft et al., 2014). During the study period, mean annual temperature and annual precipitation were 6.6°C and 732mm in 2010 and 8.91°C and 724mm in 2011, respectively (Keuter et al., 2013).

A fully randomized plot design of 20 blocks each with six plots containing each 25 poplar trees was applied by Kleemann (2010) (Figure 2). The trees were planted in four different diversity levels, including monocultures, a mixture of two poplar tree demes, a mixture of four, and a mixture of eight poplar tree demes. To avoid edge effects, each block was surrounded by a row of additional poplar trees. The plant material used (seeds or small plants) derived from Austria, Germany (three poplar demes), Poland, Sweden, Switzerland, and the USA. Seven of the *Populus tremula* demes originating from Europe and one closely related deme *P. tremuloides* from North America. These two poplars are considered as sister species (Cervera et al., 2005; Pakull et al., 2009) or as conspecific subspecies (Stettler et al., 1996), depending on the criteria of relatedness applied. The genus *Populus* was chosen due to its wide range of positive attributes such as rapid growth, high tolerance to different climatic conditions, minor requirements to soil fertilities, and because of its fully sequenced genome (Tuskan et al., 2006). In this study, the influence of two different poplar demes (Geismar2 and Geismar8), soil properties, and season on the total and active bacterial community composition and diversity was investigated.

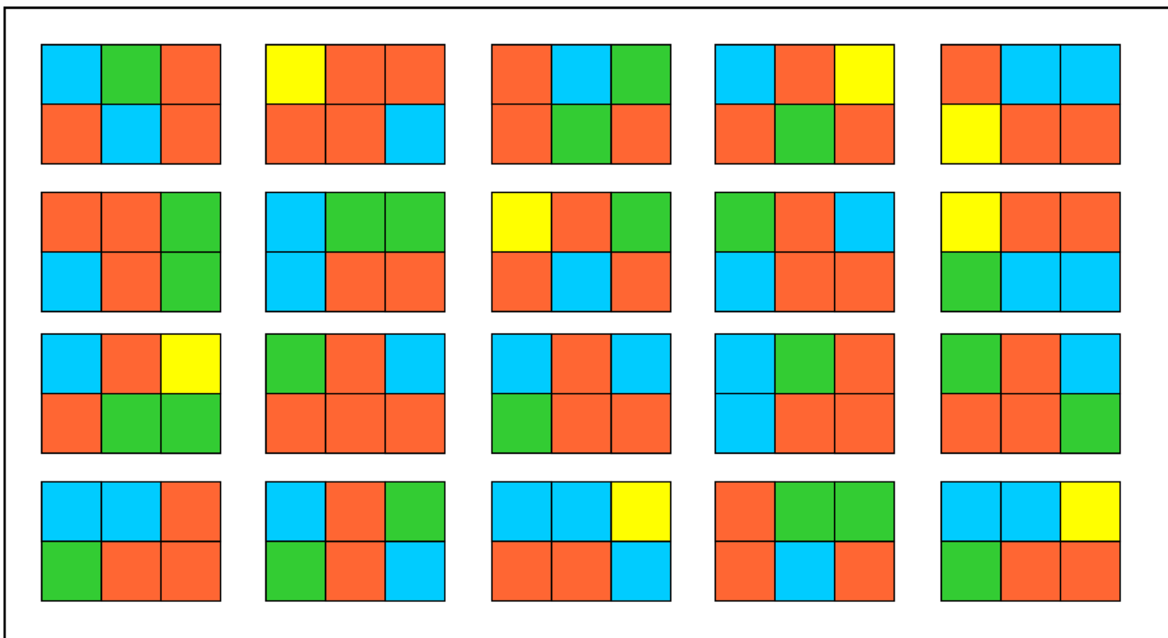

Figure 2. Simplified plot design of the poplar diversity experiment. Eight poplar demes were planted in different diversity levels. Depicted were level 1: Monocultures (green), level 2: a mixture of two poplar demes (red), level 3: a mixture of four poplar demes (blue), and level 4: a mixture of eight poplar demes (yellow) in every plot.

1.5. The GrassMan Experiment

The Grassland Management Experiment (GrassMan) was also established in June 2008 within the Göttingen cluster of excellence “Functional Biodiversity Research”. The experimental area is located in the Solling mountains, Lower Saxony, Germany (51°44′ N, 9°32′′E, 490m a.s.l.). This area has been traditionally used as a pasture for hay-making or grazing. The three-factorial design of this experiment included two mowing frequencies (once per year in July vs. three times per year in May, July, and September) and two fertilizer treatments (no fertilizer vs. NPK fertilizer application) (Figure 3). The N fertilizer was applied as calcium ammonium nitrate N27 in two equal doses (180kg N ha^{-1} yr^{-1}) in April and end of May. Additionally, 30kg P ha^{-1} yr^{-1} and 105kg K ha^{-1} yr^{-1} as Thomaskali® (8% P_2O_5, 15% K_2O, and 20% CaO) were applied at the end of May. The mowing and fertilization regimes started in 2009. A third parameter manipulated was the sward composition. This was achieved by selective herbicide application targeting either dicots (monocot-reduced) or monocots (dicot-reduced). Species-rich plots were left untreated and used as control. Each treatment (12 different combinations) was replicated six times, resulting in 72 plots of 15 x 15m size arranged in a Latin rectangle. The soil of the experimental area is a stony Haplic Cambisol, developed on sediments of loess on the middle bunter formation (Keuter et al., 2013). During the study period, mean annual temperature and annual precipitation were 6.6°C and 732mm in 2010 and 8.91°C and 724mm in 2011, respectively (Keuter et al., 2013).

In this study, the influence of season, fertilization, and soil properties on the total and active soil bacterial community composition and diversity was investigated. Furthermore, the impact of management regimes and herbivory on the total rhizospheric bacterial community composition was examined.

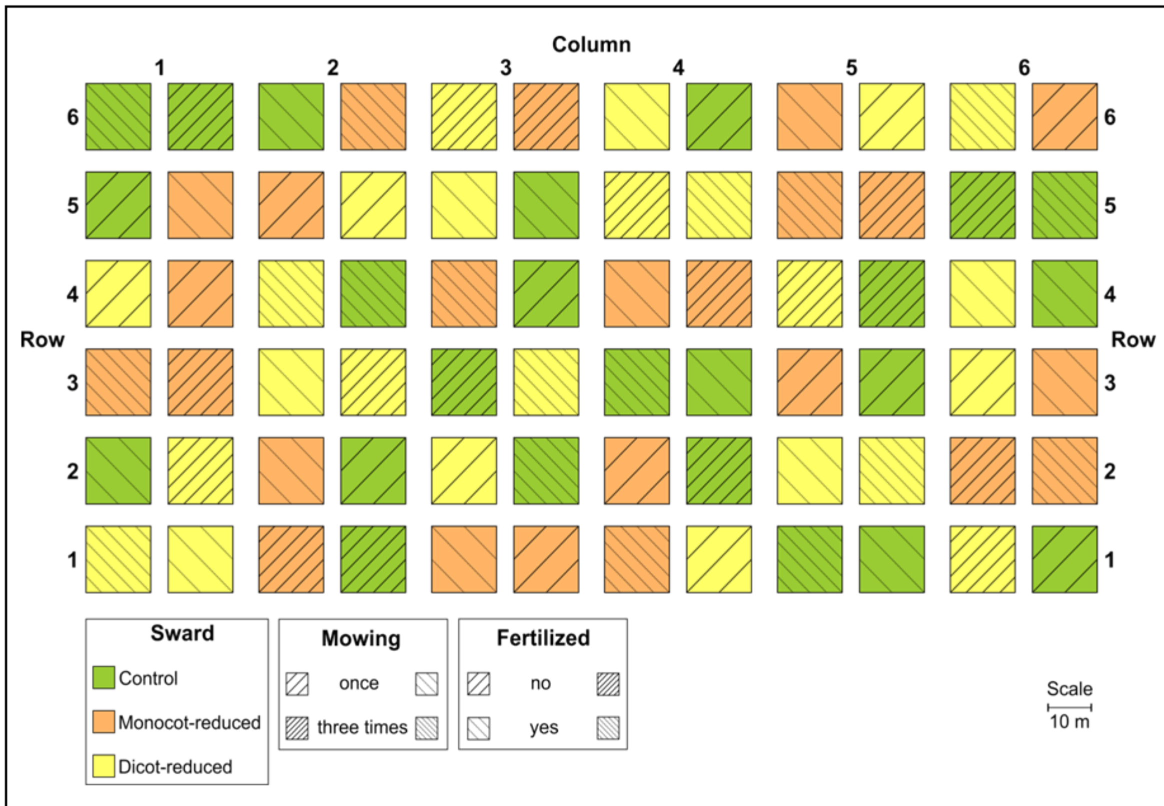

Figure 3: Simplified three-factorial plot design of the GrassMan study site. Depicted were 72 plots with twelve different treatments replicated each six times (Wemheuer, 2013).

1.6. Aim of this study

The aim of this study was to characterize the total and metabolic active soil bacterial community and diversity under different management regimes for a better understanding of the ecological role of soil bacterial communities. Thus, the impact of fertilizer application, poplar demes, season, and soil properties on total and active bacterial community composition and diversity were analyzed. Additionally, effects of different management regimes such as mowing frequencies and sward composition on plant-associated bacteria in the plant rhizosphere in permanent grasslands were investigated.

Chapter II examined the versatile impact of fertilization and sampling time on the total and active bacterial community composition in German grassland soil. In Chapter III differences of active and total bacterial community structure between two genetic different poplar demes were analyzed. Additionally, the effect of seasonality was studied. For both chapters (II and III) 216 soil samples were collected over two years (2010 and 2011) in April, July, and September. DNA and RNA were co-isolated and the RNA reversed transcribed to cDNA. Parts of the 16S rRNA gene and gene transcript were further

amplified and via 454-pyrosequencing technique analyzed. The focus of this research was to evaluate the influence of sampling time, poplar demes, soil properties, and fertilization on the total and active soil bacterial community composition.

In Chapter IV the mixed effects of management regimes and above-ground herbivory on bacterial community composition in the rhizosphere of permanent grassland was analyzed. In more detail, a lysimeter experiment was conducted on the GrassMan experimental site. After two weeks of herbivory exposure (snails and grasshopper), samples were taken in summer 2011 and analyzed by 454-pyrosequencing technique and DGGE. Additionally to the influence of herbivory on the rhizospheric bacterial community composition, samples were analyzed with respect to different mowing frequency and fertilizer application.

1.7. References

Baldrian, P., Kolařík, M., Stursová, M., Kopecký, J., Valášková, V., Větrovský, T., Zifčáková, L., Snajdr, J., Rídl, J., Vlček, C., and Voříšková, J. (2012). Active and total microbial communities in forest soil are largely different and highly stratified during decomposition. *ISME J.* 6**,** 248-258.

Barnard, R.L., Osborne, C.A., and Firestone, M.K. (2015). Changing precipitation pattern alters soil microbial community response to wet-up under a Mediterranean-type climate. *ISME J.* 9**,** 946-957.

Bastida, F., Moreno, J., Nicolas, C., Hernandez, T., and Garcia, C. (2009). Soil metaproteomics: a review of an emerging environmental science. Significance, methodology and perspectives. *Eur. J. Soil Sci*. 60**,** 845-859.

Berg, G., and Smalla, K. (2009). Plant species and soil type cooperatively shape the structure and function of microbial communities in the rhizosphere. *Fems Microbiol. Ecol.* 68**,** 1-13.

Bulgarelli, D., Rott, M., Schlaeppi, K., Van Themaat, E., Ahmadinejad, N., Assenza, F., Rauf, P., Huettel, B., Reinhardt, R., Schmelzer, E., Peplies, J., Gloeckner, F., Amann, R., Eickhorst, T., and Schulze-Lefert, P. (2012). Revealing structure and assembly cues for Arabidopsis root-inhabiting bacterial microbiota. *Nature.* 488**,** 91-95.

Cervera, M., Storme, V., Soto, A., Ivens, B., Van Montagu, M., Rajora, O., and Boerjan, W. (2005). Intraspecific and interspecific genetic and phylogenetic relationships in the genus Populus based on AFLP markers. *Theor. App. Gene.* 111**,** 1440-1456.

Cruz-Martinez, K., Suttle, K., Brodie, E., Power, M., Andersen, G., and Banfield, J. (2009). Despite strong seasonal responses, soil microbial consortia are more resilient to long-term changes in rainfall than overlying grassland. *ISME J.* 3**,** 738-744.

Dean, S.L., Farrer, E.C., Porras-Alfaro, A., Suding, K.N., and Sinsabaugh, R.L. (2014). Assembly of root-associated bacteria communities: interactions between abiotic and biotic factors. *Environ. Microbiol. Rep*. 7, 102-110.

Fierer, N., Lauber, C., Ramirez, K., Zaneveld, J., Bradford, M., and Knight, R. (2012). Comparative metagenomic, phylogenetic and physiological analyses of soil microbial communities across nitrogen gradients. *ISME J.* 6**,** 1007-1017.

Hawkes, C.V., Deangelis, K.M., and Firestone, M.K. (2007). Root interactions with soil microbial communities and processes. *The Rhizosphere, an Ecological Perspective***,** 1-29.

Hoeft, I., Keuter, A., Quinones, C., Schmidt-Walter, P., Veldkamp, E., and Corre, M. (2014). Nitrogen retention efficiency and nitrogen losses of a managed and phytodiverse temperate grassland. *Basic Appl. Ecol.* 15**,** 207-218.

Janssen, P. (2006). Identifying the dominant soil bacterial taxa in libraries of 16S rRNA and 16S rRNA genes. *Appl. Environ. Microbiol.* 72**,** 1719-1728.

Jorquera, M.A., Martínez, O.A., Marileo, L.G., Acuña, J.J., Saggar, S., and Mora, M.L. (2013). Effect of nitrogen and phosphorus fertilization on the composition of rhizobacterial communities of two Chilean Andisol pastures. *World J. Microbiol. Biotechnol*. 30, 99-107

Kennedy, N., Gleeson, D., Connolly, J., and Clipson, N. (2005). Seasonal and management influences on bacterial community structure in an upland grassland soil. *Fems Microbiol. Ecol.* 53**,** 329-337.

Keuter, A., Hoeft, I., Veldkamp, E., and Corre, M. (2013). Nitrogen response efficiency of a managed and phytodiverse temperate grassland. *Plant Soil.* 364**,** 193-206.

Kirchman, D., Cottrell, M., and Lovejoy, C. (2010). The structure of bacterial communities in the western Arctic Ocean as revealed by pyrosequencing of 16S rRNA genes. *Environ. Microbiol.* 12**,** 1132-1143.

Kleemann, F. (2010). *Bedeutung von intraspezifischer Diversität für Ökophysiologie und organismische Interaktionen bei der Pappel.*Göttingen: Cuvillier, E.

Koeberl, M., Schmidt, R., Ramadan, E., Bauer, R., and Berg, G. (2013). The microbiome of medicinal plants: diversity and importance for plant growth, quality, and health. *Front. Microbiol.* 4, doi: 10.3389/fmicb.2013.00400

Kuffner, M., Hai, B., Rattei, T., Melodelima, C., Schloter, M., Zechmeister-Boltenstern, S., Jandl, R., Schindlbacher, A., and Sessitsch, A. (2012). Effects of season and experimental warming on the bacterial community in a temperate mountain forest soil assessed by 16S rRNA gene pyrosequencing. *Fems Microbiol. Ecol.* 82**,** 551-562.

Landesman, W.J., Nelson, D.M., and Fitzpatrick, M.C. (2014). Soil properties and tree species drive ß-diversity of soil bacterial communities. *Soil Biol. Biochem.* 76**,** 201-209.

Lauber, C.L., Hamady, M., Knight, R., and Fierer, N. (2009). Pyrosequencing-based assessment of soil pH as a predictor of soil bacterial community structure at the continental scale. *Appl. Environ. Microbiol.* 75**,** 5111-5120.

Lauber, C.L., Ramirez, K.S., Aanderud, Z., Lennon, J., and Fierer, N. (2013). Temporal variability in soil microbial communities across land-use types. *ISME J.*

Lennon, J., and Jones, S. (2011). Microbial seed banks: the ecological and evolutionary implications of dormancy. *Nature Rev. Microbiol.* 9**,** 119-130.

Lundberg, D., Lebeis, S., Paredes, S., Yourstone, S., Gehring, J., Malfatti, S., Tremblay, J., Engelbrektson, A., Kunin, V., Del Rio, T., Edgar, R., Eickhorst, T., Ley, R., Hugenholtz, P., Tringe, S., and Dangl, J. (2012). Defining the core *Arabidopsis thaliana* root microbiome. *Nature* 488**,** 86-90.

Lynch, J.M., and Whipps, J.M. (1991). "Substrate flow in the rhizosphere," in *The rhizosphere and plant growth*. West Sussex: Springer, 15-24.

Mardis, E. (2008). Next-generation DNA sequencing methods. *Annu. Rev. Genomics. Hum. Genet.* 9**,** 387-402.

McHugh, T., and Schwartz, E. (2015). Changes in plant community composition and reduced precipitation have limited effects on the structure of soil bacterial and fungal communities present in a semiarid grassland. *Plant Soil.* 388**,** 175-186.

Mendes, R., Kruijt, M., De Bruijn, I., Dekkers, E., Van Der Voort, M., Schneider, J., Piceno, Y., Desantis, T., Andersen, G., Bakker, P., and Raaijmakers, J. (2011). Deciphering the Rhizosphere Microbiome for Disease-Suppressive Bacteria. *Science* 332**,** 1097-1100.

Nacke, H., Thurmer, A., Wollherr, A., Will, C., Hodac, L., Herold, N., Schoning, I., Schrumpf, M., and Daniel, R. (2011). Pyrosequencing-Based Assessment of Bacterial Community Structure Along Different Management Types in German Forest and Grassland Soils. *PloSs One* 6. doi: 10.1371/journal.pone.0017000

Pakull, B., Groppe, K., Meyer, M., Markussen, T., and Fladung, M. (2009). Genetic linkage mapping in aspen (*Populus tremula* L. and *Populus tremuloides* Michx.). *Tree Genet. Genomes.* 5**,** 505-515.

Pfeiffer, B., Fender, A.-C., Lasota, S., Hertel, D., Jungkunst, H.F., and Daniel, R. (2013). Leaf litter is the main driver for changes in bacterial community structures in the rhizosphere of ash and beech. *Appl. Soil Ecol.* 72**,** 150-160.

Pinton, R., Varanini, Z., and Nannipieri, P. (2007). *The rhizosphere: biochemistry and organic substances at the soil-plant interface.* New York: CRC press.

Portillo, M., Leff, J., Lauber, C., and Fierer, N. (2013). Cell Size Distributions of Soil Bacterial and Archaeal Taxa. *Appl. Environ. Microbiol.* 79**,** 7610-7617.

Rampelotto, P.H., De Siqueira Ferreira, A., Barboza, A.D., and Roesch, L.F. (2013). Changes in Diversity, Abundance, and Structure of Soil Bacterial Communities in Brazilian Savanna Under Different Land Use Systems. *Microb Ecol*. 3, 593-607

Rasche, F., Knapp, D., Kaiser, C., Koranda, M., Kitzler, B., Zechmeister-Boltenstern, S., Richter, A., and Sessitsch, A. (2011). Seasonality and resource availability control bacterial and archaeal communities in soils of a temperate beech forest. *ISME J.* 5**,** 389-402.

Rovira, A.D. (1969). Plant root exudates. *Bot.l Rev.* 35**,** 35-57.

Röske, I., Sabra, W., Nacke, H., Daniel, R., Zeng, A.-P., Antranikian, G., and Sahm, K. (2014). Microbial community composition and dynamics in high-temperature biogas reactors using industrial bioethanol waste as substrate. *Appl. Microbiol. Biotechnol.* 98**,** 9095-9106.

Schneider, D., Arp, G., Reimer, A., Reitner, J., and Daniel, R. (2013). Phylogenetic analysis of a microbialite-forming microbial mat from a hypersaline lake of the kiritimati atoll, central pacific. *PloS one* 8**,** e66662. doi: 10.1371/journal.pone.0066662

Schostag, M., Stibal, M., Jacobsen, C., Baelum, J., Tas, N., Elberling, B., Jansson, J., Semenchuk, P., and Prieme, A. (2015). Distinct summer and winter bacterial communities in the active layer of Svalbard permafrost revealed by DNA- and RNA-based analyses. *Front. Microbiol.* 6. doi: 10.3389/fmicb.2015.00399

Shade, A., Caporaso, J., Handelsman, J., Knight, R., and Fierer, N. (2013). A meta-analysis of changes in bacterial and archaeal communities with time. *ISME J.* 7**,** 1493-1506.

Sikorski, J. (2015). The Prokaryotic Biology of Soil. *Soil Org*. 87, 1-28

Silva, M., Dias, A., Van Elsas, J., and Salles, J. (2012). Spatial and Temporal Variation of Archaeal, Bacterial and Fungal Communities in Agricultural Soils. *PloS One* 7. doi: 10.1371/journal.pone.0051554

Simon, C., Wiezer, A., Strittmatter, A., and Daniel, R. (2009). Phylogenetic Diversity and Metabolic Potential Revealed in a Glacier Ice Metagenome. *Appl. Environ. Microbiol.* 75**,** 7519-7526.

Singh, G., and Mukerji, K. (2006). "Root Exudates as Determinant of Rhizospheric Microbial Biodiversity," in *Microbial Activity in the Rhizoshere,* eds. K.G. Mukerji, C. Manoharachary & J. Singh. Berlin: Springer, 39-53.

Smalla, K., Wieland, G., Buchner, A., Zock, A., Parzy, J., Kaiser, S., Roskot, N., Heuer, H., and Berg, G. (2001). Bulk and rhizosphere soil bacterial communities studied by denaturing gradient gel electrophoresis: plant-dependent enrichment and seasonal shifts revealed. *Appl. Environ. Microbiol.*67**,** 4742-4751.

Stettler, R.F., Bradshaw, H.D.J., Heilman, P.E., and Hinckley, T.M. (1996). "Biology of *Populus* and its implications for management and conservation," in *Biology of Populus*, Ottawa: NRC Research Press,

Stotzky, G. (1997). Soil as an environment for microbial life. *Modern soil microbiology***,** New York: CRC Press,

Sturz, A.V., and Nowak, J. (2000). Endophytic communities of rhizobacteria and the strategies required to create yield enhancing associations with crops. *Appl. Soil. Ecol.* 15**,** 183-190.

Thoms, C., Gattinger, A., Jacob, M., Thomas, F., and Gleixner, G. (2010). Direct and indirect effects of tree diversity drive soil microbial diversity in temperate deciduous forest. *Soil Biol. Biochem.* 42**,** 1558-1565.

Thomson, B., Tisserant, E., Plassart, P., Uroz, S., Griffiths, R., Hannula, S., Buee, M., Mougel, C., Ranjard, L., Van Veen, J., Martin, F., Bailey, M., and Lemanceau, P. (2015). Soil conditions and land use intensification effects on soil microbial communities across a range of European field sites. *Soil Biol. Biochem.* 88**,** 403-413.

Thurengia, G.M.O.P.A.T.S.O. (1910). *Geological map of Prussia and the states of Thurengia 1:25.000/Topographic inventory of the royal Prussian general staff; Sievershausen*. General Staff Prussia, Berlin.

Torsvik, V., Goksøyr, J., and Daae, F.L. (1990). High diversity in DNA of soil bacteria. *Appl. Environ. Microbiol.* 56**,** 782-787.

Torsvik, V., Øvreås, L., and Thingstad, T.F. (2002). Prokaryotic diversity--magnitude, dynamics, and controlling factors. *Science.* 296**,** 1064-1066.

Tripathi, B., Kim, M., Singh, D., Lee-Cruz, L., Lai-Hoe, A., Ainuddin, A., Go, R., Rahim, R., Husni, M., Chun, J., and Adams, J. (2012). Tropical Soil Bacterial Communities

in Malaysia: pH Dominates in the Equatorial Tropics Too. *Microbial Ecol.*64**,** 474-484.

Tuskan, G., Difazio, S., Jansson, S., Bohlmann, J., Grigoriev, I., Hellsten, et al. (2006). The genome of black cottonwood, Populus trichocarpa (Torr. & Gray). *Science* 313**,** 1596-1604.

Urich, T., Lanzen, A., Qi, J., Huson, D., Schleper, C., and Schuster, S. (2008). Simultaneous Assessment of Soil Microbial Community Structure and Function through Analysis of the Meta-Transcriptome. *PloS One* 3. doi: 10.1371/journal.pone.0002527

Uroz, S., Buee, M., Murat, C., Frey-Klett, P., and Martin, F. (2010). Pyrosequencing reveals a contrasted bacterial diversity between oak rhizosphere and surrounding soil. *Environ. Microbiol. Rep.* 2**,** 281-288.

Voroney, R., P. (2007). "The soil habitat," in *Soil Microbiology and Biochemistry,* ed. E. Paul, A. 3, UK: Elsevier,

Wemheuer, B., Gullert, S., Billerbeck, S., Giebel, H., Voget, S., Simon, M., and Daniel, R. (2014). Impact of a phytoplankton bloom on the diversity of the active bacterial community in the southern North Sea as revealed by metatranscriptomic approaches. *Fems Microbiol. Ecol.* 87**,** 378-389.

Wemheuer, F. (2015). Influence of Grassland Management and Herbivory on Diversity and Ecology of plant-associated Bacterial Communities. PhD-Thesis, Georg-August-University Göttingen. Availible: http://hdl.handle.net/11858/00-1735-0000-0022-5FFE-F

Will, C., Thurmer, A., Wollherr, A., Nacke, H., Herold, N., Schrumpf, M., Gutknecht, J., Wubet, T., Buscot, F., and Daniel, R. (2010). Horizon-Specific Bacterial Community Composition of German Grassland Soils, as Revealed by Pyrosequencing-Based Analysis of 16S rRNA Genes. *Appl. Environ. Microbiol.* 76**,** 6751-6759.

Chapter II

2.1. Impact of Fertilization and Sampling Time on Composition and Diversity of Total and Active Soil Bacterial Communities in German Grassland Soils

Impact of Fertilization and Sampling Time on Composition and Diversity of Total and Active Soil Bacterial Communities in German Grassland Soils

(In vice revision for PlosOne with the title: Effects of Fertilization and Sampling Time on Composition and Diversity of Entire and Active Bacterial Communities in German Grassland Soils)

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Abstract

Soil bacteria play a major role in driving and regulation of ecosystem processes. The identification of factors shaping the diversity and structure of soil bacterial communities is crucial for understanding bacterial-mediated processes such as nutrient transformation and cycling. As most studies targeted only the entire soil bacterial community, the response of active bacterial communities to environmental changes is still poorly understood. The objective of this study was to investigate the effect of fertilizer application and sampling time on structure and diversity of the active (RNA level) and the entire (DNA level) bacterial communities in a grassland soil. Analysis of more than 2.3 million 16S rRNA transcripts and gene sequences derived from amplicon-based sequencing of 16S rRNA genes revealed that fertilization and sampling time significantly altered the diversity and composition of total and active bacterial communities. Although the composition of both the entire and the active bacterial community was correlated with environmental factors such as pH or C/N ratio, the active community showed a higher sensitivity to environmental changes than the entire community. Functional analyses were performed based on the prediction of functional content from 16S rRNA genes and gene transcripts. Genes encoding the uptake of nitrate/nitrite, nitrification, and denitrification were more abundant and significantly up-regulated in fertilized plots compared to non-fertilized plots. This study provided insights into changes in dynamics and functions of soil bacterial communities as response to season and fertilizer application.

Introduction

Soil bacteria play important roles in ecosystem functioning and processes such as biogeochemical cycles and nutrient transformation [1-3]. Moreover, they have a severe impact on plant productivity (reviewed in [1, 4]). Thus, the identification of key factors shaping the diversity and structure of soil bacterial communities is crucial for understanding how these communities support the stability of ecosystem processes [5-7]. It is well-known that different soil properties influence bacterial communities in grassland soils [8-10]. It has been shown that bacterial community structure in German grassland soils was largely driven by soil pH [11]. This is consistent with the results of Lauber et al. [10], who showed that the overall bacterial community composition in 88 soils from across South and North America correlated with differences in soil pH.

Previous studies showed that the structure of bacterial communities in grassland soils is altered by sampling time and season [12-15]. The bacterial community structure in an upland grassland soil analyzed by automated ribosomal intergenic spacer analysis (ARISA) was influenced by season [15]. This result was supported by a study of Habekost et al. [12], who observed distinct seasonal variations in microbial community structure of a temperate grassland soil. The authors suggest that these changes are driven by the availability and quality of organic resources. The analysis of soil microbial communities across different land-use types revealed that temporal shifts in community composition were often correlated with temperature conditions and soil moisture, which directly or indirectly regulate the structure of soil bacterial communities [16].

Recently, the influence of different management regimes on bacterial community composition and diversity in grassland soils has been frequently addressed [11, 16-18]. Fierer et al. (2012) investigated soil microbial communities across nitrogen gradients by amplicon-based analysis. N amendment did not affect the soil bacterial diversity but significantly altered the community composition. Nacke et al. [11] investigated German grassland soils under different management regimes. They observed the highest diversity of soil bacteria in fertilized intensely managed grasslands. However, the majority of these studies used DNA-based approaches. Thus, they focused on the total bacterial community, which also contains dead cells, extracellular DNA, and dormant microorganisms [19]. Correspondingly, still little is known about the active (rRNA-based) bacterial communities in grassland soils and their responses to changing environmental conditions.

The aim of this study was to investigate the influence of fertilizer application and sampling time on the bacterial community in a grassland soil. Therefore, soil samples were taken in April, July, and September over two consecutive years (2010 and 2011). We applied large-scale amplicon-based analysis of the V2-V3 region of the 16S rRNA genes and gene transcripts to assess the diversity and structure of entire (DNA) and active (RNA) bacterial communities. We hypothesized that the entire and active community are differently influenced by fertilizer application (hypothesis I). We further hypothesized that the community diversity remained consistent throughout the year, whereas the structure is shaped by season (hypothesis II). Moreover, we used this unique dataset to perform functional predictions with Tax4Fun and examined soil microbial functions and metabolic capabilities of the entire and the active bacterial communities. We hypothesized that fertilization changes the community structure and this is accompanied by changes in bacterial functions (hypothesis III).

Material and Methods

Study site

This study was carried out within the GrassMan experiment, an interdisciplinary project investigating the relationships between land-use intensity, biodiversity and ecosystems functions. This experiment was established on former moderately species-rich, semi-natural grassland in June 2008 [20]. The experimental area was located in the Solling Uplands, Lower Saxony, Germany (51°44′ N, 9°32′′E, 490 m a.s.l.). The sampling area is a field site belonging to the institution (Georg-August-University) of the researchers conducting this study. Therefore, no special permit was required for soil sampling. As soil bacterial communities were sampled, endangered species were not affected by the study. The three-factorial design of GrassMan experiment included three levels of sward compositions (species-rich, monocot-reduced, and dicot-reduced), two mowing frequencies (once or three times per year), and two fertilizer treatments. Fertilizer treatments included NPK fertilizer application (nitrogen 180 kg ha^{-1} yr^{1}, phosphorus 30 kg ha^{-1} yr^{-1}, and potassium 100 kg ha^{-1} yr^{-1}) and as reference without fertilizer application. Each treatment was set up with 6 replicates in a full factorial design (72 plots; 15 m × 15 m each) arranged in a Latin rectangle. The experimental setup is further described by Petersen et al. [20]. The soil of the experimental area is a stony Haplic Cambisol, developed on sediments of loess on the middle bunter formation [21]. During the study period, mean annual temperature and annual precipitation were 6.6°C and 732 mm in 2010 and 8.91°C and 724 mm in 2011, respectively (S1 Table).

Sampling and soil characterization

Soil samples were collected from three fertilized (fe) and three non-fertilized (nf), species-rich plots mown once a year. Three soil cores (8 cm in diameter, depth 20 cm) per plot were taken and then pooled. To analyze the effect of sampling time, samples were collected in spring (April; Apr), summer (July; Jul), and autumn (September; Sep) 2010 (10) and 2011 (11). Soil samples were shock- frozen in liquid nitrogen and stored at -80°C until analysis. For determination of soil properties, subsamples from the pooled soil samples were dried at 60°C for seven days and sieved to < 2mm. Soil organic carbon (C) and total nitrogen (N) concentrations were determined from dried soil with a CN elemental

analyzer (Elemental Analyzer EA 1108, Carlo Erba Instruments, Rodano, Italy). The gravimetric soil water content (%) was calculated from oven-dried subsamples. Soil pH values were measured from a soil water suspension ratio of 1:2 (water contains 0.1 M KCl).

Extraction of nucleic acids from soil and reverse transcription

Total environmental RNA and DNA were co-extracted from 0.5 g soil per sample employing the RNA PowerSoil total RNA isolation kit and the RNA PowerSoil DNA elution accessory kit, respectively, as recommended by the manufacturer (MoBio Laboratories, Carlsbad, CA, USA). For RNA purification, residual DNA was removed with the TURBO DNA-free™ kit (Ambion Applied Biosystems, Darmstadt, Germany) from the extracted RNA. The absence of DNA was confirmed by PCR as described by Wemheuer et al. [22]. The DNA-free RNA was purified and concentrated using the RNeasy MinElute cleanup kit (Qiagen GmbH, Hilden, Germany). Isolated DNA was purified with the PowerClean DNA cleanup kit (MoBio Laboratories). DNA and RNA concentrations were determined using a NanoDrop ND-1000 spectrophotometer (Peqlab Biotechnologie GmbH, Erlangen, Germany). Approximately 500 ng of purified RNA was converted to cDNA using the SuperScript™ III reverse transcriptase and the reverse primer V3rev [23] of the subsequent PCR reaction, as recommended by the supplier (Invitrogen, Karlsruhe, Germany).

Amplification of 16S rRNA gene regions and sequencing

The V2-V3 region of the 16S rRNA gene was amplified by PCR. The PCR reaction mixture (25 µl) contained 5-fold Phusion GC buffer, 200 µM of each of the four deoxynucleoside triphosphates, 5% DMSO, 0.4 µM of each primer, 0.5 U of Phusion Hot Start HF DNA polymerase (Fisher Scientific GmbH, Schwerte, Germany), and 25 ng of isolated DNA or cDNA as template. The V2-V3 region was amplified with the following set of primers modified by Schmalenberger [23] containing the Roche 454-pyrosequencing adaptors, key sequences and one unique MID (underlined) per sample: V2for 5’-CGTATCGCCTCCCTCGCGCCATCAG- $(dN)_{10}$- AGTGGCGGACGGGTGAGTAA- 3’ and V3rev 5’-CTATGCGCCTTGCCAGCCCGCTCAG-$(dN)_{10}$-CGTATTACCGCGGCT

GCTGG-3'. The following cycling conditions were used for the amplification of cDNA: initial denaturation at 98°C for 5 min and 25 cycles of denaturation at 98°C for 10 s, annealing at 72°C for 10 s and extension at 72°C for 10 s, followed by a final extension at 72°C for 5 min. For DNA amplification, the following cycling scheme was used: initial denaturation at 98°C for 5 min and 25 cycles of denaturation at 98°C for 45 s, annealing at 72°C for 30 s and extension at 72°C for 30 s, followed by a final extension at 72°C for 10 min. PCR reactions were performed in triplicate for each sample. The resulting PCR products were pooled in equal amounts and purified using the peqGold gel extraction kit (Peqlab Biotechnologie GmbH, Erlangen, Germany). Obtained PCR products were quantified using the Quant-iT dsDNA HS assay kit and a Qubit fluorometer (Invitrogen GmbH) as recommended by the manufacturer. The Göttingen Genomics Laboratory determined the sequences of the partial 16S rRNA genes employing the Roche GS-FLX 454 pyrosequencer with Titanium chemistry as recommended by the manufacturer (Roche, Mannheim, Germany).

Processing of 16S rRNA sequence data

Pyrosequencing-derived 16S rRNA gene (DNA) and transcript (RNA) datasets were processed and analyzed using the QIIME software package version 1.6 [24]. Sequences shorter than 200 bp, low quality sequences, and sequences with homopolymers (> 8 bp) were removed from the datasets. Pyrosequencing noise was removed using Acacia 1.52 [25]. Primer sequence residues were truncated using cutadapt version 1.0 [26]. Chimeric sequences were detected and eliminated using UCHIME 7.0.190 in *de novo* and in reference mode with the Silva SSURef 119 NR database as reference database [27, 28]. All remaining sequences were subsequently clustered in operational taxonomic units (OTUs) at 3 and 20% genetic distance using the QIIME pick_otus.py script and uclust [27]. OTUs represented by only a single sequence in the entire dataset (singletons) were removed (see [29]). Taxonomic assignment was performed via BLAST alignment against the most recent SILVA database (SSURef NR 119) [28]. Rarefaction curves, alpha diversity indices (Chao1, Shannon, Simpson, and Michaelis-Menten-Fit), and beta diversity (Principle Component analyses) were determined using QIIME according to Wemheuer et al. [30]. Functional predictions for each sample were performed in R (version 3.2.0; R Development Core Team 2015 [http://www.R-project.org/]) using Tax4Fun [31].

Statistical analysis

T-test for normal distributed data or the Mann-Whitney-test for not normal distributed data were performed using SigmaPlot version 11.0 (Systat Software GmbH, Erkrath, Germany). To compare taxonomic groups with soil properties, Spearman's rank correlation coefficient was determined in SigmaPlot version 11.0. All other statistical analyses were conducted employing R version 3.2 [32]. Effects of fertilizer application on environmental parameters and bacterial community were tested as described by Wemheuer et al [30]. Changes in community structure and significant differences between samples and treatments were examined employing the metaMDS and RDA as well as envfit functions within the vegan package [33] as described by Wietz et al. [34]. Total and active bacterial communities were analyzed separately as DNA and RNA were extracted from the same soil samples and thus represent spatial pseudo-replicates. The results of the statistical tests were regarded as significant at P values ≤ 0.05.

Sequence data deposition

Sequence data were deposited in the Sequence Read Archive (SRA) of the National Center for Biotechnology Information (NCBI) under the accession number SRP041803.

Results and Discussion

Soil properties

In this study, the influence of season and fertilizer application on bacterial communities was assessed. Therefore, soil samples from fertilizer and non-fertilizer treatments were collected in April, July, and September over two constitutive years (2010 and 2011). Several soil properties from the sampling area including total N or C content, C/N ratio, water content, and pH were determined (Table 1). Water content varied between 12.6 and 34.0% with the highest content in September 2011 and the lowest content in July 2010. In 2010, water content was twofold higher in April and September than in July due to higher temperatures and dryer conditions during summer time in this year (S1 Table). The soil pH values ranged from 4.6 to 4.9. Statistical analysis revealed no significant differences of pH values between fertilized and non-fertilized plots. The carbon/nitrogen (C/N) ratio explains

the ability to use carbon and nitrogen in soil for microbial processes including the decomposition of soil organic matter [35]. As consequence, it is an indicator of soil microbial activity [36]. The C/N ratios were relatively constant among the sampling plots during the sampling period (2010 and 2011). They varied between 11.1 and 15.2, which is typically for field conditions with a soil texture of loamy silt that was determined for the study site by Keuter et al. [21].

Table 1: Soil parameter determination in fertilized and non-fertilized samples.

Sample/ plot	Season	Plot Treatment	pH-value (+KCl) ± SD	Water content ± SD (%)	C/N
fe1.apr10	spring 2010	fertilized	4.9±0.2	27.8±2.3	14.2
fe2.apr10	spring 2010	fertilized	4.8±0.1	27.6±1.9	12.3
fe3.apr10	spring 2010	fertilized	4.2±0.4	28.9±1.4	13.3
fe1.jul10	summer2010	fertilized	5.4±0.6	16.3±4.7	13.5
fe2.jul10	summer 2010	fertilized	4.6±0.2	13.1±1.2	12.0
fe3.jul10	summer 2010	fertilized	4.3±0.2	13.4±1.0	12.3
fe1.sep10	autumn 2010	fertilized	4.6±0.9	24.9±0.7	14.5
fe2.sep10	autumn 2010	fertilized	4.5±0.0	23.9±1.9	13.6
fe3.sep10	autumn 2010	fertilized	4.6±0.2	24.9±0.7	13.0
nf1.apr10	spring 2010	non-fertilized	4.8±0.1	28.4±1.5	13.3
nf2.apr10	spring 2010	non-fertilized	4.8±0.2	28.2±0.6	15.2
nf3.apr10	spring 2010	non-fertilized	4.6±0.1	28.1±0.8	14.6
nf1.jul10	summer2010	non-fertilized	4.9±0.2	12.6±1.1	11.8
nf2.jul10	summer 2010	non-fertilized	4.5±0.1	13.6±0.5	11.1
nf3.jul10	summer 2010	non-fertilized	4.9±0.2	13.6±3.4	11.9
nf1.sep10	autumn 2010	non-fertilized	4.7±0.2	24.4±0.8	13.1
nf2.sep10	autumn 2010	non-fertilized	4.8±0.3	25.1±2.6	13.8
nf3.sep10	autumn 2010	non-fertilized	4.5±0.1	23.9±1.8	13.1
fe1.apr11	spring 2011	fertilized	6.2±1.2	25.2±1.1	13.3
fe2.apr11	spring 2011	fertilized	4.6±0.1	25.6±1.4	12.7
fe3.apr11	spring 2011	fertilized	4.7±0.1	24.6±1.1	13.7
fe1.jul11	summer2011	fertilized	4.8±0.0	26.5±0.1	13.8
fe2.jul11	summer 2011	fertilized	4.9±0.0	25.5±0.3	11.7
fe3.jul11	summer 2011	fertilized	4.5±0.0	24.9±0.4	13.4
fe1.sep11	autumn 2011	fertilized	6.1±1.2	33.0±1.1	14.5
fe2.sep11	autumn 2011	fertilized	4.4±0.0	33.8±1.6	12.5
fe3.sep11	autumn 2011	fertilized	4.5±0.1	33.2±0.7	12.9
nf1.apr11	spring 2011	non-fertilized	4.7±0.2	23.8±0.7	14.1
nf2.apr11	spring 2011	non-fertilized	4.5±0.2	24.7±0.4	13.3
nf3.apr11	spring 2011	non-fertilized	4.3±0.1	25.0±1.1	13.9
nf1.jul11	summer2011	non-fertilized	4.7±0.0	24.5±0.6	12.6
nf2.jul11	summer 2011	non-fertilized	4.7±0.0	23.8±0.8	13.4
nf3.jul11	summer 2011	non-fertilized	4.6±0.0	25.5±0.4	14.7
nf1.sep11	autumn 2011	non-fertilized	4.9±0.2	31.9±1.2	12.7
nf2.sep11	autumn 2011	non-fertilized	4.8±0.1	34.0±0.8	11.7
nf3.sep11	autumn 2011	non-fertilized	4.6±0.2	32.7±2.1	14.7

General characteristics of the 16S rRNA datasets

To analyze and compare active and total bacterial community structure and diversity DNA and RNA were isolated from a total of 72 soil samples. Subsequently, bacterial community composition and diversity were assessed by amplicon-based analyses of the V2-V3 region of the 16S rRNA gene and the corresponding transcript. After quality filtering, denoising, and removal of potential chimeras and non-bacterial sequences, 2,386,234 high-quality sequences with an average read length of 359 bp were used for analyses (S2 Table). All sequences could be classified below phylum level. The number of sequences per sample ranged from 11,804 to 72,754 (DNA level) and from 17,919 to 72,380 (RNA level). To perform analysis at equal surveying effort 11,800 sequences per sample were randomly selected and subsequently clustered into operational taxonomic units (OTUs) at 3 and 20% genetic distance (S1 and S2 Figs.).

Diversity of active and entire bacterial community

Diversity and richness indices were determined for the entire (DNA level) and the active (RNA level) bacterial community in fertilizer and non-fertilizer treatments. Calculated rarefaction curves reached saturation at 20% genetic distance (phylum level), indicating that the surveying effort covered almost the full taxonomic diversity at DNA and RNA level (S1 Fig.). Comparison of rarefaction analyses with the number of OTUs determined by Chao1 richness estimator at 20% genetic divergence indicated that 69 to 79% of the estimated taxonomic richness was covered by the surveying effort at DNA and RNA level (S3 and S4 Tables). At 3% genetic distance, the richness estimator indicated coverage of 34 to 44% (S5 and S6 Tables). Furthermore, the maximal number of OTUs was determined by using non-linear regression based on Michaelis-Menten-Fit metrics at 20 and 3% genetic distance at DNA and RNA level. Coverage of 80 to 87% and 40 to 54% were determined at 20 and 3% genetic distance, respectively (S3 to S6 Tables).

Additionally, we evaluated Shannon (H´) and Simpson indices at 20 and 3% genetic distance (S3 to S6 Tables), as these indices provide a higher accuracy and robustness than Chao1 values due to their insensitivity for presence of rare species and a stronger valuation of non-rare species [37]. The Shannon index varied from 2.03 to 4.2 and 4.69 to 7.19, while Simpson indices varied from 0.66 to 0.97 and 0.88 to 0.94 at 20 and 3% genetic distance, respectively. Similar Shannon indices for total bacterial communities in soils

were predicted by Nacke et al. (2011), who determined Shannon indices up to 5.92 in grassland soil samples at DNA level. According to Roesch et al. [38], nonparametric diversity estimators such as Chao1 overestimate the number of species below genus level (5% genetic distance), whereas rarefaction analyses underestimate the number of species. Taking this into account, a substantial part of the bacterial diversity within the individual soil samples was assessed by the surveying effort.

Composition of active and entire bacterial communities

Obtained sequences were assigned to 41 bacterial phyla, 150 classes, and 374 families (Fig. 1). Five dominant phyla (> 1% abundance) were present in each soil sample and accounted for more than 96% of all bacterial sequences analyzed in this study. Rare phyla are shown in S3 Fig. *Proteobacteria* were predominant across all samples (DNA 31.2%, RNA 45.3%). The active bacterial community was dominated by *Alphaproteobacteria* (37.2%) and *Firmicutes* (36.0%) whereas the total bacterial community was dominated by *Firmicutes* (27.4%), *Alphaproteobacteria* (15.9%), *Chloroflexi* (17%), *Acidobacteria* (13.3%), and *Gammaproteobacteria* (7.7%). These results were in agreement with previous studies on bacterial community composition in grassland soils [11, 16, 39]. As most previous researchers used only DNA as template, studies investigating the active bacterial community in addition to the total community in grassland soils are rare.

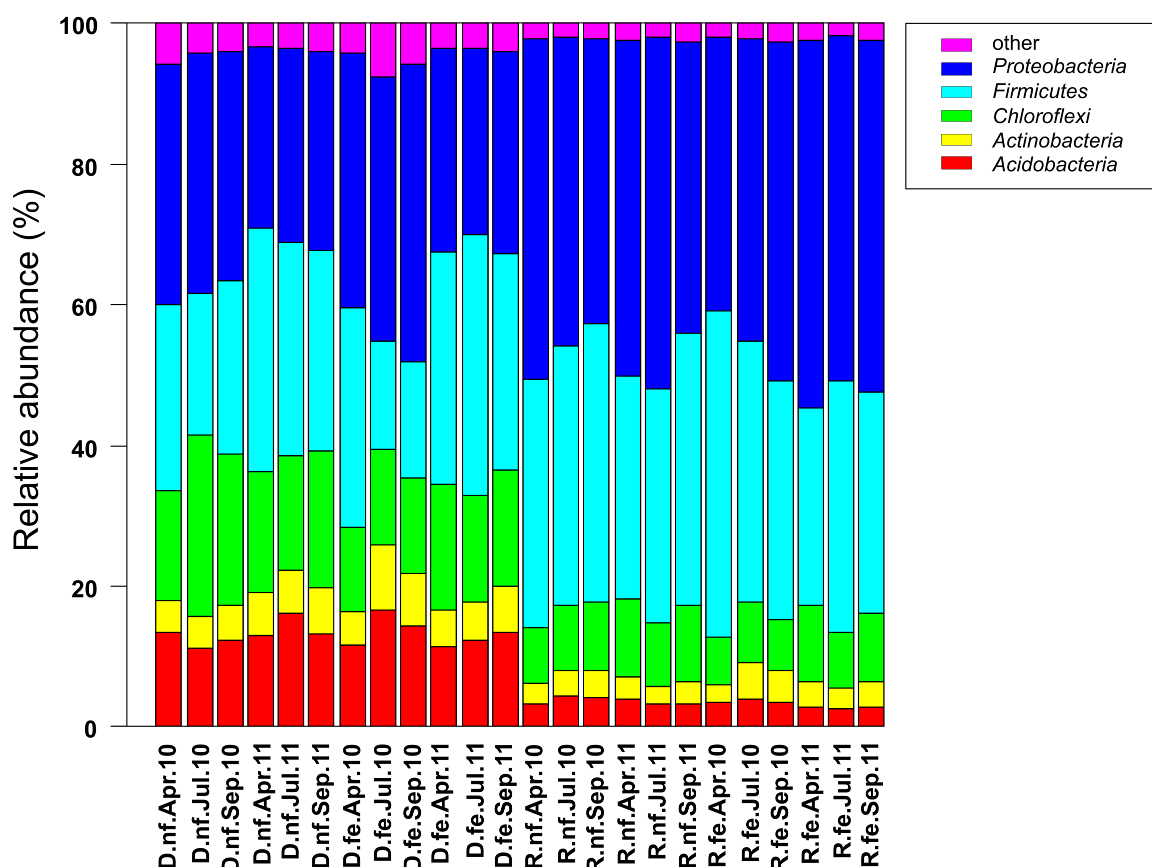

Figure 1: Relative abundances of bacterial phyla (> 1%) derived from the analyzed soil samples. Phyla accounting < 1% of all sequences are summarized in the group "other". Fertilized (fe) and non-fertilized (nf) samples are shown. Samples were taken in April (Apr), July (Jul), and September (Sep) in 2010 (10) and 2011 (11). The entire (D) and active (R) bacterial communities were analyzed.

We found significant differences between the number of OTUs derived from 16S rRNA genes and 16S rRNA transcripts (Fig. 2). At 20% and 3% genetic distance, the number of OTUs at DNA level (358 and 3,159 OTUs, respectively) was significantly higher (p <0.001) compared to RNA level (292 and 2,674 OTUs, respectively). In conclusion, the active community was less diverse than the entire community. This is consistent with the results of Baldrian et al. [40], who investigated the active and the total bacterial community in forest soils. They found a stronger dominance of fewer phyla in the RNA dataset compared to the DNA-derived dataset. Moreover, they encountered 1,500 (DNA level) and 1,200 OTUs (RNA level) at 3% genetic distance. This is in accordance with a study on prokaryotic communities in dryland soils [41]. In this study, the differences between soil samples were much higher in total rather than in active communities.

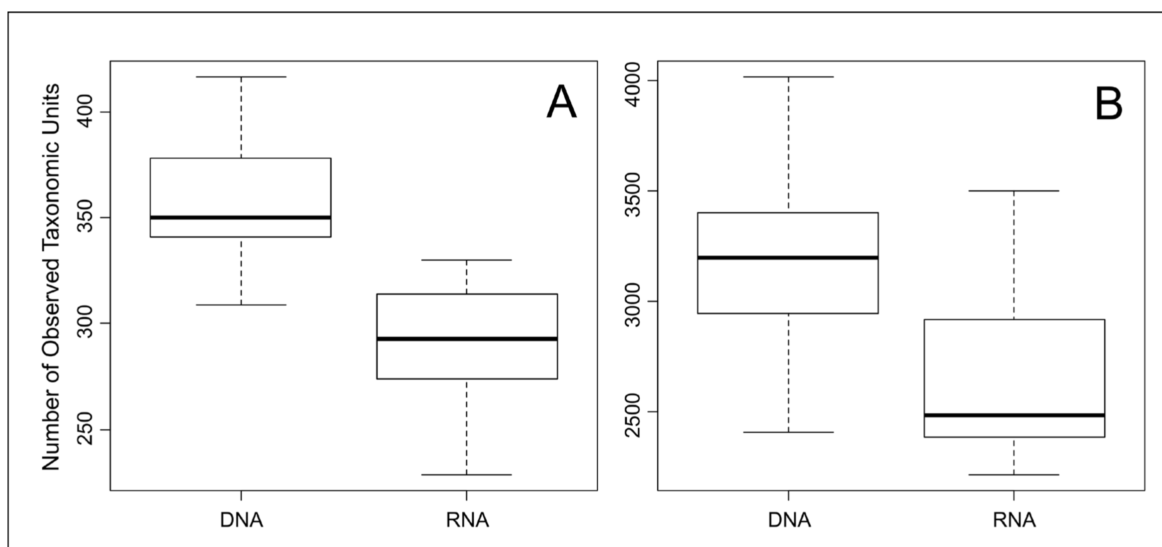

Figure 2: Boxplot diagram of the number of observed taxonomic units in the entire and active bacterial community. A. estimated OTUs at 20 % genetic distance and **B**. estimated OTUs at 3 % genetic distance. Depicted were estimated OUTs of the entire (D) and active (R) bacterial community.

Analysis of bacterial community composition revealed that 11,038 OTUs were shared between the entire and active bacterial community in fertilizer and non-fertilizer treatments. This core community comprised approximately 90% of all analyzed sequences (Fig. 3). More than 21,632 OTUs were unique (present at DNA or RNA level or in fertilized or non-fertilized plots). These OTUs represented only 1% of all analyzed sequences.

The most abundant OTU in the active and entire bacterial community belonged to the genus *Bacillus* (phylum *Firmicutes*), which comprised 15.3% (RNA level) and 12.5% (DNA level) of all analyzed sequences. Members of *Bacillus* are known as spore-forming bacteria, which are well adapted to heat, UV radiation, and oxidizing agents [42]. *Bacillus* strains are most common in grassland soils and well adapted to this environment [43]. Members of the *Bacillus* genus improve plant health due to their ability to produce substances that suppress pests and pathogens [44]. At RNA level, the second most abundant OTU (12.5%) was classified as member of the *Acetobacteraceae* (*Proteobacteria*). This family is recognized by their ability to oxidize ethanol to acidic acid in acidic and neutral media [45]. As members of this family can use a wide range of substrates such as glucose, ethanol, lactate or glycerol as energy source, they are important microorganisms in food industry such as the vinegar production [46]. Furthermore,

members of this family exhibit optimal growth conditions at low pH values [47] as observed in our study.

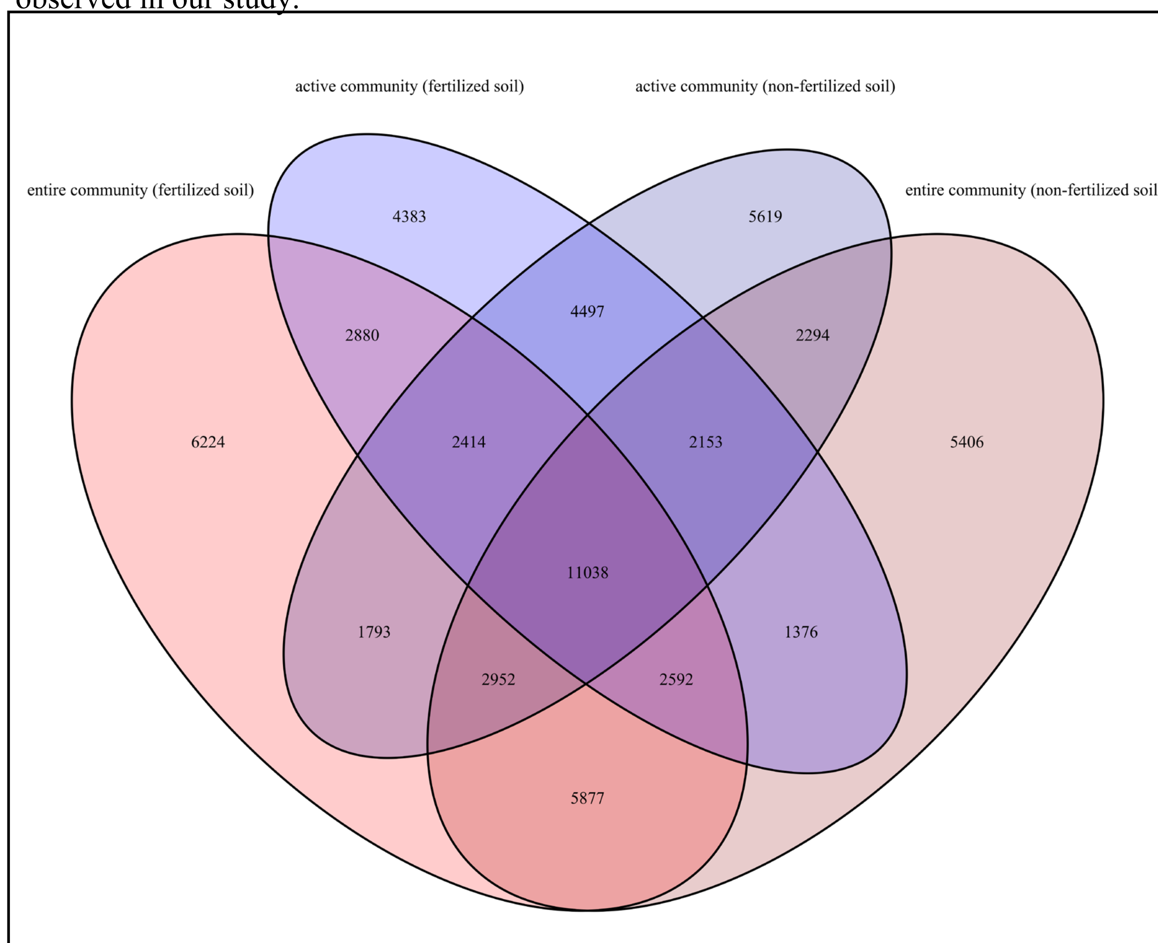

Figure 3: Venn diagram of all analyzed OTUs in fertilized and non-fertilized soils at entire and active bacterial community level. Depicted were OTUs estimated at entire community level (fertilized soil), active community level (fertilized soil), entire community level (non-fertilized soil), and active community level (non-fertilized soil) and all other possible interfaces.

Correlation between abundant bacterial groups and soil properties in fertilized and non-fertilized soils

We used Spearman's rank correlation coefficients to analyze the relationship between soil properties and relative abundances of the most abundant phyla, proteobacterial classes, and orders (Tables 2-5). We tested all phylogenetic groups with more than 1% abundance in the complete dataset. At phylum level, several phyla and proteobacterial classes correlated with environmental parameters (Tables 2 and 3). In the fertilized plots, the active part of the *Chloroflexi* correlated significant positively with pH and C/N. In addition, *Firmicutes* showed a significant negative correlation with C/N, whereas a significant positive

correlation existed between *Alphaproteobacteria* and the C/N ratio. The *Deltaproteobacteria* correlated significantly positively with pH and C/N in active and entire communities (Fig. 4).

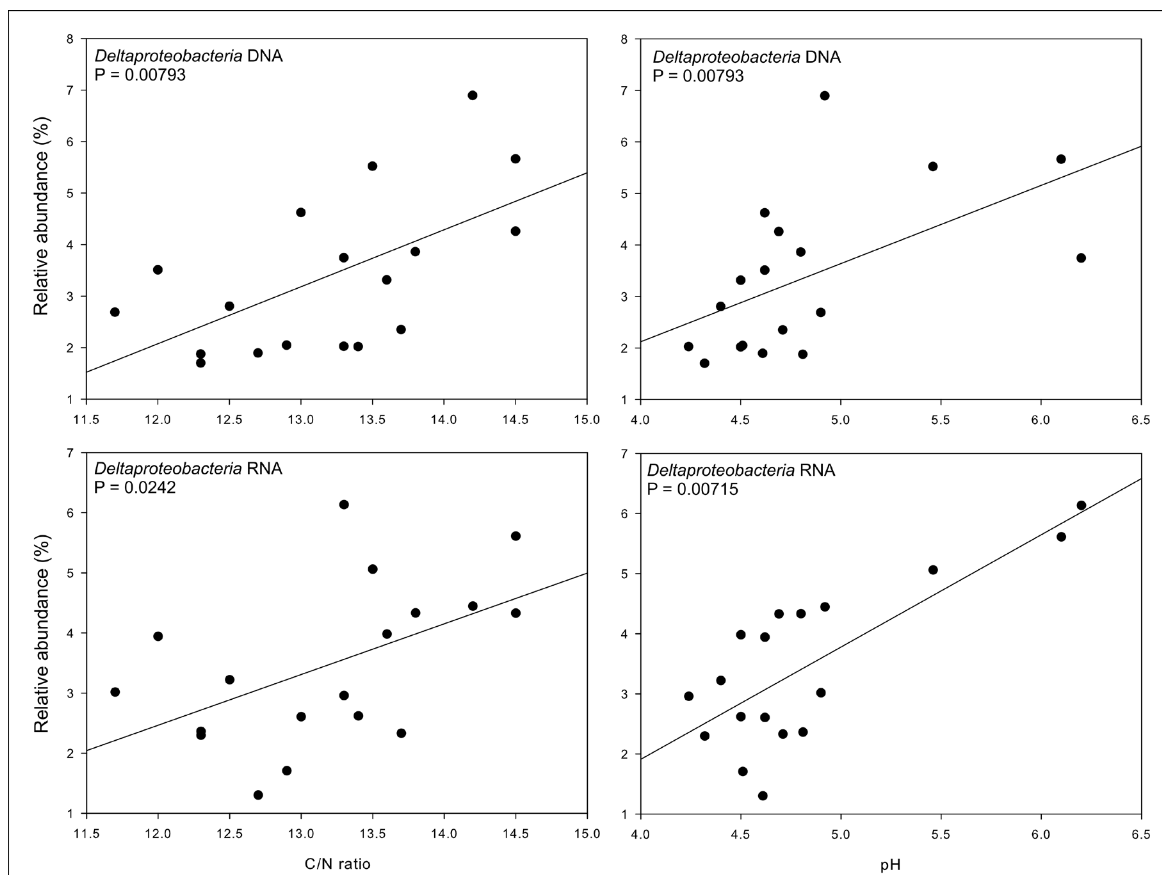

Figure 4: Spearman´s rank correlations between relative abundances of the class *Deltaproteobacteria* derived from DNA and RNA dataset with pH and C/N ratio in the fertilizer treatment**. A regression line was included and P values are shown for the active (RNA) and entire (DNA)** ***Deltaproteobacteria*****.**

In the non-fertilized plots, the Gammaproteobacteria correlated significant negatively with the water content at entire community level, while Deltaproteobacteria correlated significantly positively with pH at active community level. These results indicate that the active bacterial community is more sensitive to soil parameters than the entire community. Moreover, the bacterial community is stronger influenced by soil properties in fertilized compared to non-fertilized soils.

Table 2. Spearman´s Rank correlations of the abundance of the most abundant phyla, proteobacterial classes and soil properties in fertilized soils. Relative abundances derived from the active (RNA) and entire (DNA) bacterial community were separately analyzed. Bold numbers indicate P values < 0.05.

Group	Correlation					
	pH		Water content		C/N	
	DNA	RNA	DNA	RNA	DNA	RNA
Acidobacteria	0.302	0.347	-0.188	-0.155	0.170	0.375
Actinobacteria	0.139	0.394	-0.373	-0.413	0.258	0.418
Chloroflexi	0.085	**0.480**	0.123	-0.131	-0.148	**0.489**
Firmicutes	-0.299	-0.333	0.298	0.149	-0.260	**-0.621**
Alphaproteobacteria	-0.363	0.013	-0.226	0.023	-0.086	**0.481**
Betaproteobacteria	0.244	0.246	-0.319	-0.079	0.326	0.407
Gammaproteobacteria	-0.001	0.149	0.004	0.045	0.137	0.125
Deltaproteobacteria	**0.604**	**0.611**	0.039	0.010	**0.604**	**0.528**

Table 3. Spearman´s Rank correlations of the abundance of the most abundant phyla, proteobacterial classes and soil properties in non-fertilized soils. **Relative abundances derived from the active (RNA) and entire (DNA) bacterial community were separately analyzed. Bold numbers indicate P values < 0.05.**

Group	Correlation					
	pH		Water content		C/N	
	DNA	RNA	DNA	RNA	DNA	RNA
Acidobacteria	-0.323	-0.176	0.102	0.110	0.238	0.388
Actinobacteria	-0.096	-0.043	-0.158	-0.110	-0.033	0.121
Chloroflexi	-0.437	0.076	-0.309	-0.238	-0.309	0.377
Firmicutes	0.020	-0.298	0.156	0.323	0.003	-0.322
Alphaproteobacteria	-0.187	0.083	0.088	-0.282	0.166	0.304
Betaproteobacteria	0.347	0.390	-0.247	-0.117	-0.205	0.095
Gammaproteobacteria	0.344	0.373	**-0.515**	0.273	-0.047	-0.009
Deltaproteobacteria	0.279	**0.544**	0.102	0.158	-0.437	-0.002

At order level, the most abundant orders of the active bacterial community in the fertilizer-treated soils were strongly correlated with soil properties (Tables 4 and 5). Active community members of the order *Acidobacteriales* (subgroup 1) were significant negatively correlated with pH. This is consistent with the results of a DNA-based study [48]. *Myxococcales* (*Deltaproteobacteria*) were significant positively correlated with pH and C/N (Fig. 5). This is in line with a study of myxobacterial communities in different soils by Zhou et al. [49]. The authors observed a strong correlation between pH and the relative abundance of *Myxobacteria*. This group plays a key role in the carbon turnover in soils [50].

Table 4. Spearman´s Rank correlations of the abundance of the most abundant orders and soil properties in fertilized soils. **Relative abundances derived from the active (RNA) and entire (DNA) bacterial community were separately analyzed. Bold numbers indicate P values < 0.05.**

Group	Correlation					
	pH		Water content		C/N	
	DNA	RNA	DNA	RNA	DNA	RNA
Acidobacteriales	-0.342	**-0.568**	0.177	0.151	-0.09	-0.291
Subgroup 3	-0.004	0.206	0.034	-0.201	-0.141	0.374
Subgroup 7	0.278	**0.618**	-0.053	-0.163	0.013	0.410
Frankiales	0.039	0.290	-0.313	-0.418	0.051	**0.487**
S085_uncultured bacterium	0.361	0.286	-0.305	0.040	0.330	**0.644**
Ktedonobacterales	-0.316	0.321	0.219	-0.332	-0.391	**0.624**
AG30-KF-AS9	-0.358	-0.444	0.104	-0.136	-0.457	-0.424
JG37_AG-4_uncultered bacterium	0.222	0.087	0.125	0.236	-0.061	0.100
D4-96_unculutred bacterium	**0.523**	**0.539**	-0.258	-0.260	0.319	0.453
Bacillales	-0.285	-0.339	0.305	0.171	-0.332	**-0.645**
Clostridiales	-0.197	-0.227	-0.158	-0.255	0.302	-0.036
Myxococcales	**0.591**	**0.536**	0.052	0.012	**0.623**	**0.494**
Burkholderiales	0.129	0.173	-0.132	0.056	**0.479**	0.217
Caulobacterales	-0.221	-0.336	-0.201	-0.034	0.116	0.259
Rhizobiales	0.377	0.041	-0.180	-0.336	0.349	0.186
Rhodospirillales	-0.457	0.061	-0.146	0.035	-0.277	**0.500**
Xanthomonadales	0.120	0.147	-0.177	-0.033	-0.085	0.130

Table 5. Spearman´s Rank correlations of the abundance of the most abundant orders and soil properties in non-fertilized soils**. Relative abundances derived from the active (RNA) and entire (DNA) bacterial community were separately analyzed. Bold number: P < 0.05.**

Group	Correlation					
	pH		Water content		C/N	
	DNA	RNA	DNA	RNA	DNA	RNA
Acidobacteriales	**-0.469**	-0.347	0.075	0.0114	0.171	0.378
Subgroup 3	-0.380	-0.189	0.077	-0.075	0.159	0.308
Subgroup 7	0.131	0.067	-0.009	-0.209	0.236	0.084
Frankiales	-0.193	-0.244	0.146	-0.307	0.008	0.086
S085_uncultured bacterium	0.345	0.388	-0.410	0.0568	-0.503	**-0.137**
Ktedonobacterales	-0.231	-0.004	0.009	-0.366	-0.009	0.194
AG30-KF-AS9	-0.253	-0.193	-0.006	-0.197	-0.248	0.024
JG37_AG-4_uncultered bacterium	-0.002	-0.285	-0.383	-0.110	-0.180	**0.491**
D4-96_unculutred bacterium	0.023	**0.534**	-0.284	-0.309	-0.453	-0.110
Bacillales	-0.045	-0.399	0.214	0.187	0.023	-0.349
Clostridiales	**0.685**	**0.480**	0.012	0.391	-0.130	-0.01
Myxococcales	0.253	**0.558**	0.133	0.162	-0.443	-0.013
Burkholderiales	0.227	0.093	-0.172	0.100	-0.042	0.164
Caulobacterales	0.095	0.115	-0.226	0.216	-0.117	0.448
Rhizobiales	-0.182	-0.135	0.168	0.096	0.217	0.374
Rhodospirillales	-0.215	0.064	0.100	-1.430	0.093	0.170

Xanthomonadales	0.275	0.375	-0.104	0.057	-0.129	-0.144

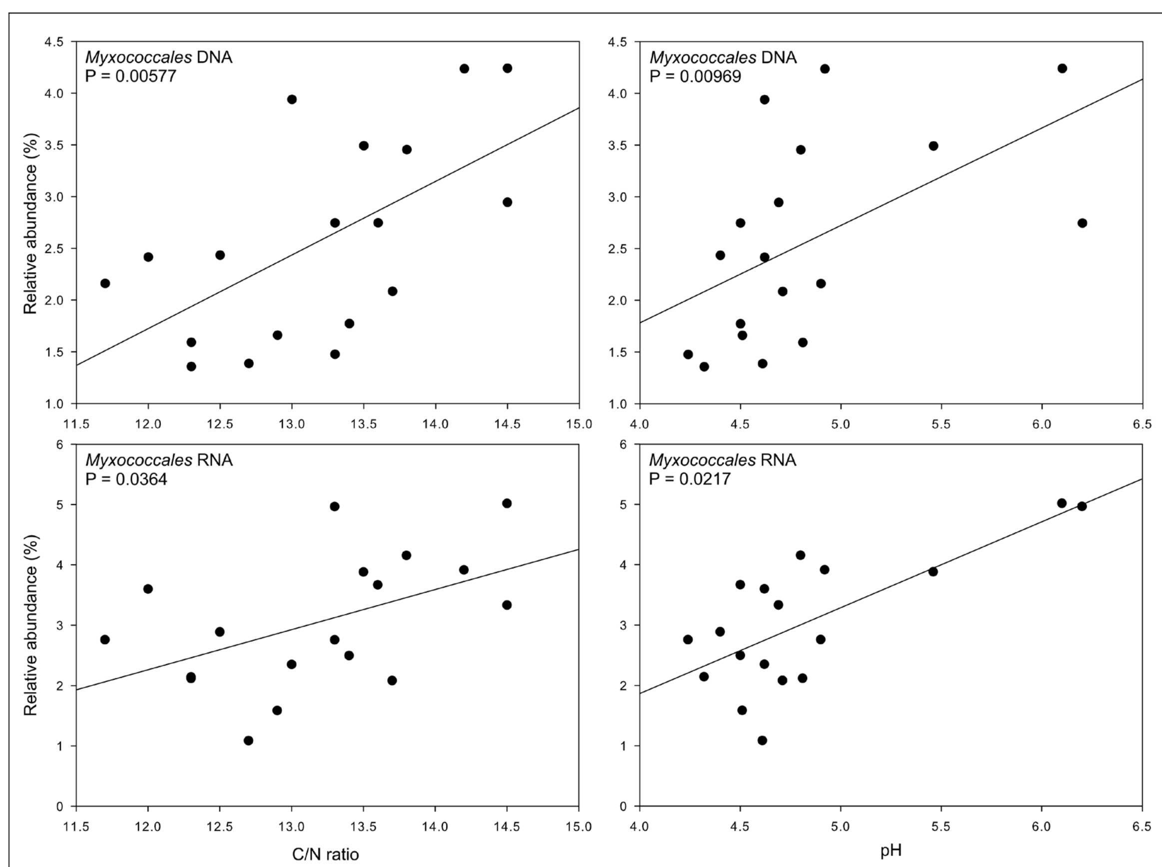

Figure 5: Spearman´s rank correlations between relative abundances of the order *Myxococcales* derived from DNA and RNA dataset with pH and C/N ratio in fertilizer soils. **A regression line was included and P values are shown for the active (RNA) and entire (DNA) *Myxococcales*.**

Fertilizer application changed the bacterial community composition

To analyze the influence of fertilizer amendment on the bacterial community structure, we collected and analyzed samples from non-fertilized and fertilized plots over two consecutive years. We observed a higher number of *Actinobacteria*, *Betaproteobacteria*, and *Gammaproteobacteria* in the fertilized soils whereas *Acidobacteria*, *Chloroflexi*, *Firmicutes*, *Alphaproteobacteria*, and *Deltaproteobacteria* were more abundant in the non-fertilized plots (Fig. 6).

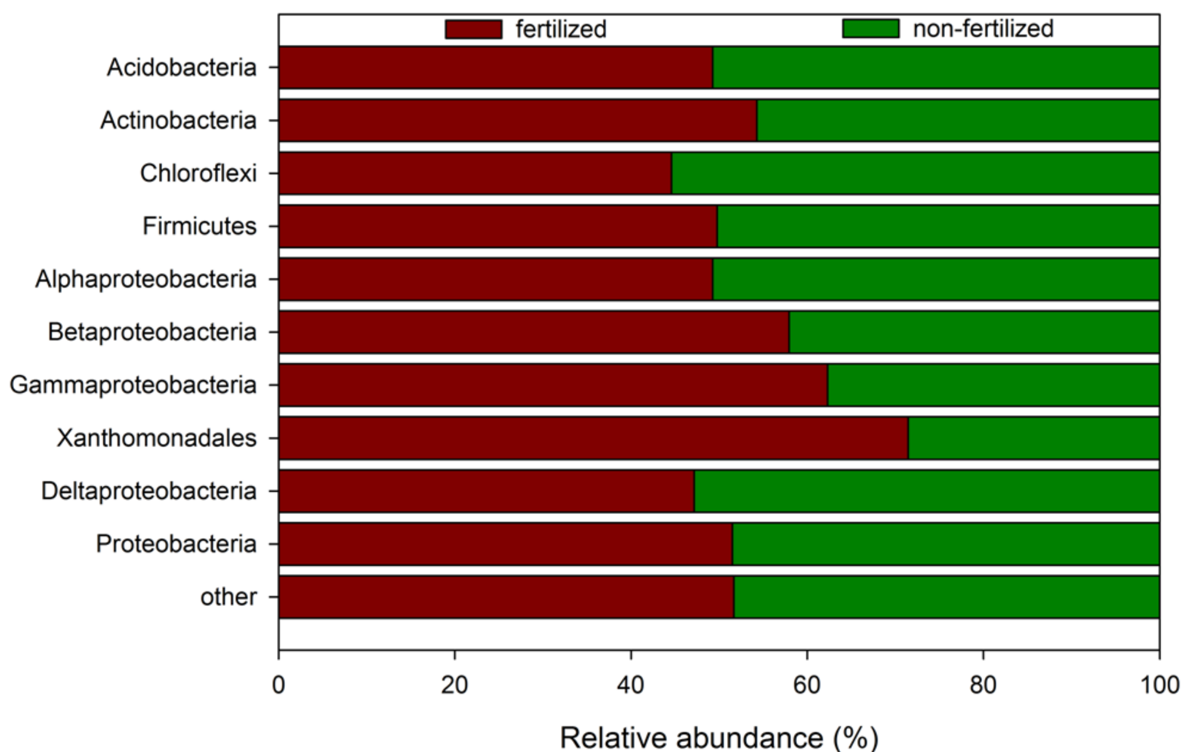

Figure 6: Relative abundances of bacterial phyla, proteobacterial classes, and *Xanthomonadales* derived from the fertilizer (red) and non-fertilizer (green) treatment. **Depicted were phyla with more than 1% abundance. All other rare phyla were summarized in the group "other".**

Especially, members of *Xanthomonadales* (*Gammaproteobacteria*) were significantly more dominant in the fertilized plots at entire and active community level. The order *Xanthomonadales* includes many members, which are able to use ammonium salts as nitrogen sources. These organisms could also be beneficial for plant growth by increasing sulfur availability via oxidation and phosphate via solubilization [51]. This is consistent with previous studies investigating the impact of nitrogen fertilization on soil bacterial communities. *Gammaproteobacteria* increased with rising N inputs [52-54] or with long-term fertilization [55].

At entire bacterial community level, we found higher numbers of OTUs at 3% and 20% genetic distance in fertilized soils (3,265 and 363 OTUs, respectively) than in non-fertilized plots (3,053 and 352 OTUs, respectively). These results were in accordance with previous studies [11, 56, 57]. Nacke et al. [11] found similar OTU values at 3% genetic distance in fertilized and non-fertilized grasslands. In contrast to this, we observed higher numbers of OTUs at the active bacterial community level in non-fertilized plots at 3 and 20% genetic distance. The active bacterial community showed an opposite behavior in

non-fertilized plots with 5 and 3% more OTUs at 3% and 20% genetic distance, respectiviely.

Until now, very little is known on RNA-based analysis of the active bacterial community composition in soils by using next-generation-sequencing-technologies [40, 58]. Baldrian et al. [40] described differences between the active and total bacterial community in forest soils and Pfeiffer et al. [58] investigated the active and total bacterial community in a soil mesocosm experiment using beech and ash with and without litter overlay. Our study showed that fertilizer amendment impacts the active bacterial community. This yielded a diversity loss and resulted in a higher activity of fewer groups, which can use N compounds in respiratory processes. On the other hand, it is of great importance for maintaining nutrient cycles to stabilze soil pH of fertilized soils [59] In our study, fertilizer application was combined with phosphorus, potassium oxide and calcium oxide (lime), which lead to stable soil pH values. In contrast, Kennedy et al. [60] investigated in a microcosm experiment the impact of lime and nitrogen amendment on bacterial community structure. They observed that a combined amendment of lime and nitrogen increased microbial activity whereas nitrogen amendment alone lead to a significant decrease of microbial activity compared to non-treated soils. However, it is difficult to compare our data with the results of recent studies due to the fact that the number of analyzed sequences impacts the estimated number of OTUs [61]. In most of these studies, fewer sequences and other regions of the 16S rRNA gene have been analyzed [17, 56, 62] and different methods were used [60].

Sampling time influence the soil bacterial communities in different ways

To analyze the effect of sampling time on soil bacterial community structure, soil samples were collected in spring (April), summer (July), and autumn (September) over two consecutive years (2010 and 2011). *Acidobacteria*, *Actinobacteria*, *Firmicutes*, and *Betaproteobacteria* showed significant different abundances with respect to sampling times (Fig. 7).

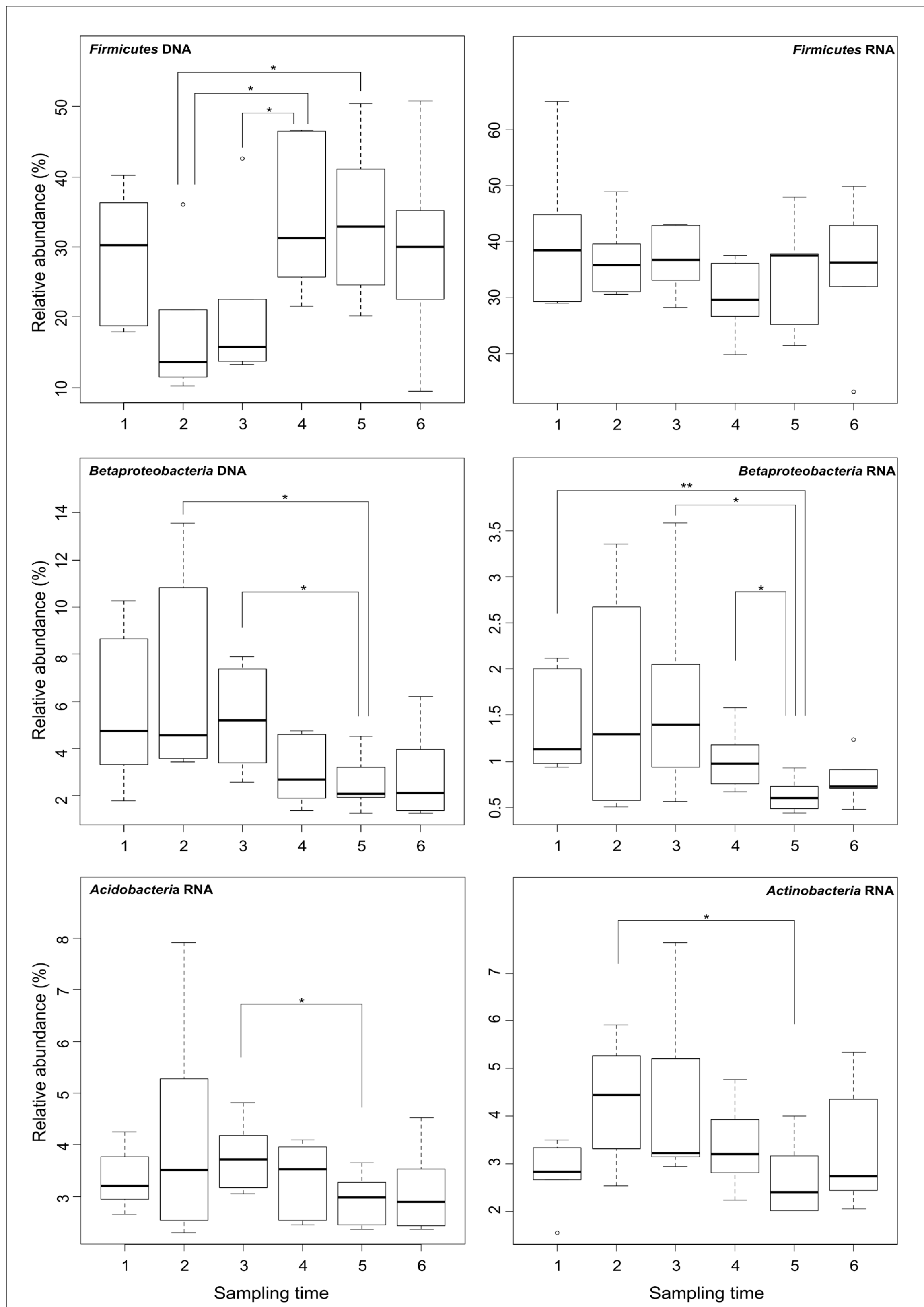

Figure 7: Boxplot diagram showing relative abundances of main phyla and proteobacterial classes over sampling time. **1**; April 2010, **2**; July 2010, **3**; September 2010, **4**; April 2011, **5**; July 2011, and **6**; September 2011. Asterisks indicate significant differences between sampling times in the active (RNA) and entire (DNA) bacterial phyla and proteobacterial classes.

Firmicutes showed only significant differences at entire bacterial community level, while *Betaproteobacteria* showed significant differences at active and entire community level. For *Actinobacteria* and *Acidobacteria,* a seasonal effect was determined at active community level but not at entire community level. *Chloroflexi*, *Alphaproteobacteria*, *Gammaproteobacteria* and *Deltaproteobacteria* were not affected by sampling time, which indicates that these groups are more recalcitrant to environmental changes.

Moreover, we observed significant differences of OTU numbers at 20% and 3% genetic distance within the different sampling times at active bacterial community level but not at entire bacterial community level (Fig. 8). Especially at active community level, the summer samples in 2010 were significant different from the summer samples in 2011 at 20% and 3% genetic distance. An explanation for is that the bacterial community is altered as response to seasonal changes of temperature, water availability, and plant growth activity. This is in line with the results of other studies. A rainfall manipulating experiment showed little differences in soil bacterial community composition in grasslands after 5 years of manipulation [63]. Nevertheless, changes in microbial abundance and composition in response to extreme weather conditions were recorded. Interestingly, repeated sampling across seasons and years showed that these changes were only short-lived. Smit et al. [64] analyzed samples of an agricultural soil taken in all seasons and determined the bacterial community composition by cultivation and denaturing gradient gel electrophoresis (DGGE). The authors showed that the bacterial community in summer (July) differs from that in other seasons. They concluded that a stable microbial community existed, although parameters such as humidity and nutrient supply shape the bacterial communities. Our study showed minor differences of the entire bacterial community diversity and structure between the sampling times, but significant differences of the active bacterial community. We concluded that the response of changing environmental conditions were more pronounced and earlier visible at active than at entire bacterial community level.

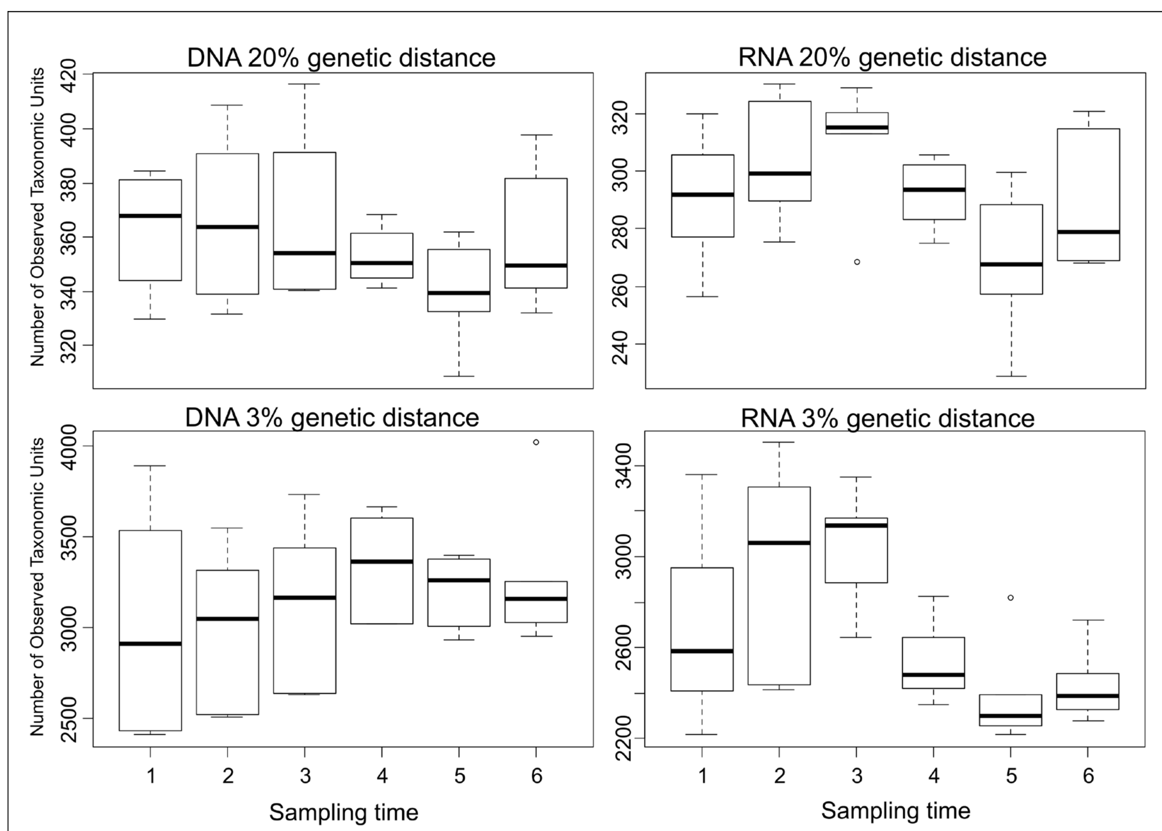

Figure 8: **Boxplot diagram of the number of taxonomic units (OTUs) at 20% and 3% genetic distance over sampling time at DNA and RNA level**. **1**; April 2010, **2**; July 2010, **3**; September 2010, **4**; April 2011, **5**; July 2011, and **6**; September 2011.

Functional Analysis

We used Tax4Fun analyses to predict metabolism pathways from 16S rRNA marker genes and gene transcripts [31]. Redundancy analysis (RDA) of the complete functional profile showed no significant difference between the soil communities in fertilized and non-fertilized plots but between entire and active bacterial communities (Fig. 9). Analysis revealed that 1,421 genes significantly increased at DNA level. Fifty-four % of these were significantly more abundant in the fertilized plots and 46% in non-fertilized plots. At RNA level, approximately 74% of the analyzed genes were significantly more abundant in the fertilized plots compared to non-fertilized plots (26%). Especially, higher abundances of genes encoding subunits for nitrate reductases (*nar*IJ) and nitrite reductase (*nir*B) were observed in fertilized plots at active bacterial community level. Furthermore, genes facilitating the first step of the nitrification reaction (*amo*ABC) were more abundant in fertilized soils. In summary, fertilizer application increased nitrate/nitrite uptake, denitrification, and nitrification steps in the bacterial community composition. Thus,

fertilizer application enhanced most of the nitrogen-related metabolism except nitrogen fixation pathways.

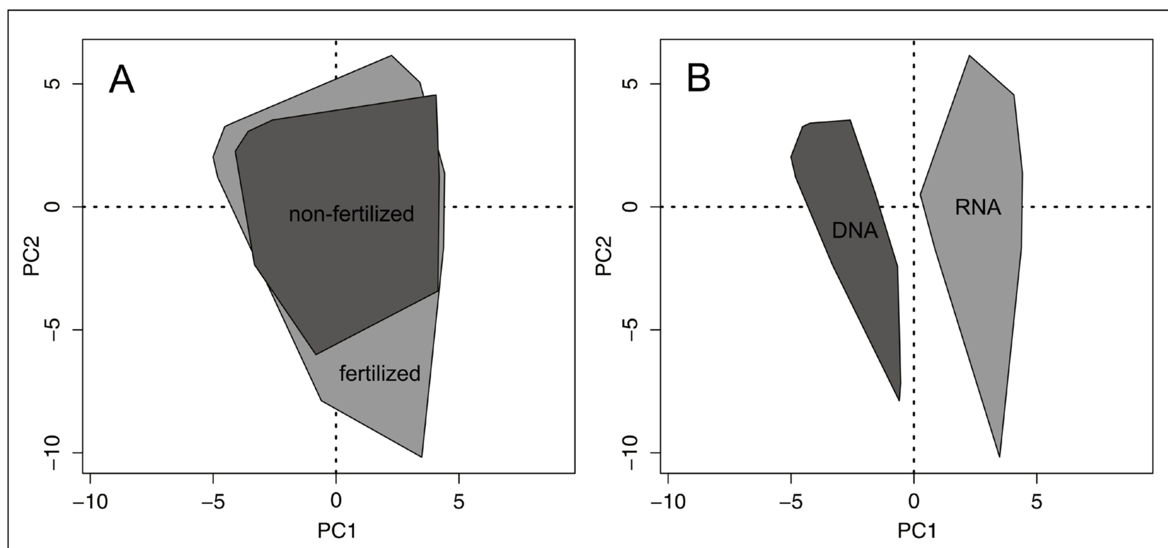

Figure 9: Redundancy analysis (RDA) of the functional bacterial community profiles derived from fertilizer and non-fertilizer treatments (A)**, and from the entire (DNA) and active (RNA) bacterial communities** (B)**.**

Conclusion

Due to the importance of soil bacterial communities for ecosystem functioning, it is of crucial importance to analyze the main drivers of these communities. In this study, the active and total bacterial communities in fertilized and non-fertilized grassland soils were investigated over two constitutive years. According to our hypothesis I, we showed that fertilizer application altered the structure and the diversity of the total and active bacterial community. This alteration was stronger at active bacterial community level, and leads to a diversity loss and a shift to taxonomic groups, which are able to use N compounds for respiratory processes. In accordance with our hypotheses I and III fertilizer amendment increased phylogenetic groups performing nitrate/nitrite uptake, denitrification and nitrification steps, with higher abundances of these genes in the active bacterial community. In contrast to hypothesis II, we could show that sampling year impacts bacterial diversity, but only at active bacterial community level. Sampling time affected only a few phyla and orders and changes of environmental conditions were earlier detectable in the active bacterial community. We suggest that there is a stable core community, which is able to adapt to environmental changes. Correlation analyses of soil properties and the relative abundances of bacterial phyla and orders suggest that soil pH

and C/N ratio were good predictors for bacterial community composition and diversity. The analysis showed stronger correlations of the active bacterial community in fertilized than in non-fertilized soils. The observed changes in dynamics and functions of bacterial soil communities as response to season and fertilizer application could contribute to a better understanding of ecosystem services provided by soil bacteria.

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References

1. van der Heijden MG, Bardgett RD, van Straalen NM. The unseen majority: soil microbes as drivers of plant diversity and productivity. Ecol Lett. 2008; 11:296-310.
2. Mooshammer M, Wanek W, Hämmerle I, Fuchslueger L, Hofhansl F, Knoltsch A, et al. Adjustment of microbial nitrogen use efficiency to carbon: nitrogen imbalances regulates soil nitrogen cycling. Nat Commun. 2014; 5:3694.
3. Alexander M. Introduction to Soil Microbiology. Soil Sci. 1978; 125:331.
4. Hayat R, Ali S, Amara U, Khalid R, Ahmed I. Soil beneficial bacteria and their role in plant growth promotion: a review. Ann Microbiol. 2010; 60:579-598.
5. Nannipieri P, Ascher J, Ceccherini M, Landi L, Pietramellara G, Renella G. Microbial diversity and soil functions. Eur J Soil Sci. 2003; 54:655-670.
6. Barberan A, Casamayor E, Fierer N. The microbial contribution to macroecology. Front Microbiol. 2014; 5:203. doi: 10.3389/fmicb.2014.00203
7. Prosser J. Ecosystem processes and interactions in a morass of diversity. FEMS Microbiol Ecol. 2012; 81:507-519.
8. de Vries F, Manning P, Tallowin J, Mortimer S, Pilgrim E, Harrison K, et al. Abiotic drivers and plant traits explain landscape-scale patterns in soil microbial communities. Ecol Lett. 2012; 15: 1230-1239.
9. Fierer N, Jackson RB. The diversity and biogeography of soil bacterial communities. Proc Natl Acad Sci USA. 2006; 103:626-631.
10. Lauber CL, Hamady M, Knight R, Fierer N. Pyrosequencing-based assessment of soil pH as a predictor of soil bacterial community structure at the continental scale. Appl Environ Microbiol. 2009; 75:5111-5120.
11. Nacke H, Thurmer A, Wollherr A, Will C, Hodac L, Herold N, et al. Pyrosequencing-based assessment of bacterial community structure along different management types in German forest and grassland soils. PloS ONE. 2011; 6:e17000. doi: 10.1371/journal.pone.0017000
12. Habekost M, Eisenhauer N, Scheu S, Steinbeiss S, Weigelt A, Gleixner G. Seasonal changes in the soil microbial community in a grassland plant diversity gradient four years after establishment. Soil Biol Biochem. 2008; 40:2588-2595.
13. Griffiths RI, Whiteley AS, O'Donnell AG, Bailey MJ. Influence of depth and sampling time on bacterial community structure in an upland grassland soil. FEMS Microbiol Ecol. 2003; 43:35-43.
14. Bardgett RD, Lovell RD, Hobbs PJ, Jarvis SC. Seasonal changes in soil microbial communities along a fertility gradient of temperate grasslands. Soil Biol Biochem. 1999; 31:1021-1030.
15. Kennedy NM, Gleeson DE, Connolly J, Clipson NJ. Seasonal and management influences on bacterial community structure in an upland grassland soil. FEMS Microbiol Ecol. 2005; 53:329-337.
16. Lauber CL, Ramirez KS, Aanderud Z, Lennon J, Fierer N. Temporal variability in soil microbial communities across land-use types. ISME J. 2013; 7:1641-1650.

17. Rampelotto PH, de Siqueira Ferreira A, Barboza AD, Roesch LF. Changes in diversity, abundance, and structure of soil bacterial communities in Brazilian Savanna under different land use systems. Microb Ecol. 2013; 66:593-607.
18. Fierer N, Lauber C, Ramirez K, Zaneveld J, Bradford M, Knight R. Comparative metagenomic, phylogenetic and physiological analyses of soil microbial communities across nitrogen gradients. ISME J. 2012; 6:1007-1017.
19. Lennon J, Jones S. Microbial seed banks: the ecological and evolutionary implications of dormancy. Nat Rev Microbiol. 2011; 9:119-130.
20. Petersen U, Wrage N, Kohler L, Leuschner C, Isselstein J. Manipulating the species composition of permanent grasslands- A new approach to biodiversity experiments. Basic Appl Ecol. 2012; 13:1-9.
21. Keuter A, Hoeft I, Veldkamp E, Corre M. Nitrogen response efficiency of a managed and phytodiverse temperate grassland. Plant Soil. 2013; 364:193-206.
22. Wemheuer B, Wemheuer F, Daniel R. RNA-based assessment of diversity and composition of active archaeal communities in the German Bight. Archaea. 2012; 2012:695826. doi: 10.1155/2012/695826
23. Schmalenberger A, Schwieger F, Tebbe C. Effect of primers hybridizing to different evolutionarily conserved regions of the small-subunit rRNA gene in PCR-based microbial community analyses and genetic profiling. Appl Environ Microb. 2001; 67:3557-3563.
24. Caporaso J, Kuczynski J, Stombaugh J, Bittinger K, Bushman F, Costello E, et al. QIIME allows analysis of high-throughput community sequencing data. Nat Methods. 2010; 7:335-336.
25. Bragg L, Stone G, Imelfort M, Hugenholtz P, Tyson G. Fast, accurate error-correction of amplicon pyrosequences using Acacia. Nat Methods. 2012; 9:425-426.
26. Martin M. Cutadapt removes adapter sequences from high-throughput sequencing reads. EMBnet.journal. 2011; 17:10-12
27. Edgar R. Search and clustering orders of magnitude faster than BLAST. Bioinformatics. 2010; 26: 2460-1.
28. Quast C, Pruesse E, Yilmaz P, Gerken J, Schweer T, Yarza P, et al. The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. Nucleic Acids Res. 2013; 41:D590-D596.
29. Schneider D, Arp G, Reimer A, Reitner J, Daniel R. Phylogenetic analysis of a microbialite-forming microbial mat from a hypersaline lake of the Kiritimati Atoll, Central Pacific. PLoS ONE. 2013; 8:e66662. doi: 10.1371/journal.pone.0066662
30. Wemheuer B, Gullert S, Billerbeck S, Giebel H, Voget S, Simon M, et al. Impact of a phytoplankton bloom on the diversity of the active bacterial community in the southern North Sea as revealed by metatranscriptomic approaches. FEMS Microbiol Ecol. 2014; 87:378-389.
31. Aßhauer KP, Wemheuer B, Daniel R, Meinicke P. Tax4Fun: predicting functional profiles from metagenomic 16S rRNA data. Bioinformatics. 2015; 31:2882-2884.

32. Team RDC. R: A Language and environment for statistical computing. version 3.1.2; R Development Core Team 2012; Availible: http://www.R-project.org/
33. Oksanen J, Blanchet FG, Kindt R, Legendre P, Minchin PR, O'Hara RB, et al. VEGAN: Community ecology package R package version 2.0-10: 2013; Available: http://cran.r-project.org, http://vegan.r-forge.r-project.org/
34. Wietz M, Wemheuer B, Simon H, Giebel H-A, Seibt MA, Daniel R, et al. Bacterial community dynamics during polysaccharide degradation at contrasting sites in the Southern and Atlantic Oceans. Environ Microbiol. 2015; doi: 10.1111/1462-2920.12842
35. Wardle DA. A comparative assessment of factors which influence microbial biomass carbon and nitrogen levels in soil. Biol Rev. 1992; 67:321-358.
36. He Z, Wu J, O´Donnell A, Syers J. Seasonal responses in microbial biomass carbon, phosphorus and sulphur in soils under pasture. Biol Ferti Soils. 1997; 24:421-428.
37. Haegeman B, Hamelin J, Moriarty J, Neal P, Dushoff J, Weitz J. Robust estimation of microbial diversity in theory and in practice. ISME J. 2013; 7:1092-1101.
38. Roesch LF, Fulthorpe RR, Riva A, Casella G, Hadwin AK, Kent AD, et al. Pyrosequencing enumerates and contrasts soil microbial diversity. ISME J. 2007; 1:283-290.
39. Will C, Thürmer A, Wollherr A, Nacke H, Herold N, Schrumpf M, et al. Horizon-specific bacterial community composition of German grassland soils, as revealed by pyrosequencing-based analysis of 16S rRNA genes. Appl Environ Microbiol. 2010; 76:6751-6759.
40. Baldrian P, Kolařík M, Stursová M, Kopecký J, Valášková V, Větrovský T, et al. Active and total microbial communities in forest soil are largely different and highly stratified during decomposition. ISME J. 2012; 6:248-258.
41. Angel R, Pasternak Z, Soares M, Conrad R, Gillor O. Active and total prokaryotic communities in dryland soils. FEMS Microbiol Ecol. 2013; 86:130-138.
42. Popham DL, Senguta S, Seltow P. Heat, hydrogen-peroxide, and UV resistance of *Bacillis subtilis s*pores with increased core water-content and with or without major DNA-binding proteins. Appl Environ Microbiol. 1995; 61:3633-3638.
43. Garbeva P, van Veen J, van Elsas J. Predominant *Bacillus spp*. in agricultural soil under different management regimes detected via PCR-DGGE. Microb Ecol. 2003; 45: 302-16.
44. Gardener B. Ecology of *Bacillus* and *Paenibacillus spp*. in agricultural systems. Phytopathology. 2004; 94:1252-1258.
45. Ruiz A, Poblet M, Mas A, Guillamon J. Identification of acetic acid bacteria by RFLP of PCR-amplified 16S rDNA and 16S-23S rDNA intergenic spacer. Int J Syst Evol Microbiol. 2000; 50:1981-1987.
46. Sengun IY, Karabiyikli S. Importance of acetic acid bacteria in food industry. Food Control. 2011; 22: 647-656.

47. Sievers M, Swings J. Family II *Acetobacteraceae*. In: Garrity, GM, editor. Bergey´s Manual of Systematic Bacteriology, second edition, volume 2. New York: Springer; 2005. pp. 41-50.
48. Jones R, Robeson M, Lauber C, Hamady M, Knight R, Fierer N. A comprehensive survey of soil acidobacterial diversity using pyrosequencing and clone library analyses. ISME J. 2009; 3:442-453.
49. Zhou X, Li S, Li W, Jiang D, Han K, Wu Z, et al. Myxobacterial community is a predominant and highly diverse bacterial group in soil niches. Environ Microbiol Rep. 2014; 6:45-56.
50. Lueders T, Kindler R, Miltner A, Friedrich M, Kaestner M. Identification of bacterial micropredators distinctively active in a soil microbial food web. Appl Environ Microbiol. 2006; 72:5342-5348.
51. Anandham R, Gandhi P, Madhaiyan M, Sa T. Potential plant growth promoting traits and bioacidulation of rock phosphate by thiosulfate oxidizing bacteria isolated from crop plants. J Basic Microbiol. 2008; 48 439-447.
52. Ramirez KS, Lauber CL, Knight R, Bradford MA, Fierer N. Consistent effects of nitrogen fertilization on soil bacterial communities in contrasting systems. Ecology. 2010; 91:3463-3470.
53. Broszat M, Nacke H, Blasi R, Siebe C, Huebner J, Daniel R, et al. Wastewater irrigation increases the abundance of potentially harmful *Gammaproteobacteria* in soils in Mezquital Valley, Mexico. Appl Environ Microbiol. 2014; 80:5282-5291.
54. Udikovic-Kolic N, Wichmann F, Broderick N, Handelsman J. Bloom of resident antibiotic-resistant bacteria in soil following manure fertilization. Proc Natl Acad Sci USA. 2014; 111:15202-15207.
55. Campbell B, Polson S, Hanson T, Mack M, Schuur E. The effect of nutrient deposition on bacterial communities in Arctic tundra soil. Environ Microbiol. 2010; 12:1842-1854.
56. Shange R, Ankumah R, Ibekwe A, Zabawa R, Dowd S. Distinct soil bacterial communities revealed under a diversely managed agroecosystem. PloS ONE. 2012; 7:e40338. doi: 10.1371/journal.pone.0040338
57. Poulsen PHB, Al-Soud WA, Bergmark L, Magid J, Hansen LH, Sørensen SJ. Effects of fertilization with urban and agricultural organic wastes in a field trial – Prokaryotic diversity investigated by pyrosequencing. Soil Biol Biochem. 2013; 57:784-793.
58. Pfeiffer B, Fender A-C, Lasota S, Hertel D, Jungkunst HF, Daniel R. Leaf litter is the main driver for changes in bacterial community structures in the rhizosphere of ash and beech. Appl Soil Ecol. 2013; 72:150-160.
59. Stevens R, Laughlin R, Malone J. Soil pH affects the processes reducing nitrate to nitrous oxide and di-nitrogen. Soil Biol Biochem. 1998; 30:1119-1126.
60. Kennedy N, Brodie E, Connolly J, Clipson N. Impact of lime, nitrogen and plant species on bacterial community structure in grassland microcosms. Environ Microbiol. 2004; 6:1070-1080.

61. Schloss P, Handelsman J. Status of the microbial census. Microbiol Mol Biol Rev. 2004; 68:686-691.
62. Tripathi B, Kim M, Singh D, Lee-Cruz L, Lai-Hoe A, Ainuddin A, et al. Tropical soil bacterial communities in Malaysia: pH dominates in the equatorial tropics too. Microbial Ecol. 2012; 64:474-484.
63. Cruz-Martinez K, Suttle K, Brodie E, Power M, Andersen G, Banfield J. Despite strong seasonal responses, soil microbial consortia are more resilient to long-term changes in rainfall than overlying grassland. ISME J. 2009; 3:738-744.
64. Smit E, Leeflang P, Gommans S, van den Broek J, van Mil S, Wernars K. Diversity and seasonal fluctuations of the dominant members of the bacterial soil community in a wheat field as determined by cultivation and molecular methods. Appl Environ Microbiol. 2001; 67:2284-2291.

2.2. Supplemental information

Table S1: climatic conditions during sample periods in 2010 and 2011

month	Mean temperature [°C]		mean precipitation [mm]	
	2010	2011	2010	2011
January	-4.72	0.15	n.d.	19.4
February	-2.62	0.05	n.d.	35.5
March	1.93	4.42	53.13	6
April	**7.78**	**11.26**	**14.84**	**41.75**
May	8.45	12.71	113.39	23.25
June	15.65	15.17	26.45	60.5
July	**19.87**	**14.48**	**47.27**	**110.85**
August	15.36	16.62	181.70	125.25
September	**11.42**	**14.75**	**102.12**	**54.75**
October	7.65	9.24	37.835	69.5
November	3.24	5.52	155.135	15.25
December	-4.81	2.51	n.d.	162

Bold: sample time in 2010 and 2011

Table S2: Number of 16S rRNA gene sequences derived from the analyzed GrassMan soil samples

Sample/plot	**Season**	**Year**	**Plot Treatment**	**Type**	**No. of sequences ≥ 200 bp**
fe.1.apr10.D	April	2010	fertilized	DNA	33037
fe.2.apr10.D	April	2010	fertilized	DNA	51918
fe.3.apr10.D	April	2010	fertilized	DNA	63036
fe.1.jul10.D	July	2010	fertilized	DNA	42250
fe.2.jul10.D	July	2010	fertilized	DNA	23727
fe.3.jul10.D	July	2010	fertilized	DNA	20785
fe.1.sep10.D	September	2010	fertilized	DNA	68334
fe.2.sep10.D	September	2010	fertilized	DNA	32079
fe.3.sep10.D	September	2010	fertilized	DNA	22040
fe.1.apr11.D	April	2011	fertilized	DNA	11804
fe.2.apr11.D	April	2011	fertilized	DNA	26113
fe.3.apr11.D	April	2011	fertilized	DNA	26294
fe.1.jul11.D	July	2011	fertilized	DNA	24935
fe.2.jul11.D	July	2011	fertilized	DNA	29163
fe.3.jul11.D	July	2011	fertilized	DNA	27321
fe.1.sep11.D	September	2011	fertilized	DNA	16582
fe.2.sep11.D	September	2011	fertilized	DNA	25557
fe.3.sep11.D	September	2011	fertilized	DNA	23785
fe.1.apr10.R	April	2010	fertilized	RNA	23464
fe.2.apr10.R	April	2010	fertilized	RNA	39332
fe.3.apr10.R	April	2010	fertilized	RNA	47063
fe.1.jul10.R	July	2010	fertilized	RNA	30060
fe.2.jul10.R	July	2010	fertilized	RNA	38149
fe.3.jul10.R	July	2010	fertilized	RNA	34804
fe.1.sep10.R	September	2010	fertilized	RNA	28644
fe.2.sep10.R	September	2010	fertilized	RNA	47588
fe.3.sep10.R	September	2010	fertilized	RNA	33842

Sample/plot	Season	Year	Plot Treatment	Type	No. of sequences ≥ 200 bp
fe.1.apr11.R	April	2011	fertilized	RNA	28592
fe.2.apr11.R	April	2011	fertilized	RNA	21720
fe.3.apr11.R	April	2011	fertilized	RNA	34974
fe.1.jul11.R	July	2011	fertilized	RNA	28413
fe.2.jul11.R	July	2011	fertilized	RNA	19965
fe.3.jul11.R	July	2011	fertilized	RNA	32437
fe.1.sep11.R	September	2011	fertilized	RNA	29549
fe.2.sep11.R	September	2011	fertilized	RNA	26764
fe.3.sep11.R	September	2011	fertilized	RNA	41785

Table S3: Chao1, michaelis-menten-fit (MMF), number of OTUs, Shannon-, Simpson-indices, and the coverage of chao1 and MMF in % at 3% genetic distance calculated for fertilized soil samples

Sample	Obs. OTUs	MMF	coverage MMF %	Chao1	coverage Chao1 %	Shannon corr.	Simpson
fe.1.apr10.D	3,533.50	7,443.72	47	8,352.01	42	6.83	0.99
fe.1.apr10.R	3,362.60	8,377.43	40	9,350.91	36	6.16	0.98
fe.1.apr11.D	3,661.00	8,273.90	44	9,543.51	38	6.75	0.99
fe.1.apr11.R	2,485.30	5,171.73	48	6,180.17	40	5.60	0.96
fe.1.jul10.D	3,549.90	7,135.18	50	8,192.97	43	7.01	0.99
fe.1.jul10.R	3,502.20	8,610.54	41	9,708.60	36	6.06	0.96
fe.1.jul11.D	3,397.00	7,386.63	46	8,243.70	41	6.60	0.99
fe.1.jul11.R	2,215.90	4,924.85	45	5,882.24	38	5.05	0.94
fe.1.sep10.D	3,730.40	8,137.87	46	9,269.85	40	6.93	0.99
fe.1.sep10.R	3,348.20	8,030.63	42	9,260.66	36	6.16	0.97
fe.1.sep11.D	4,017.70	8,818.65	46	9,885.08	41	7.19	1.00
fe.1.sep11.R	2,485.50	5,220.93	48	6,490.45	38	5.65	0.96
fe.2.apr10.D	2,433.40	5,075.13	48	6,357.31	38	5.67	0.97
fe.2.apr10.R	2,216.40	5,056.07	44	6,021.05	37	4.69	0.88
fe.2.apr11.D	3,024.10	7,005.35	43	8,325.98	36	5.97	0.97
fe.2.apr11.R	2,350.60	5,361.83	44	6,195.84	38	5.13	0.94
fe.2.jul10.D	3,314.70	6,785.17	49	8,416.30	39	6.87	1.00
fe.2.jul10.R	2,856.80	6,488.34	44	7,889.27	36	5.83	0.97
fe.2.jul11.D	2,932.20	6,764.50	43	7,989.55	37	5.78	0.95
fe.2.jul11.R	2,301.20	5,243.51	44	6,093.79	38	4.88	0.91
fe.2.sep10.D	3,300.90	7,005.74	47	8,543.74	39	6.71	0.99
fe.2.sep10.R	2,887.00	6,745.33	43	7,970.75	36	5.77	0.97
fe.2.sep11.D	3,025.10	6,804.79	44	7,999.27	38	6.06	0.97
fe.2.sep11.R	2,280.10	5,177.16	44	6,214.42	37	4.91	0.92
fe.3.apr10.D	2,410.80	4,875.85	49	6,174.85	39	5.73	0.97
fe.3.apr10.R	2,407.90	5,357.96	45	6,448.21	37	5.20	0.94
fe.3.apr11.D	3,327.00	8,053.37	41	9,271.08	36	5.93	0.94
fe.3.apr11.R	2,471.30	5,688.88	43	6,828.62	36	5.37	0.96
fe.3.jul10.D	3,161.90	6,534.74	48	8,005.74	39	6.57	0.99
fe.3.jul10.R	2,435.10	5,778.43	42	6,872.45	35	5.11	0.94
fe.3.jul11.D	3,249.70	7,683.24	42	8,773.27	37	5.96	0.95
fe.3.jul11.R	2,301.70	5,289.29	44	6,249.79	37	5.07	0.94
fe.3.sep10.D	3,439.70	7,595.30	45	8,608.86	40	6.71	0.99
fe.3.sep10.R	2,644.50	6,357.24	42	7,579.46	35	5.43	0.96
fe.3.sep11.D	3,254.90	7,617.83	43	8,901.00	37	6.01	0.95
fe.3.sep11.R	2,376.60	5,520.48	43	6,495.67	37	5.21	0.95

Table S3 continued: Chao1, michaelis-menten-fit (MMF), observed OTUs, Shannon-, Simpson-indices, and the coverage of chao1 and MMF in % at 3% genetic distance calculated for non-fertilized soil samples

Sample	Obs. OTUs	MMF	coverage MMF %	Chao1	coverage Chao1 %	Shannon corr.	Simpson
nf.1.apr10.D	2,880.50	6,374.64	45	7,631.01	38	5.98	0.97
nf.1.apr10.R	2,435.70	5,128.78	47	6,267.89	39	5.54	0.96
nf.1.apr11.D	3,600.00	8,344.67	43	9,629.26	37	6.59	0.98
nf.1.apr11.R	2,419.70	5,353.16	45	6,388.14	38	5.42	0.96
nf.1.jul10.D	2,938.80	6,409.38	46	7,744.69	38	6.08	0.98
nf.1.jul10.R	3,259.20	7,750.36	42	8,626.49	38	6.18	0.98
nf.1.jul11.D	3,376.30	7,529.48	45	8,930.88	38	6.51	0.98
nf.1.jul11.R	2,394.10	5,908.84	41	6,950.88	34	4.90	0.92
nf.1.sep10.D	3,032.90	6,858.83	44	8,108.52	37	5.93	0.96
nf.1.sep10.R	3,112.20	7,287.10	43	8,723.44	36	6.03	0.97
nf.1.sep11.D	3,239.90	7,151.90	45	8,733.32	37	6.39	0.98
nf.1.sep11.R	2,401.40	5,400.19	44	6,368.17	38	5.28	0.95
nf.2.apr10.D	2,950.20	6,044.58	49	7,178.80	41	6.43	0.99
nf.2.apr10.R	2,731.70	5,895.18	46	7,040.79	39	5.90	0.97
nf.2.apr11.D	3,402.70	8,023.85	42	9,263.07	37	6.33	0.97
nf.2.apr11.R	2,643.30	6,209.03	43	7,535.76	35	5.46	0.96
nf.2.jul10.D	2,521.40	4,686.95	54	5,794.76	44	6.33	0.99
nf.2.jul10.R	2,413.70	5,337.76	45	6,506.61	37	5.38	0.96
nf.2.jul11.D	3,006.40	6,854.37	44	8,309.71	36	6.10	0.97
nf.2.jul11.R	2,256.50	5,149.60	44	6,264.86	36	5.10	0.95
nf.2.sep10.D	2,636.20	4,989.75	53	6,535.58	40	6.24	0.98
nf.2.sep10.R	3,167.20	7,413.04	43	8,623.58	37	6.08	0.97
nf.2.sep11.D	3,078.60	6,902.05	45	8,050.91	38	6.11	0.97
nf.2.sep11.R	2,325.70	5,727.57	41	6,665.32	35	4.83	0.92
nf.3.apr10.D	3,891.40	8,703.98	45	0,062.35	39	7.01	0.99
nf.3.apr10.R	2,952.20	6,921.75	43	8,134.66	36	5.84	0.97
nf.3.apr11.D	3,023.90	7,490.27	40	8,839.03	34	5.69	0.95
nf.3.apr11.R	2,823.80	6,707.73	42	7,384.02	38	5.59	0.96
nf.3.jul10.D	2,506.20	4,947.26	51	6,186.17	41	5.92	0.98
nf.3.jul10.R	3,306.70	8,004.72	41	8,706.97	38	6.08	0.97
nf.3.jul11.D	3,272.80	7,811.78	42	8,807.20	37	6.22	0.98
nf.3.jul11.R	2,817.20	6,887.81	41	7,675.72	37	5.50	0.95
nf.3.sep10.D	2,630.50	5,442.65	48	6,742.54	39	6.07	0.99
nf.3.sep10.R	3,162.80	7,590.92	42	8,698.21	36	5.91	0.96
nf.3.sep11.D	2,956.30	6,610.75	45	7,512.26	39	6.03	0.98
nf.3.sep11.R	2,722.00	5,999.56	45	6,976.53	39	5.72	0.97

Table S4: Chao1, michaelis-menten-fit (MMF), number of OTUs, Shannon-, Simpson-indices, and the coverage of chao1 and MMF in % at 20% genetic distance calculated for fertilized soil samples

Sample	Obs. OTUs	MMF	coverage MMF %	Chao1	coverage Chao1 %	Shannon corr.	Simpson
fe.1.apr10.D	329.90	393.10	84	437.507	75	3.43	0.911
fe.1.apr10.R	319.80	382.31	84	430.709	74	2.94	0.84
fe.1.apr11.D	345.10	408.86	84	444.849	78	3.39	0.889
fe.1.apr11.R	283.30	341.05	83	386.429	73	2.76	0.825
fe.1.jul10.D	380.70	438.87	87	483.727	79	4.04	0.958
fe.1.jul10.R	305.90	361.79	85	413.385	74	2.85	0.827
fe.1.jul11.D	361.90	430.12	84	473.574	76	3.36	0.867
fe.1.jul11.R	288.20	350.55	82	381.538	76	2.65	0.804
fe.1.sep10.D	360.70	414.97	87	452.882	80	3.91	0.953
fe.1.sep10.R	268.40	324.02	83	358.117	75	2.72	0.821
fe.1.sep11.D	346.90	412.49	84	447.326	78	3.25	0.845
fe.1.sep11.R	273.50	330.65	83	371.841	74	2.56	0.789
fe.2.apr10.D	344.00	406.41	85	458.145	75	3.49	0.911
fe.2.apr10.R	256.60	329.38	78	360.876	71	2.03	0.659
fe.2.apr11.D	346.10	410.10	84	448.556	77	3.28	0.871
fe.2.apr11.R	274.90	334.73	82	366.959	75	2.48	0.762
fe.2.jul10.D	390.90	454.21	86	513.136	76	3.93	0.951
fe.2.jul10.R	292.50	349.67	84	384.654	76	2.68	0.794
fe.2.jul11.D	335.80	390.81	86	421.219	80	3.45	0.899
fe.2.jul11.R	257.50	309.40	83	360.998	71	2.51	0.779
fe.2.sep10.D	416.40	480.95	87	542.397	77	4.20	0.965
fe.2.sep10.R	314.40	370.67	85	403.574	78	2.97	0.84
fe.2.sep11.D	397.60	457.80	87	510.454	78	3.99	0.954
fe.2.sep11.R	314.60	379.36	83	414.781	76	2.82	0.808
fe.3.apr10.D	381.40	451.12	85	504.026	76	3.41	0.866
fe.3.apr10.R	287.80	353.93	81	390.033	74	2.48	0.761
fe.3.apr11.D	354.90	418.62	85	457.392	78	3.37	0.874
fe.3.apr11.R	294.60	342.84	86	382.745	77	2.93	0.837
fe.3.jul10.D	408.60	473.83	86	542.977	75	4.09	0.961
fe.3.jul10.R	330.10	394.74	84	438.216	75	2.99	0.841
fe.3.jul11.D	308.60	365.04	85	404.501	76	3.05	0.832
fe.3.jul11.R	228.70	283.85	81	321.617	71	2.16	0.732
fe.3.sep10.D	391.50	449.44	87	494.759	79	3.99	0.95
fe.3.sep10.R	313.10	376.32	83	410.953	76	2.94	0.846
fe.3.sep11.D	332.30	389.26	85	445.925	75	3.35	0.88
fe.3.sep11.R	268.00	323.90	83	359.501	75	2.53	0.79

Table S4 continued: Chao1, michaelis-menten-fit (MMF), number of OTUs, Shannon-, Simpson-indices, and the coverage of chao1 and MMF in % at 20% genetic distance calculated for non-fertilized soil samples

Sample	Obs. OTUs	MMF	coverage MMF %	Chao1	coverage Chao1 %	Shannon corr.	Simpson
nf.1.apr10.D	360.00	422.16	85	477.4	75	3.49	0.902
nf.1.apr10.R	305.70	368.37	83	415.49	74	2.83	0.83
nf.1.apr11.D	368.20	430.12	86	482.098	76	3.25	0.823
nf.1.apr11.R	305.50	364.59	84	406.166	75	3.01	0.855
nf.1.jul10.D	331.60	392.58	84	437.343	76	3.48	0.916
nf.1.jul10.R	289.50	346.89	83	395.266	73	2.70	0.81
nf.1.jul11.D	355.70	414.37	86	458.683	78	3.63	0.918
nf.1.jul11.R	263.50	316.36	83	363.799	72	2.49	0.758
nf.1.sep10.D	348.00	411.87	84	474.055	73	3.57	0.921
nf.1.sep10.R	315.60	379.17	83	414.391	76	2.93	0.842
nf.1.sep11.D	381.60	444.95	86	486.216	78	3.77	0.933
nf.1.sep11.R	320.60	373.80	86	438.322	73	3.29	0.889
nf.2.apr10.D	375.80	447.55	84	488.227	77	3.53	0.903
nf.2.apr10.R	295.90	355.86	83	389.304	76	2.79	0.829
nf.2.apr11.D	341.50	396.03	86	442.91	77	3.51	0.917
nf.2.apr11.R	292.70	351.37	83	421.47	69	2.67	0.806
nf.2.jul10.D	339.10	401.16	85	464.493	73	3.56	0.931
nf.2.jul10.R	275.50	337.29	82	367.932	75	2.50	0.765
nf.2.jul11.D	342.90	399.02	86	438.666	78	3.52	0.915
nf.2.jul11.R	299.50	362.24	83	405.644	74	2.54	0.777
nf.2.sep10.D	340.40	407.87	83	439.648	77	3.39	0.891
nf.2.sep10.R	320.20	386.88	83	429.841	74	2.87	0.827
nf.2.sep11.D	341.50	404.73	84	448.923	76	3.26	0.875
nf.2.sep11.R	268.90	334.93	80	368.097	73	2.40	0.765
nf.3.apr10.D	384.20	451.95	85	492.195	78	3.79	0.943
nf.3.apr10.R	277.10	335.78	83	365.574	76	2.62	0.802
nf.3.apr11.D	361.60	422.34	86	468.792	77	3.52	0.899
nf.3.apr11.R	302.10	366.67	82	408.509	74	2.58	0.782
nf.3.jul10.D	346.80	413.84	84	467.899	74	3.64	0.941
nf.3.jul10.R	324.10	389.60	83	419.702	77	2.99	0.85
nf.3.jul11.D	332.50	391.18	85	454.457	73	3.29	0.874
nf.3.jul11.R	271.80	335.95	81	379.09	72	2.41	0.756
nf.3.sep10.D	340.80	406.60	84	455.856	75	3.66	0.945
nf.3.sep10.R	329.10	395.05	83	430.621	76	2.88	0.804
nf.3.sep11.D	352.20	414.40	85	447.24	79	3.50	0.904
nf.3.sep11.R	283.90	339.19	84	383.635	74	2.67	0.809

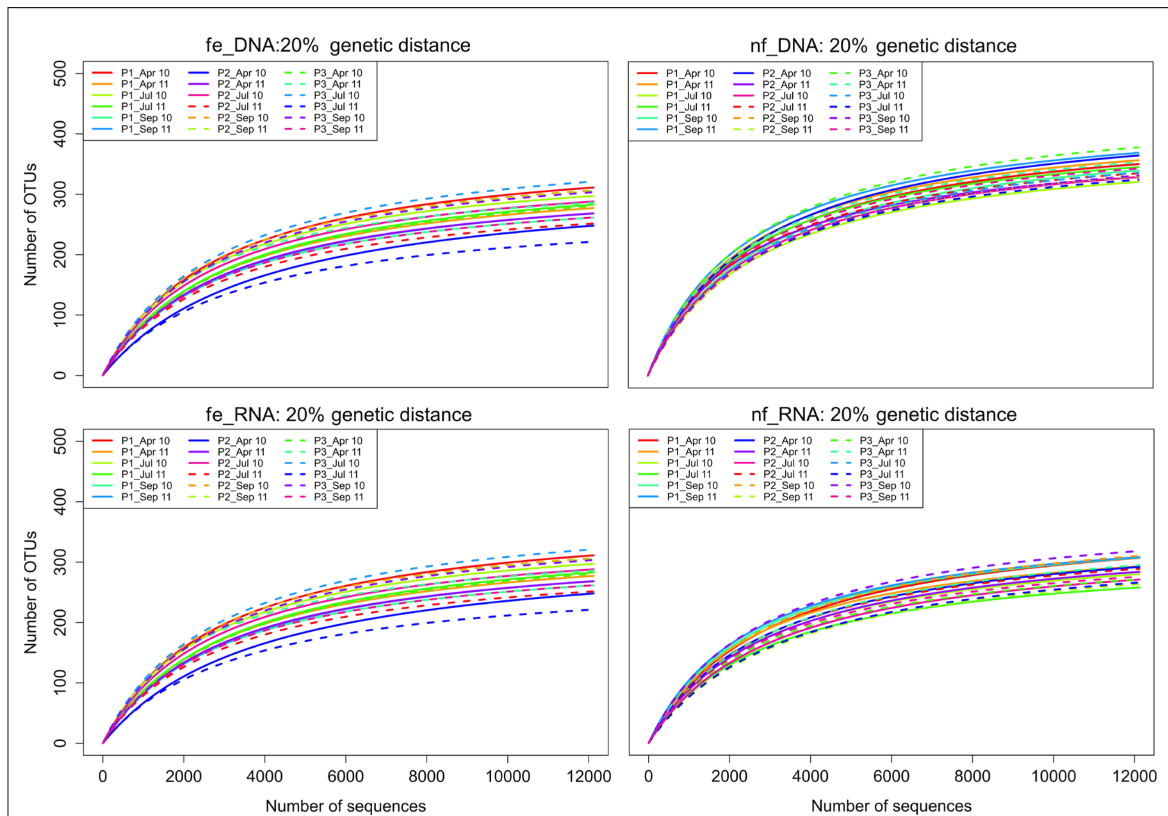

Figure S1: Rarefaction curves at 20% genetic distance calculated for the entire bacterial community in fertilized plots (fe_DNA:20%), active bacterial community in fertilized plots (fe_RNA:20%), entire bacterial community in non-fertilized plots (nf_DNA:20%), and active bacterial community in non-fertilized plots (nf_RNA:20%).

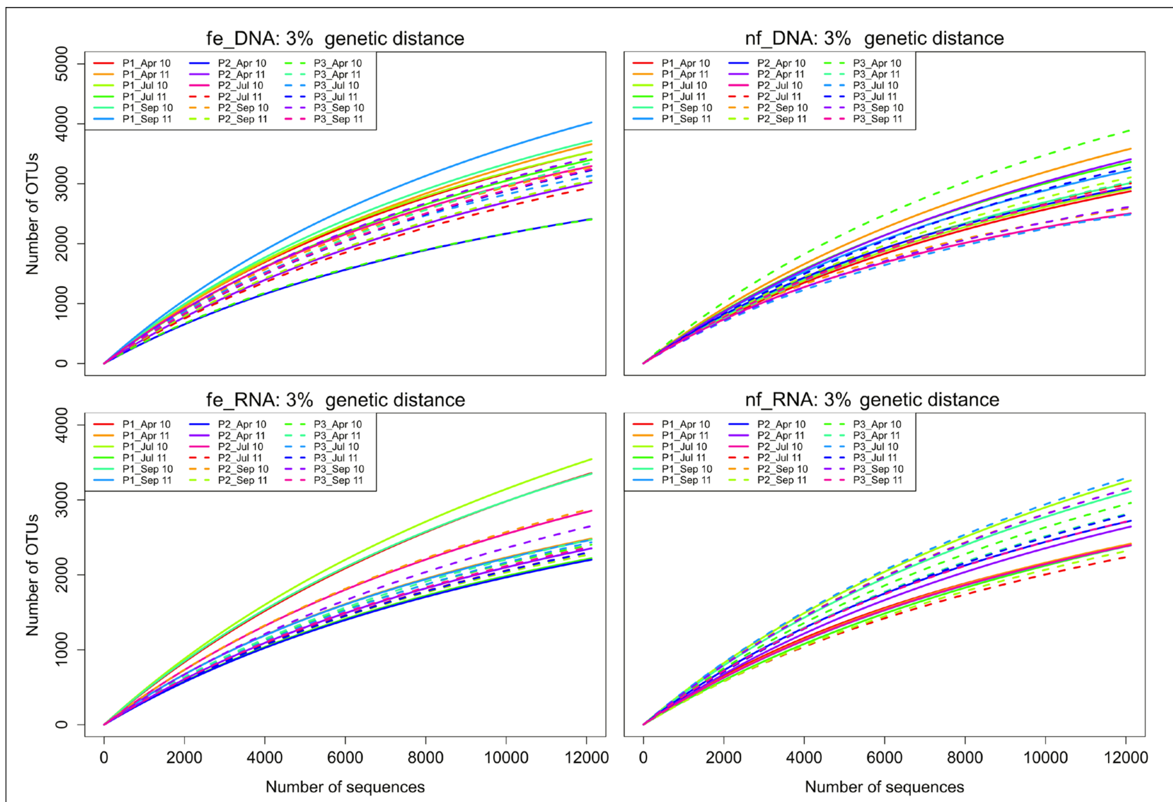

Figure S2: Rarefaction curves at 3% genetic distance calculated for the entire bacterial community in fertilized plots (fe_DNA:3%), active bacterial community in fertilized plots (fe_RNA:3%), entire bacterial community in non-fertilized plots (nf_DNA:3%), and active bacterial community in non-fertilized plots (nf_RNA:3%).

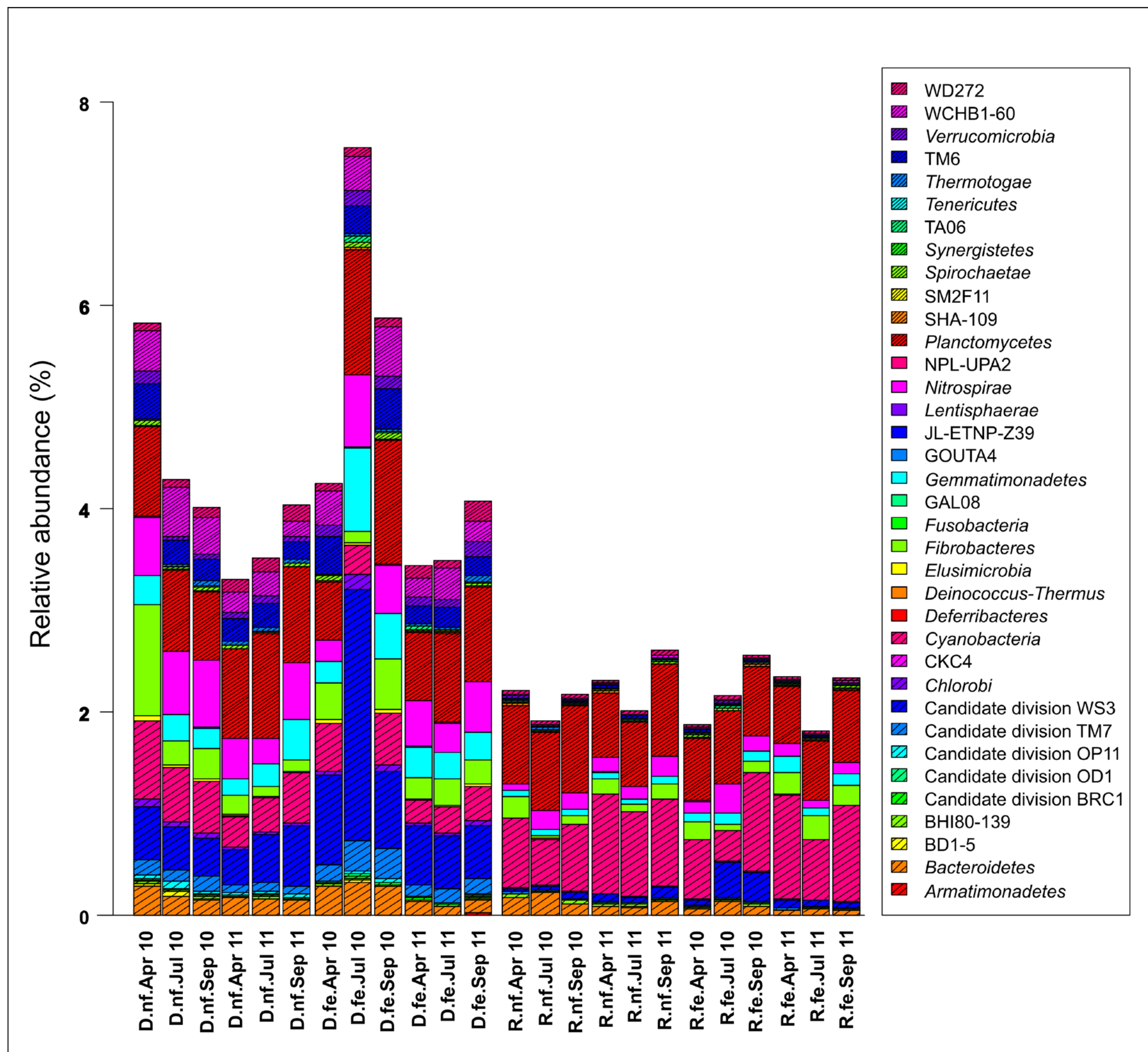

Figure S3: Relative abundances of rare phyla (< 1% abundance) derived from the analyzed soil samples. Fertilized (fe) and non-fertilized (nf) samples are shown in this figure. Samples were taken in April (Apr), July (Jul), and September (Sep) in 2010 (10) and 2011 (11) and the total (D) and active (R) bacterial communities were analyzed.

Chapter III

3.1. Drivers shaping the structure and functions of total and active bacterial communities in soil of two Aspen demes

For submission to Frontiers in Microbiology (Terrestrial Microbiology)

Drivers shaping the structure and functions of total and active bacterial communities in soil of two Aspen demes

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Running title: Bacterial communities and aspen demes

Key words: active vs. total bacterial communities, diversity, structure, pyrotag sequencing, functional predictions

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Abstract

Despite the ecological and environmental importance of *Populus,* little is known about effects of aspen demes on soil bacterial communities. In this study, the active and total bacterial communities of two *Populus tremula* demes (Geismar 2 and 8) were examined by amplicon-based pyrosequencing. Analysis of 2.3 million high quality 16S rRNA sequences exhibit a significant influence of aspen demes on diversity and composition of the active communities. *Firmicutes* was significant more abundant in the active Geismar 8 samples, while the *Chloroflexi*, and *Deltaproteobacteria* were significant more abundant in the active Geismar 2 sample. Correlation analysis with relative abundances and soil properties revealed at phylum as well as at order level more significant interactions in Geismar 8 compared to Geismar 2. Analysis of functional composition revealed that 36 and 941 genes were found with higher abundances in either aspen deme Geismar 2 or Geismar 8 at DNA and RNA level, respectively. At DNA level, 97% of all significant genes were higher abundant in Geismar 2, while at RNA level 75% of all significant genes were predominant in Geismar 8. We tried to link environmental parameters to observed active and total bacterial community structures by fitting multinomial regression modes via neural networks. Obtained results suggest that the total bacterial community is mainly driven by long-term effects such as environmental conditions, whereas the active bacterial community was influenced by the two aspen demes.

Introduction

The genus *Populus* (poplars, aspens and cottonwoods) consists of approximately 30 species (Taylor, 2002). Trembling aspen including the European species *Populus tremula* L. (European Common Aspen) are among the most widespread tree species in temperate forest and circumpolar boreal regions (Hultén and Fries, 1988). They are widely used in fiber, wood, and energy production due to their fast growth, the relatively low nutrient demand and the high tolerance to different climatic conditions such as drought (Bradshaw et al., 2000; Taylor, 2002; Dickmann and Kuzovkina, 2008). In addition, members of the genus *Populus* play an important role for the phytoremediation of contaminated soils (El-Gendy et al., 2009; Hur et al., 2011; Mukherjee et al., 2015). Thus, this genus is of high economic and ecological importance.
Several poplar species served as model tree systems in ecological and genetic studies due to many reasons such as their vegetative propagation and the small genome size (Bradshaw et al., 2000; Taylor, 2002). Recently, the complete genome sequence of *Populus trichocarpa* genotype "Nisqually-1" was published (Tuskan et al., 2006). In the last years, poplars have been intensively studied in a wide range of research areas. This includes plant physiology traits (e.g. Kleemann et al., 2011; Hajek et al., 2013; Müller et al., 2013) or interactions of poplar trees with other organisms such as arthropods (Zhang et al., 2015) or soil microbial communities (Gamalero et al., 2012; Baum et al., 2013).
Bacteria are the most abundant and diverse group of microorganisms in soil ecosystems and mediate nearly all biogeochemical cycles (Whitman et al., 1998; Torsvik et al., 2002; Fierer et al., 2007). Recent studies showed that tree species and tree identity are major drivers of composition and diversity of bacterial communities in forest soils (Nacke et al., 2011; Sun et al., 2014; Scheibe et al., 2015; Urbanova et al., 2015). The diversity of bacterial communities in different peat forest soils analyzed by pyrosequencing of 16S rRNA genes was influenced by the tree species (Sun et al., 2014). Fang et al. (2013) showed that microbial activity in the rhizosphere of poplar, alder, and willow growing in different tree species mixtures was significantly affected by the trees. In another study with different poplar clones, both presence and size of these clones affected the soil bacterial community structure (Gamalero et al., 2012).
However, only little is known about the interaction of poplar trees with soil bacterial community structure and functions. Most previous studies used DNA as template, thereby focusing on the total bacterial community (Gamalero et al., 2012; Baum et al., 2013;

Winder et al., 2013). The abundance of rRNA is a widely used indicator for the potential activity in microbial communities (Blazewicz et al., 2013). Previous studies found differences between DNA- and RNA-derived bacterial populations in soils (Baldrian et al., 2012; Barnard et al., 2015; Felsmann et al., 2015). For example, the analysis of bacterial communities in a *Picea abies* forest revealed a higher abundance of *Actinobacteria* in the active compared to the total community (Baldrian et al., 2012). This is in line with the results of Felsmann et al. (2015). Here, the comparison of active and total soil bacterial community structure in forest ecosystems revealed a higher abundance of *Actinobacteria* in the active than in the total community, whereas *Acidobacteria* were less abundant. Barnard et al. (2015) found a significant effect of environmental changes in the active, but not in the total bacterial community. Therefore, it is of great importance to analyze driving forces and ecological functions of both the metabolic active and the total bacterial community in soil ecosystems.

So far, the effect of two different aspen demes on both the active (RNA level) and the total (DNA level) bacterial community was not investigated. The aim of this study was to demonstrate how two *P. tremula* demes influence the soil bacterial community and which functions are affected in this community. The term deme was first described by Gilmour and Gregor (1939) as “any assemblage of taxonomically closely related individuals”. These demes are not necessarily equivalent to a specific taxonomic category such as species or variety (Gilmour and Heslop-Harrison, 1955). The study was performed within the Poplar Diversity Experiment (PopDiv) in Germany (Zhang et al., 2015). Sampling was performed in April, July, and September in 2010 and 2011 to include possible sampling time effects. Composition and diversity of total and active bacterial communities were assessed by pyrotag sequencing of 16S rRNA genes and transcripts, respectively. We hypothesized that the two aspen demes will have a deme-specific impact on the soil bacterial community structure and diversity. In addition, we expected that different drivers shape the active and the total bacterial communities. We employed Tax4Fun (Aßhauer et al., 2015) to predict functional profiles and link bacterial community structure with ecosystem functioning. We hypothesized that functional profiles of the two aspen demes would be similar because they are closely genetically related.

Material and Methods

Study site description and soil sampling

The study was conducted within the Poplar Diversity Experiment (PopDiv). This multidisciplinary experiment investigated the role of intraspecific diversity in aspen for selected ecosystem functions. Seven European aspen demes (*P. tremula*) and one from North America (*P. tremuloides*) were planted in plots representing either a single deme or combinations of two, four, and eight demes. A detailed description of the study site and the experimental design is given in Zhang et al. (2015). The experiment was established on a former historically documented permanent grassland (Thurengia, 1910) in 2008. The experimental area was located in the Solling uplands in Lower Saxony, Germany (51°44´56´´N, 9°32´28´´E). The predominant soil was a non-fertilized and nutrient poor Haplic Cambisol of sandy-loamy texture on Triassic sandstone (Middle Bunter) (Keuter et al., 2013). A previous study showed that the soil is homogenous across the site (Hajek et al., 2013). During the study period, mean annual temperature and annual precipitation were 6.6°C and 732 mm in 2010, and 8.91°C and 724 mm in 2011, respectively (Table 1).

Table 1: climatic conditions during sample periods in 2010 and 2011

Month	Mean temperature [°C]		mean precipitation [mm]	
	2010	2011	2010	2011
January	-4.72	0.15	n.d.	19.4
February	-2.62	0.05	n.d.	35.5
March	1.93	4.42	53.13	6
April	**7.78**	**11.26**	**14.84**	**41.75**
May	8.45	12.71	113.39	23.25
June	15.65	15.17	26.45	60.5
July	**19.87**	**14.48**	**47.27**	**110.85**
August	15.36	16.62	181.70	125.25
September	**11.42**	**14.75**	**102.12**	**54.75**
October	7.65	9.24	37.835	69.5
November	3.24	5.52	155.135	15.25
December	-4.81	2.51	n.d.	162

Bold: sampling time in 2010 and 2011; n.d, not detected

The two aspen demes Geismar 2 (G2) and Geismar 8 (G8) originated from Göttingen, Germany (51°31´N, 9°5´E). They did not differ in several plant characteristics such as root collar diameter or plant height (Hajek et al., 2014). Soil samples were collected from six mono-culture plots (three of each aspen deme). Around each tree, three soil cores (8 cm in

diameter, depth 20 cm) were taken and then pooled. Possible effects of environmental variability were minimized as all trees were grown at the same site. To analyze the effect of sampling time and year, samples were collected in spring (April), summer (July), and autumn (September) 2010 and 2011. Soil samples were shock- frozen in liquid nitrogen and stored at -80°C until analysis.

Soil properties

For determination of soil properties, subsamples from the pooled soil samples were dried at 60°C for seven days and sieved to < 2mm. Soil organic carbon (C) and total nitrogen (N) concentrations were determined from dried soil with a CN elemental analyzer (Elemental Analyzer EA 1108, Carlo Erba Instruments, Rodano, Italy). The gravimetric soil water content (%) was calculated from oven-dried subsamples. Soil pH values were measured from soil:water with 0.1 M KCl suspension (ratio of 1:2).

Extraction of nucleic acids and reverse transcription

Total environmental RNA and DNA were co-extracted from 0.5 g soil employing the RNA PowerSoil Total RNA Isolation Kit and the RNA PowerSoil DNA Elution Accessory Kit, respectively, as recommended by the manufacturer (MoBio Laboratories, Carlsbad, CA, USA). For RNA purification, residual DNA was removed with the TURBO DNA-free™ Kit (Ambion Applied Biosystems, Darmstadt, Germany) from the extracted RNA and its absence was confirmed by PCR as described by Wemheuer et al. (2012). The DNA-free RNA was purified and concentrated using the RNeasy MinElute Cleanup Kit (Qiagen GmbH, Hilden, Germany). Isolated DNA was purified with the PowerClean DNA Cleanup Kit (MoBio Laboratories). RNA and DNA concentrations were determined using a NanoDrop ND-1000 spectrophotometer (Peqlab Biotechnologie GmbH, Erlangen, Germany). Approximately 500 ng of purified RNA was converted to cDNA using the SuperScriptTM III reverse transcriptase and the reverse primer V3rev of the subsequent PCR reaction, as recommended by the supplier (Invitrogen, Karlsruhe, Germany).

Amplification and sequencing of 16S rRNA gene regions

To analyze bacterial community structures, the V2-V3 region of the 16S rRNA gene and their corresponding transcripts were amplified by PCR using three independent reactions per sample. The reaction mixture (25 µl) contained 5 µl of 5-fold Phusion GC buffer, 200 µM of each of the four deoxynucleoside triphosphates, 5% DMSO, 0.4 µM of each primer, 0.5 U of Phusion Hot Start High Fidelity DNA polymerase (Fisher Scientific GmbH, Schwerte, Germany), and approximately 25 ng of DNA or cDNA as template. Negative controls were performed by using the reaction mixture without template. The V2-V3 region was amplified with the following set of primers modified by Schmalenberger (2001) containing the Roche 454 pyrosequencing adaptors, key sequences and one unique MID (underlined) per sample: V2for 5'-CGTATCGCCTCCCTCGCGCCATCAG-$(dN)_{10}$-AGTGGCGGACGGGTGAGTAA-3' and V3rev 5'-CTATGCGCCTTGCCAGCCCGCT CAG-$(dN)_{10}$-CGTATTACCGCGGCTGCTGG-3'. For cDNA amplification, the following cycling scheme was used: initial denaturation at 98°C for 5 min and 25 cycles of denaturation at 98°C for 10 s and annealing/extension at 72°C for 10 s, followed by a final extension at 72°C for 5 min. Cycling conditions for the amplification of DNA were 98°C for 5 min; 25 cycles of 98°C for 45 s, 72°C for 30 s, 72°C for 30 s, followed by a final extension at 72°C for 10 min. The resulting PCR products of the three independent reactions were pooled in equal amounts and purified using the peqGold gel extraction kit (Peqlab Biotechnologie GmbH, Erlangen, Germany). Obtained PCR products were quantified using the Quant-iT dsDNA HS assay kit and a Qubit fluorometer (Invitrogen GmbH) as recommended by the manufacturer. The Göttingen Genomics Laboratory determined the sequences of the partial 16S rRNA genes employing the Roche GS-FLX 454 pyrosequencer with Titanium chemistry as recommended by the manufacturer (Roche, Mannheim, Germany).

Processing of 16S rRNA sequence data

Pyrosequencing derived datasets were processed and analyzed using the QIIME software package version 1.6 (Caporaso et al., 2010). Sequences shorter than 200 bp, low quality sequences, and sequences with homopolymers (> 8 bp) were removed from the datasets. Pyrosequencing noise was removed using Acacia 1.52 (Bragg et al., 2012). Primer sequence residues were truncated using cutadapt version 1.0 (Martin, 2011). Chimeric

sequences were detected and eliminated using UCHIME 7.0.190 in de novo and in reference mode with the Silva SSURef 119 NR database as reference database (Edgar, 2010; Quast et al., 2013). All remaining sequences were subsequently clustered in operational taxonomic units (OTUs) at 3 and 20% genetic distance using the QIIME pick_otus.py script and uclust (Edgar, 2010). OTUs represented by only single sequence in the entire dataset (singletons) were removed (see (Schneider et al., 2013a). Taxonomic assignment was performed via BLAST alignment against the most recent SILVA database (SSURef NR 119) (Quast et al., 2013). Rarefaction curves, alpha diversity indices (Chao1, Shannon, Simpson, and Michaelis-Menten-Fit), and beta diversity (Principle Component analyses) were determined using QIIME according to Wemheuer et al. (2013). Functional predictions for each sample were performed in R (version 3.2.0; R Development Core Team 2015 [http://www.R-project.org/]) using Tax4Fun (Aßhauer et al., 2015).

Statistical analysis

T-test for normal distributed data or the Mann-Whitney-test for not normal distributed data and Spearman’s rank correlation coefficients were performed in SigmaPlot version 11.0 (Systat Software GmbH, Erkrath, Germany). All other statistical analyses were conducted employing the R version 3.2 (Team, 2011).Changes in community structure and significant differences between samples and treatments were examined employing the metaMDS and RDA as well as envfit functions within the vegan package (Oksanen et al., 2013) as described by Wietz et al. (2015). Total and active bacterial communities were analyzed separately as DNA and RNA were extracted from the same samples and thus represent spatial pseudoreplicates. Effects of the aspen demes on environmental parameters were tested as described by Wemheuer et al (2014). The results of the statistical tests were regarded as significant at P-values ≤ 0.05.

Sequence data deposition

Sequence data was deposited in the Sequence Read Archive (SRA) of the National Center for Biotechnology Information (NCBI) under the accession number SRA162385.

Results

Soil properties

In this study, the composition of bacterial communities present in soil samples derived from growing sites of two aspen demes was assessed. Soil samples were collected from three aspen of deme G2 and G8, respectively. Several soil properties including total N or C content, C/N ratio, water content, and pH were determined (Table 2).

Table 2: Soil properties determined at the study sites (poplar demes Geismar2 and Geismar8)

Sample	Season	Aspen deme	pH (+KCL) ± SD	Water content ± SD (%)	% C	% N	C/N
G2.1.apr10.D/R	spring 2010	Geismar2	4.39±0.12	27.7±0.4	3.90	0.27	14.31
G2.2.apr10.D/R	spring 2010	Geismar2	4.90±0.23	27.5±0.2	3.47	0.27	12.95
G2.3.apr10.D/R	spring 2010	Geismar2	4.62±0.15	24.9±1.1	3.22	0.23	13.84
G2.1.jul10.D/R	summer 2010	Geismar2	5.63±0.12	12.9±1.0	4.22	0.32	13.32
G2.2.jul10.D/R	summer 2010	Geismar2	5.73±0.27	14.9±0.1	3.96	0.30	13.17
G2.3.jul10.D/R	summer 2010	Geismar2	5.58±0.34	11.5±1.4	4.23	0.31	13.74
G2.1.sep10.D/R	autumn 2010	Geismar2	4.43±0.08	25.6±1.2	4.29	0.36	12.04
G2.2.sep10.D/R	autumn 2010	Geismar2	4.96±0.27	26.2±2.1	3.89	0.32	12.23
G2.3.sep10.D/R	autumn 2010	Geismar2	5.02±0.21	24.3±1.8	4.29	0.35	12.41
G8.1.apr10.D/R	spring 2010	Geismar8	4.94±0.12	26.0±1.4	3.79	0.26	14.59
G8.2.apr10.D/R	spring 2010	Geismar8	4.45±0.15	22.8±0.7	3.35	0.22	15.27
G8.3.apr10.D/R	spring 2010	Geismar8	4.28±0.59	25.3±4.2	3.33	0.20	16.59
G8.1.jul10.D/R	summer 2010	Geismar8	5.54±0.03	13.4±2.0	4.23	0.31	13.54
G8.2.jul10.D/R	summer 2010	Geismar8	5.19±0.02	11.4±2.0	4.04	0.31	13.06
G8.3.jul10.D/R	summer 2010	Geismar8	5.65±0.03	12.8±1.3	3.59	0.25	14.48
G8.1.sep10.D/R	autumn 2010	Geismar8	5.20±0.10	25.3±1.0	4.45	0.32	14.11
G8.2.sep10.D/R	autumn 2010	Geismar8	4.64±0.11	21.5±1.4	3.91	0.29	13.57
G8.3.sep10.D/R	autumn 2010	Geismar8	4.58±0.52	25.9±0.7	4.32	0.33	13.09
G2.1.apr11.D/R	spring 2011	Geismar2	4.58±0.12	24.9±1.0	3.90	0.29	13.33
G2.2.apr11.D/R	spring 2011	Geismar2	4.59±0.15	23.6±0.9	3.10	0.26	11.84
G2.3.apr11.D/R	spring 2011	Geismar2	5.41±0.59	23.0±0.9	4.71	0.34	14.02
G2.1.jul11.D/R	summer 2011	Geismar2	4.72±0.03	24.1±0.3	3.79	0.29	12.99
G2.2.jul11.D/R	summer 2011	Geismar2	4.30±0.02	23.9±0.7	3.56	0.28	12.78
G2.3.jul11.D/R	summer 2011	Geismar2	4.57±0.03	26.9±0.9	4.53	0.32	14.27
G2.1.sep11.D/R	autumn 2011	Geismar2	4.49±0.10	34.6±1.2	3.93	0.31	12.52
G2.2.sep11.D/R	autumn 2011	Geismar2	4.56±0.11	33.6±1.1	3.44	0.28	12.21
G2.3.sep11.D/R	autumn 2011	Geismar2	5.26±0.52	33.4±1.6	4.47	0.33	13.35
G8.1.apr11.D/R	spring 2011	Geismar8	5.25±0.32	23.8±0.5	3.23	0.24	13.43
G8.2.apr11.D/R	spring 2011	Geismar8	4.31±0.02	20.9±1.7	2.90	0.22	12.94
G8.3.apr11.D/R	spring 2011	Geismar8	4.37±0.07	24.6±0.7	4.05	0.24	16.62
G8.1.jul11.D/R	summer 2011	Geismar8	4.53±0.02	25.6±0.4	3.90	0.28	14.11
G8.2.jul11.D/R	summer 2011	Geismar8	4.26±0.05	21.0±1.8	3.70	0.26	14.40
G8.3.jul11.D/R	summer 2011	Geismar8	4.49±0.05	22.8±0.4	4.57	0.28	16.42
G8.1.sep11.D/R	autumn 2011	Geismar8	5.26±0.29	33.4±1.0	3.84	0.27	14.41
G8.2.sep11.D/R	autumn 2011	Geismar8	4.28±0.04	31.1±1.6	3.69	0.28	13.28
G8.3.sep11.D/R	autumn 2011	Geismar8	4.35±0.06	34.3±1.3	4.52	0.30	14.91

The pH values ranged from 4.26 to 5.73 with the highest pH in July 2010 and the lowest pH in July 2011. Water content varied between 11.4% and 34.6%, with the lowest water content in July 2010 and the highest in September 2011. Total N content varied between 0.20 and 0.36%. C content showed high variability ranging from 2.90 to 4.71%. The C/N ratios varied between 12 and 16.8 in the analyzed soil samples, with the lowest C/N ratio in April 2010 and with the highest in April 2011.

Bacterial community diversity and richness

Composition and diversity of total and active soil bacterial communities were assessed by pyrotag sequencing of 16S rRNA genes and the transcripts, respectively. After quality filtering, denoising, and removal of potential chimeras and non-bacterial sequences, 2,370,236 high quality sequences with an average read length of 362 bp were used for analyzing bacterial community structures and diversity. The number of sequences per sample ranged from 12,139 to 68,325 (DNA level) and from 18,658 to 54,205 (RNA level) (Table S1). All sequences were classified below phylum level.
Calculated rarefaction curves at 3% (species level) and 20% genetic distance (phylum level) revealed that the majority of the bacterial community was covered by the surveying effort (Figures S1 and S2, respectively). This is supported by the calculated coverage values (Table S2 and S3). Shannon indices varied between 2.15 and 4.08 (20% genetic distance) and between 3.33 and 6.80 (3% genetic distance).

Heterogeneity in composition and diversity of total and active bacterial communities

Obtained sequences were assigned to 41 phyla and candidate division. Five dominant phyla (> 1% abundance) were present in each soil sample and accounted for 96% of all bacterial sequences at RNA and DNA level (Figure 1). *Proteobacteria* were predominant across all samples (DNA 33%, RNA 44%). Sequences assigned to this phylum mainly belonged to the *Alphaproteobacteria* (DNA 16%, RNA 37%). *Firmicutes* were the second most abundant bacterial phylum in total and active bacterial communities (DNA 27%, RNA 37%). Other abundant phyla in the bacterial community were *Chloroflexi* (DNA 16%, RNA 9%) and *Acidobacteria* (DNA 13%, RNA 4%). Rare phyla were shown in figure S3.

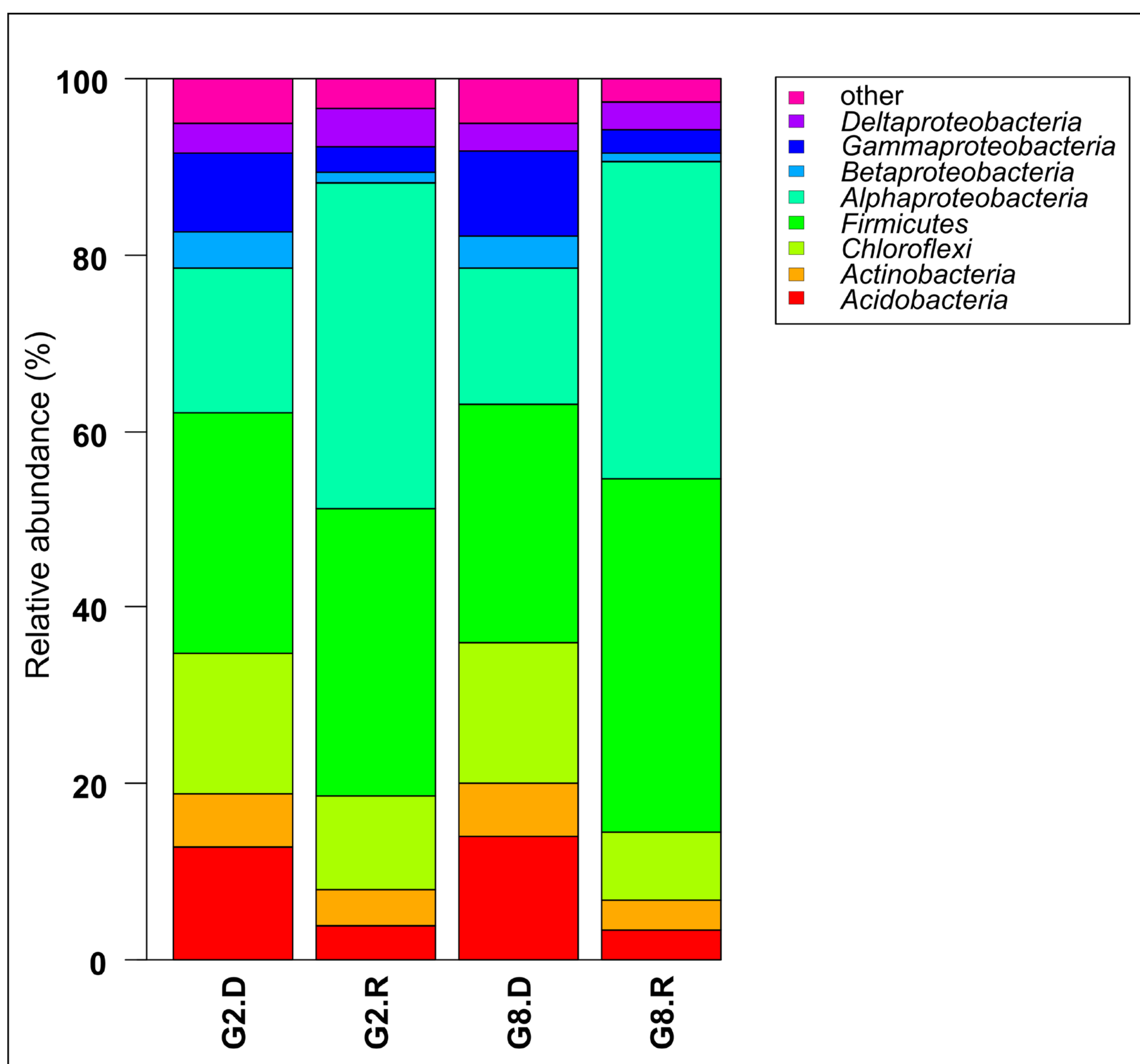

Figure 1: Relative abundances of the most abundant bacterial phyla (> 1%) and proteobacterial classes derived from the analyzed soil samples. Phyla accounting < 1% of all sequences are summarized in the group "other". Aspen deme Geismar2 (G2) and aspen deme Geismar8 (G8) samples are shown in this figure. Samples were taken in April (Apr), July (Jul), and September (Sep) in 2010 (10) and 2011 (11) and the entire (D) and active (R) bacterial community were analyzed.

At 3% genetic distance, all sequences analyzed in this study were affiliated to a total of 60,412 OTUs. The estimated number of OTUs per sample indicated that the bacterial richness was higher in the total bacterial community (3,214±322 OTUs) than in the active community level (2,660±226 OTUs). The core community represented by OTUs found in the soil community of both aspen demes at DNA and RNA level consisted of 11,601 OTUs (Figure 2). The most abundant OTU in the total and active bacterial community (13.4% and 17.3%, respectively) was *Bacillus* sp. IDA1527, a member of the phylum *Firmicutes*. The second most abundant OTU (11.7%) in the active community belonged to the family *Acetobacteraceae* (*Alphaproteobacteria*).

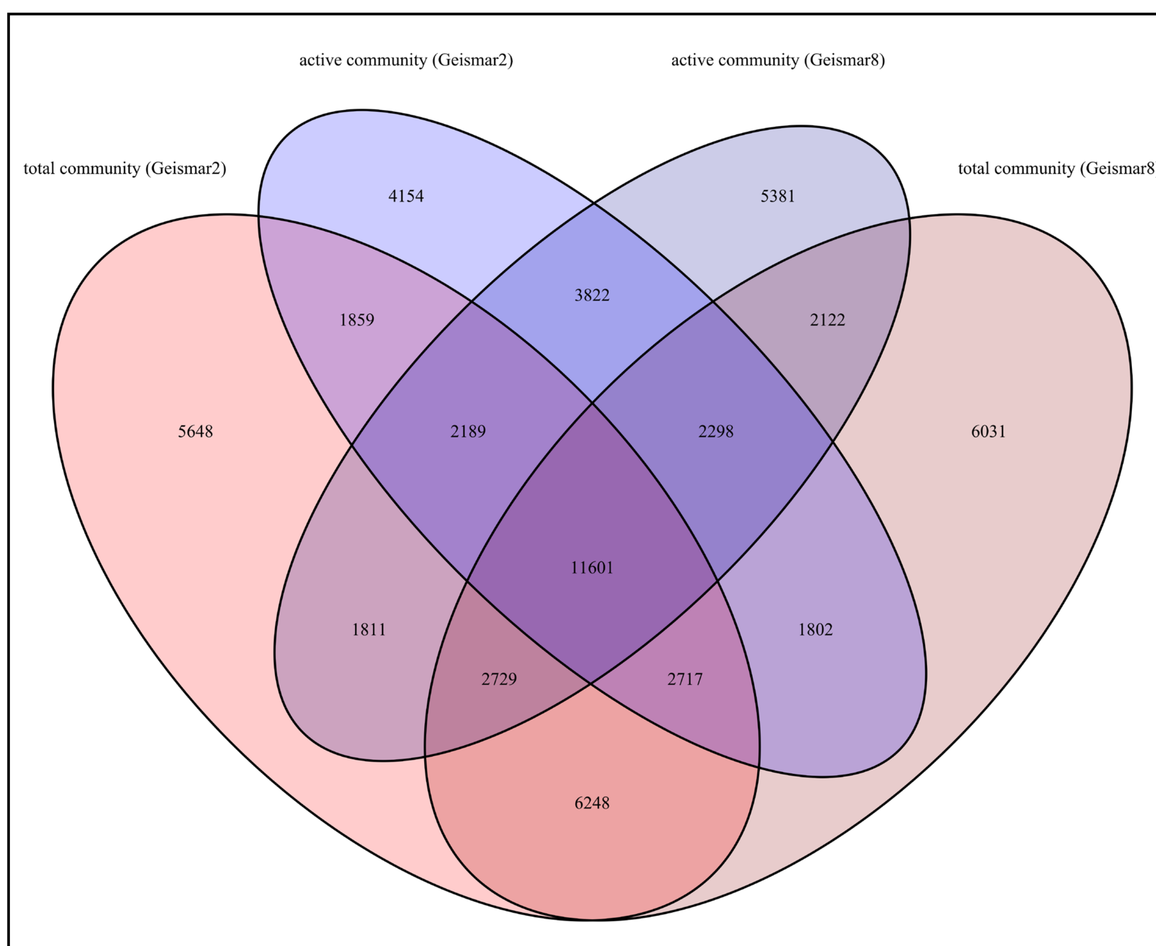

Figure 2: Venn-diagram of all analyzed OTUs showed the core community found in the fertilized, non-fertilized treatment, as well as in the entire (DNA), and active (RNA) bacterial community. Depicted were OTUs estimated at Geismar 2 DNA, Geismar 2 RNA, Geismar 8 DNA, and Geismar 8 RNA level and all other possible interfaces.

Correlation of environmental properties and relative abundances differs between both aspen demes

Spearman´s correlation was used to analyze the relationship between soil parameters and the relative abundances of the most abundant phyla, proteobacterial classes, and orders (Tables 3-6). All taxonomic groups with more than 1% abundance were included in the analysis. In aspen deme Geismar 2 plots the active part of *Chloroflexi* correlated significantly negatively with C/N and *Betaproteobacteria* correlated significantly positively with pH. *Firmicutes* (total bacterial community) correlated significantly negatively with pH and significantly positively with the water content. At order level only 2 of the 14 tested orders correlated significantly with pH in the Geismar 2 plots.

In the aspen deme Geismar 8 plots all bacterial phyla and proteobacterial classes (parts of the active or total bacterial community) correlated significantly positively or negatively with either pH or water content (Table 4).

Table 3. Spearman´s Rank correlations of the abundance of the most abundant phyla, proteobacterial classes and soil properties of the aspen deme **Geismar 2** treatment. Relative abundances derived from the active (RNA) and total (DNA) bacterial community were separately analyzed.

Group	Correlation coefficient					
	pH		Water content		C/N	
	DNA	RNA	DNA	RNA	DNA	RNA
Acidobacteria	-0.174	-0.042	0.032	-0.189	0.103	0.141
Actinobacteria	-0.102	0.362	0.007	-0.213	0.267	-0.025
Chloroflexi	-0.049	0.018	-0.268	-0.372	-0.086	**-0.476**
Firmicutes	**-0.472**	-0.313	**0.550**	0.245	0.463	0.078
Alphaproteobacteria	0.110	0.03	-0.082	0.267	-0.401	0.42
Betaproteobacteria	0.455	**0.47**	-0.114	-0.116	-0.061	-0.339
Gammaproteobacteria	0.461	0.236	-0.249	0.125	-0.441	-0.055
Deltaproteobacteria	0.249	0.375	-0.042	-0.101	0.069	0.039

Bold number: $P < 0.05$

Table 4. Spearman Rank correlations of the abundance of the most abundant phyla, proteobacterial classes and soil properties of the aspen deme **Geismar 8** treatment. Relative abundances derived from the active (RNA) and total (DNA) bacterial community were separately analyzed

Group	Correlation coefficient					
	pH		Water content		C/N	
	DNA	RNA	DNA	RNA	DNA	RNA
Acidobacteria	0.04	0.119	-0.380	**-0.535**	-0.125	-0.430
Actinobacteria	0.24	**0.779**	0.057	-0.155	0.361	-0.349
Chloroflexi	0.121	0.104	0.043	**-0.585**	-0.273	-0.365
Firmicutes	**-0.478**	0.133	0.136	0.335	0.350	-0.205
Alphaproteobacteria	-0.430	**-0.552**	-0.138	-0.135	-0.260	0.395
Betaproteobacteria	**0.822**	**0.769**	-0.057	0.202	-0.365	-0.365
Gammaproteobacteria	**0.550**	**0.546**	-0.08	0.226	-0.304	0.241
Deltaproteobacteria	**0.641**	**0.507**	-0.084	0.177	-0.409	-0.196

Bold number: $P < 0.05$

Additionally, 9 of the 14 tested orders correlated significantly positively or negatively with pH and water content. Especially, the orders *Myxococcales*, *Rhodospirillales,* and JG30-KF-AS9 correlated significantly at both, RNA and DNA level with pH (Table 6). None of these phyla, proteobacterial classes, and orders correlated significantly with C/N content in Geismar 8 plots.

Table 5. Spearman Rank correlations of the abundance of the most abundant orders and soil properties of the aspen deme **Geismar 2** treatment. Relative abundances derived from the active (RNA) and entire (DNA) bacterial community were separately analyzed.

Group	Correlation coefficient					
	pH		Water content		C/N	
	DNA	RNA	DNA	RNA	DNA	RNA
Xanthomonadales	0.193	-0.016	-0.158	0.141	-0.055	-0.133
Enterobacteriales	**0.490**	0.327	-0.089	-0.001	0.189	-0.022
Myxococcales	0.243	0.387	-0.003	-0.081	-0.03	-0.135
Caulobacterales	0.187	**0.488**	-0.165	0.026	0.053	0.170
Rhizobiales	-0.193	0.257	0.016	-0.285	0.018	-0.110
Rhodospirillales	0.024	-0.053	-0.127	0.288	0.315	0.385
Bacillales	-0.449	-0.329	0.562	0.249	0.032	0.141
Clostridiales	-0.038	0.055	0.083	0.098	-0.331	-0.315
JG30-KF-AS9	-0.401	-0.282	0.258	-0.087	-0.152	-0.232
Ktedonobacterales	-0.181	-0.392	-0.051	-0.109	-0.044	-0.220
Frankiales	-0.346	0.212	-0.062	-0.324	-0.119	-0.404
Acidobacteriales	-0.185	-0.018	-0.007	-0.261	0.230	-0.220
Subgroup 3	-0.391	-0.247	0.181	-0.173	0.020	-0.053
Subgroup 7	0.230	0.319	0.017	-0.170	0.010	-0.247

Bold number: P < 0.05

Table 6. Spearman Rank correlations of the abundance of the most abundant orders and soil properties of the aspen deme **Geismar 8** treatment. Relative abundances derived from the active (RNA) and entire (DNA) bacterial community were separately analyzed.

Group	Correlation coefficient					
	pH		Water content		C/N	
	DNA	RNA	DNA	RNA	DNA	RNA
Xanthomonadales	**0.728**	0.355	0.021	**0.490**	-0.083	-0.278
Enterobacteriales	0.405	**0.606**	-0.053	0.208	-0.032	-0.044
Myxococcales	**0.595**	**0.480**	-0.025	0.171	-0.098	-0.360
Caulobacterales	**0.589**	0.075	-0.064	0.311	0.001	0.194
Rhizobiales	-0.178	-0.127	-0.120	0.301	0.166	-0.029
Rhodospirillales	**-0.675**	**-0.601**	0.067	-0.142	0.022	0.128
Bacillales	-0.466	0.149	0.136	0.321	0.055	0.161
Clostridiales	-0.107	0.136	0.114	0.212	-0.110	-0.100
JG30-KF-AS9	**-0.497**	**-0.518**	0.150	-0.267	-0.217	0.159
Ktedonobacterales	-0.276	-0.013	-0.108	-0.363	0.051	-0.066
Frankiales	-0.163	0.469	0.133	**-0.068**	0.386	-0.320
Acidobacteriales	-0.226	-0.367	-0.281	-0.261	0.244	0.038
Subgroup 3	-0.258	-0.061	-0.350	-0.527	0.211	-0.106
Subgroup 7	0.321	**0.626**	0.116	-0.156	-0.017	-0.247

Bold number: P < 0.05

Variation in bacterial community structure as response to aspen demes

The comparison of mean Shannon indices from aspen deme Geismar2 (G2) and aspen deme Geismar8 (G8) samples revealed a significantly higher diversity in the active (RNA) soil bacterial community of G2 compared to G8 at 97% and 80% genetic similarity (P = 0.018 and P = 0.005, respectively), whereas no differences were recorded in the total bacterial community (Figure 3).

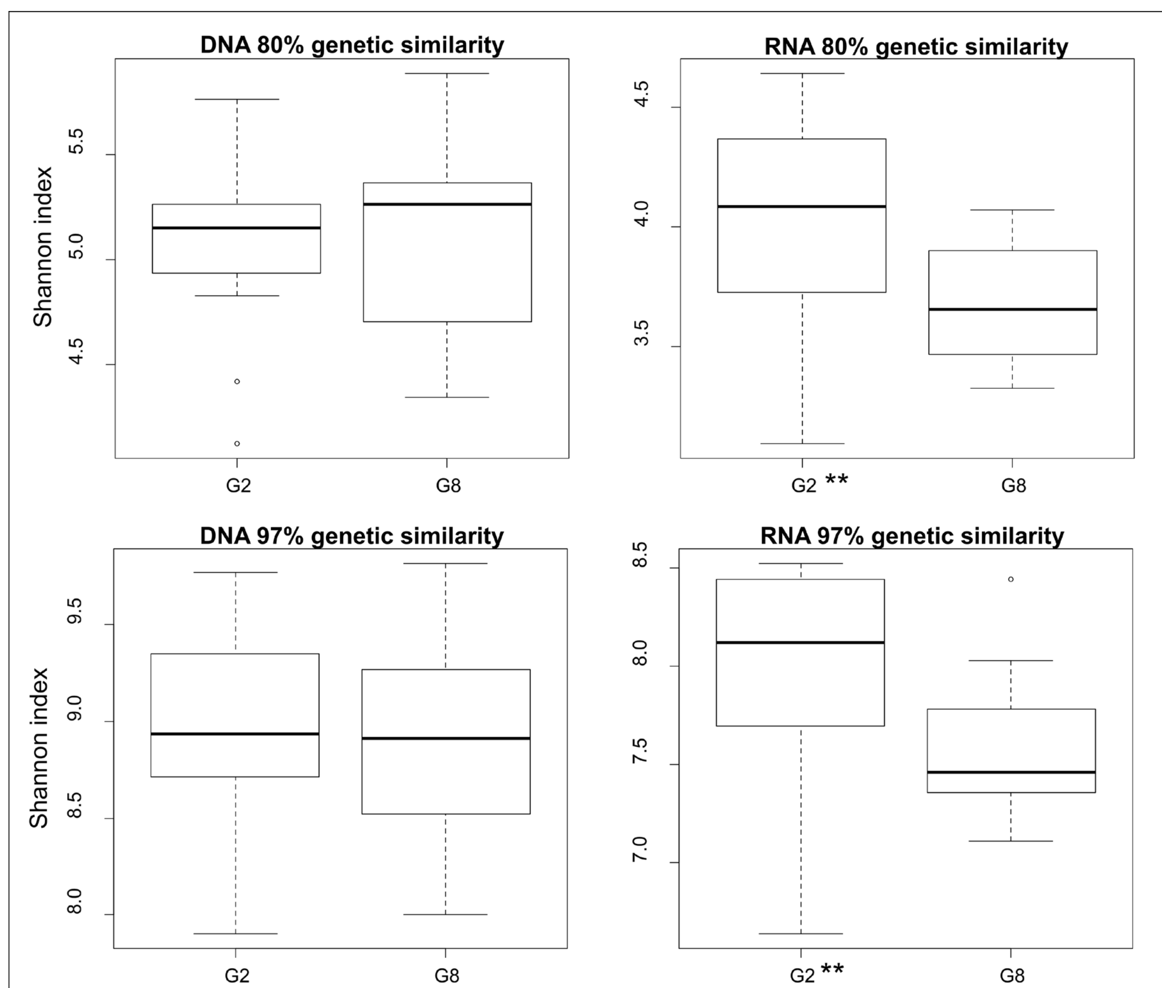

Figure 3: Boxplot diagram showing the Shannon indices of the total (DNA) and active (RNA) bacterial community derived from aspen deme Geismar2 (G2) and deme Geismar8 (G8) at 97% and 80% genetic similarity. Asterisks indicating significant differences between the two aspen demes; * P < 0.05; ** P<0.005; *** P<0.001

Differences in soil bacterial community structure of the two aspen demes were assessed by ordination analysis (Figure 4). Calculated ordination plots either based on Bray-Curtis or weighted UniFrac dissimilarity revealed no significant differences of the total bacterial community structure in two aspen demes. However, a significant impact on community composition was observed at active bacterial community level (Bray-Curtis, P = 0.039;

weighted UniFrac, P = 0.01). Especially, we observed significant higher abundances of the active part of *Chloroflexi* and *Deltaproteobacteria* in the aspen deme Geismar2, while active members of *Firmicutes* were significant more abundant in the Geismar8 plots (Table S4).

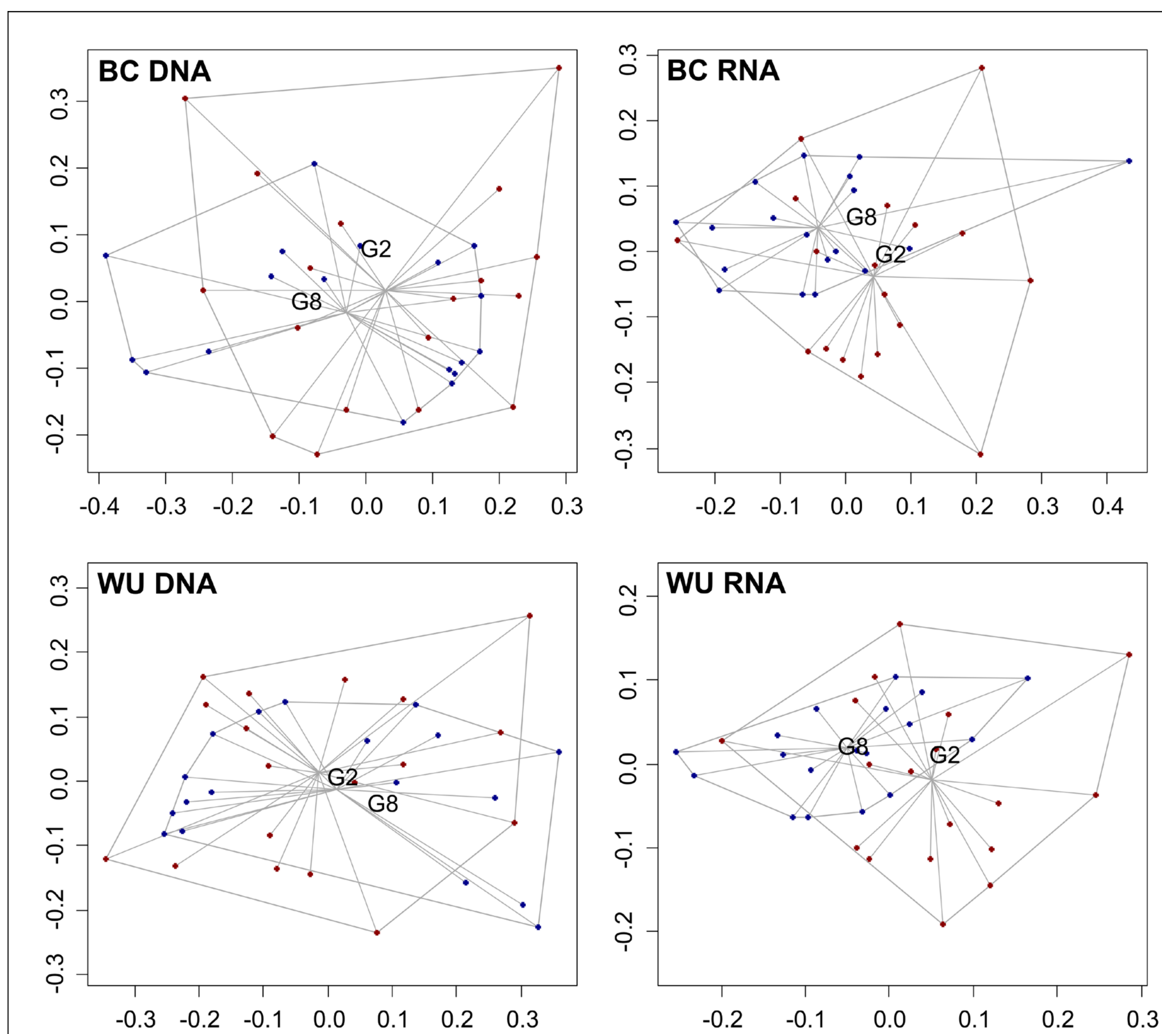

Figure 4: Calculated Bray-Curtis (BC) and weighted UniFrac dissimilarity (WU) at DNA and RNA level derived from the aspen deme Geismar2 (G2) and deme Geismar8 (G8).

Impact of aspen demes on functional profiles of the soil bacterial communities

Functional and metabolic pathways profiles were predicted from 16S rRNA data to investigate putative changes between the soil bacterial communities of G2 and G8. An initial RDA analysis of the complete functional profile of all analyzed samples showed significantly differences between the total and potentially active bacterial community (Figure 5B). Significant differences between the two aspen demes in the complete dataset were not recorded (Figure 5A). Additionally, redundancy analysis of DNA-derived or

RNA-derived datasets revealed also no significant different between both aspen demes (Figure 5C and 5D).

However, statistical analysis of single KEGG orthologs showed that only 36 genes had a significantly higher abundance in either aspen deme G2 or G8 in the DNA-based samples, whereas the abundances of 941 genes were significantly higher abundant in one of the two demes at RNA level.

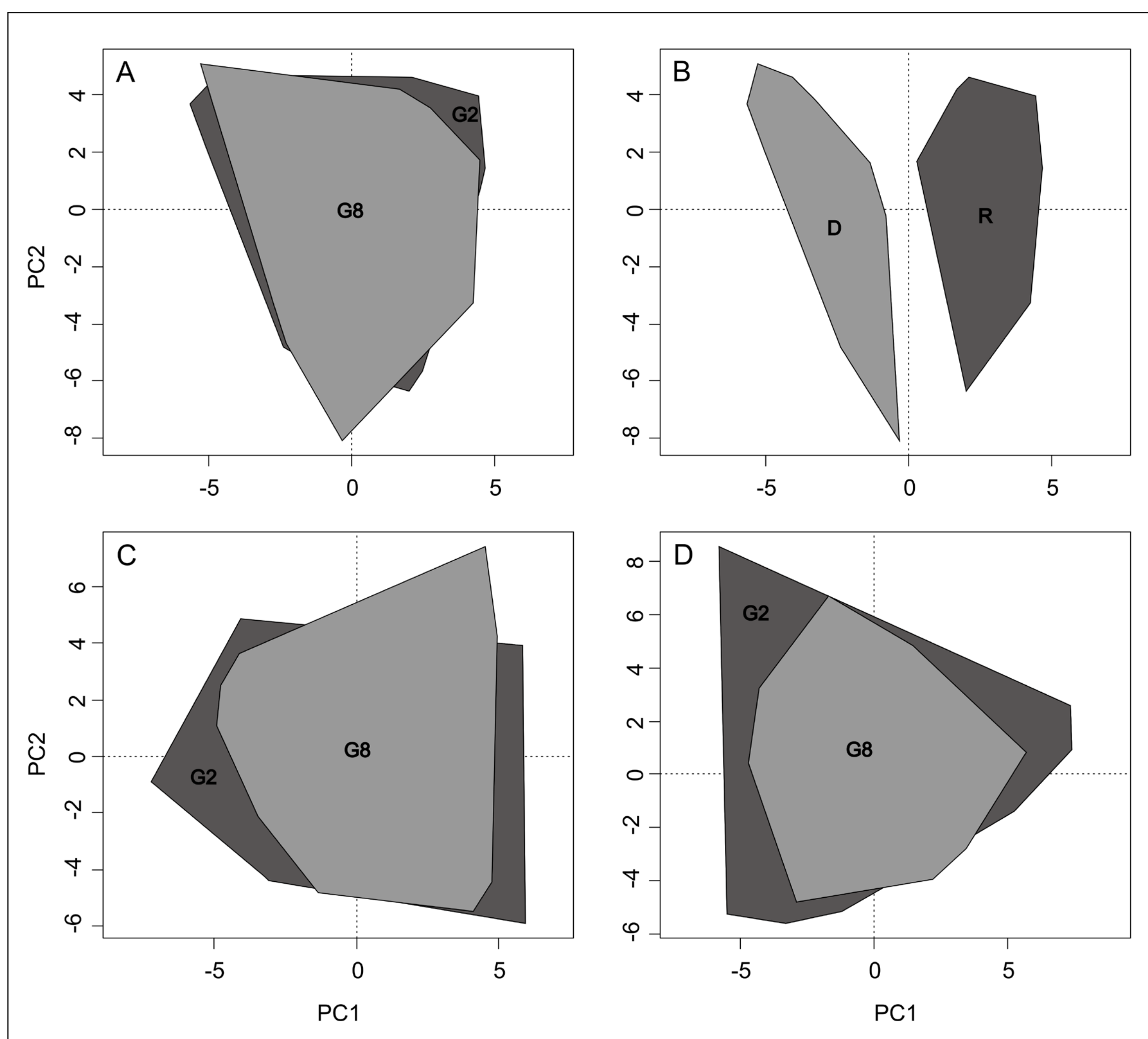

Figure 5: Redundancy Analysis (RDA) of the functional profile derived from A. complete dataset of aspen deme Geismar2 (G2) and deme Geismar8 (G8) B. total (DNA) and active (RNA) bacterial community C. only the total bacterial community and D. only the active bacterial community of both demes.

Sampling time effect differs between active and total bacterial community

To analyze the impact of sampling time and year on bacterial diversity and community composition in both aspen demes plots, sampling was performed in spring, summer and beginning of autumn in 2010 and 2011.

Bacterial diversity analyzing estimated OTUs at 3% and 20% genetic distance revealed no significant differences in the active and total bacterial community in both aspen demes. The Shannon index at 20% genetic distance indicated significant differences between the summer as well as autumn of 2010 and 2011, respectively (Figure 6).

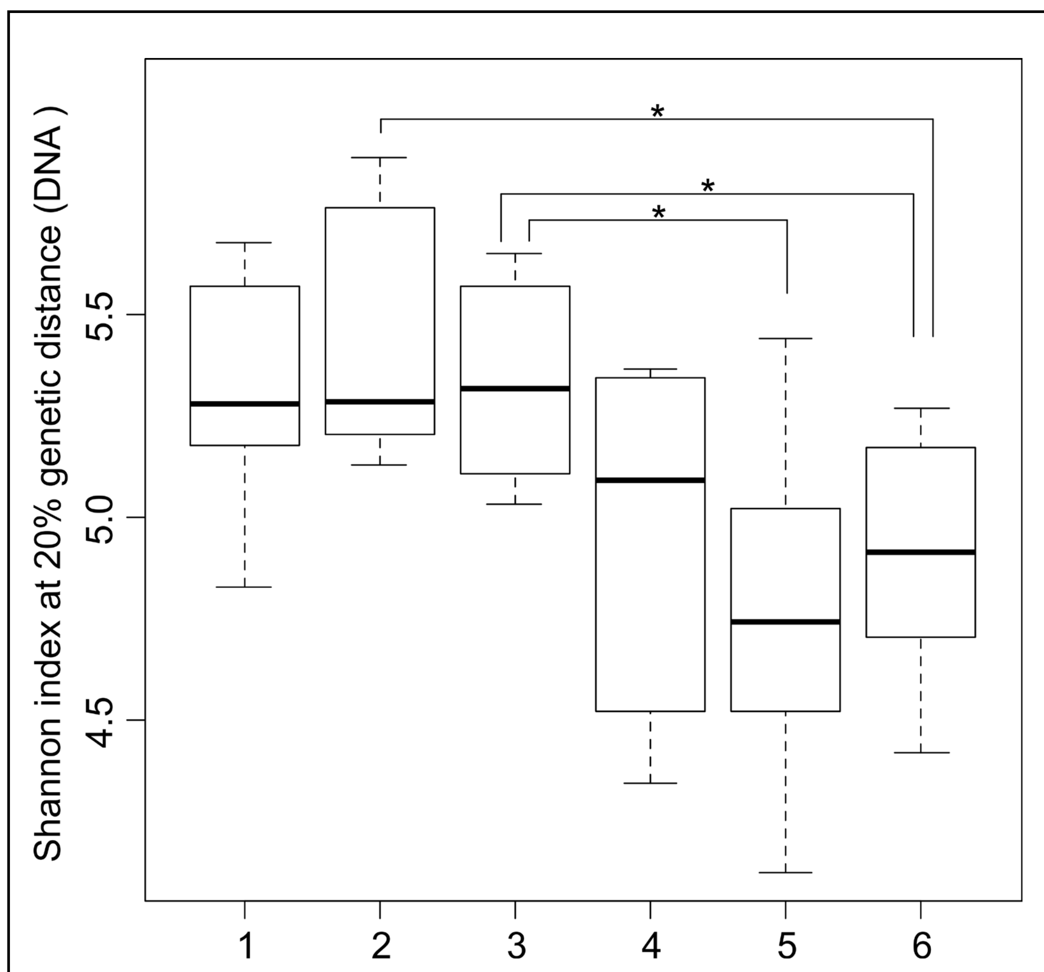

Figure 6: Boxplot depicting the Shannon index of diversity at 20% genetic distance of the total bacterial community over complete sampling period. 1; April 2010, 2; July 2010, 3; September 2010, 4; April 2011, 5; July 2011, and 6; September 2011. Asterisks indicating significant differences between sampling times in the total (DNA) bacterial community.

Significant differences in the community composition between the sampling years 2010 and 2011 were only detectable in the total bacterial community, but not in the active (Table S4). Analyzing all six sampling times, significant differences were observed in the active and total bacterial community composition. The phylum *Chloroflexi* showed significant differences at DNA and RNA level. While *Proteobacteria* and the class *Gammaproteobacteria* differs significantly at DNA level, *Actinobacteria*, *Firmicutes*, the class *Betaproteobacteria,* and the category “other *Proteobacteria*” differs significantly at RNA level.

Discussion

Active and total bacterial communities displayed different structure and diversity

The analysis of the active and the total bacterial community revealed a higher bacterial richness in the total community than in the active community. The number of OTUs at DNA level was higher than that at RNA level. This is consistent with a study of soil bacterial communities in a *P. abies* forest (Baldrian et al., 2012). The authors observed that the active bacterial community in forest soils was less evenly distributed and less diverse compared to the entire community. The higher number of generated OTUs at DNA level is that total bacterial community includes not only active cells but also dead cells, dormant cells, or extracellular DNA (Lennon and Jones, 2011).

Proteobacteria (mainly *Alphaproteobacteria*), *Firmicutes*, *Chloroflexi*, and *Acidobacteria* were abundant across all samples investigated. This is mostly in agreement with previous studies of bacterial communities in forest soils (Nacke et al., 2011; Baldrian et al., 2012; Sun et al., 2014). Nacke et al. (2011) analyzed the total bacterial community structure along different management types in forest and grassland soils and found that *Proteobacteria* dominated the German forest soils. The active bacterial community of forest soils was dominated by *Proteobacteria*, *Actinobacteria*, *Acidobacteria*, and *Planctomycetes* (Felsmann et al., 2015). Interestingly, we observed higher abundances of *Firmicutes* in soil samples compared to that mentioned in the other studies. This can be explained by the long history of the experimental site as grassland. It has been reported that *Firmicutes* exhibit higher abundance in grassland soils than in forest soils (Nacke et al., 2011; Kuramae et al., 2012; Khdhiri et al., 2015).

The majority of OTUs were observed in total and active community. This indicates that the most abundant OTUs in the total bacterial community contained active members. The most abundant OTU of the total and active bacterial community (13.4 and 17.3%) of all sequences belonged to the genus *Bacillus*, which is a member of the *Firmicutes*. *Bacilli* are spore-forming bacteria, which are common in soil. Moreover, they are well adapted to heat, UV radiation, and oxidizing agents (Popham et al., 1995). In the active community, the second most abundant OTU (11.7%) belonged to the family *Acetobacteraceae* (*Alphaproteobacteria*). Member of this family are able to oxidize ethanol to acidic acid. In

addition, these organisms can use a wide range of substrates as energy source such as glucose, ethanol, lactate, or glycerol. Nitrogen-fixing members of the *Acetobacteraceae* were first isolated from roots and stems of sugarcane in Brazil (Cavalcante and Dobereiner, 1988) and can grow at low pH values as described for the soil pH values in this study.

Environmental properties shape the bacterial communities

Spearman´s rank correlation was used to analyze the relationship between soil properties and the relative abundances of the most abundant phyla, proteobacterial classes, and orders. All phyla, proteobacterial classes, and most of the orders correlated significantly with pH and water content at DNA and RNA level in aspen deme Geismar8.

For example, the *Frankiale*s were negatively and positively correlated with pH at DNA and RNA level, respectively. Members of this order are very diverse with respect to their growth conditions (Lu and Zhang, 2012). Within the *Frankiales*, the family *Acidothermaceae* was more abundant at DNA level, whereas at RNA level *Acidothermaceae* and *Nakamurellaceae* were predominant. *Acidothermaceae* comprise fast-growing, thermophilic, acidophilic, and obligate aerobic organisms (Mohagheghi et al., 1986), whereas *Nakamurellaceae* include slow-growing, mesophilic, neutrophilic, and aerobic organisms (Yoshimi et al., 1996). In this study, we observed significantly more *Acidothermaceae* at total community level (mean abundance 1.2%) compared to active community level (mean abundance 0.6%) in the Geismar8 treatment.

Moreover, the order *Myxococcales* correlated significantly positively with pH in the active and total bacterial community in the aspen deme Geismar8. This order is described as very homogeneous group due to their high potential of secondary metabolite production (Gerth et al., 2003) and their ability of fruiting body-formation (Kaiser, 2008). Within the *Myxococcales*, the *Haliangiaceae* were the most abundant family. This is accordance with a study by Zhou et al. (2014) who observed a strong correlation with pH for *Myxobacteria*. Additionally, *Myxobacteria* were found in various soils in Russia (Chirak et al., 2012) which indicates that this group is more ubiquitous in soil than expected.

Interestingly, a correlation of the analyzed orders with the C/N content was not recorded.

Diversity, structure, and function of soil bacterial communities were influenced by aspen demes

A significant higher diversity in soil samples of aspen deme Geismar2 (G2) compared to aspen deme Geismar8 (G8) was recorded in the active but not in the total bacterial community. The same result was found for community composition as calculated ordination plots revealed no significant differences between the two aspen demes in the total bacterial community structure, but in the in the active bacterial community structure. Previous studies showed a strong effect of tree species on total bacterial community structure and diversity in forest soils (Nacke et al., 2011; Sun et al., 2014; Urbanova et al., 2015). However, most previous studies used only DNA as template. So far, the response of the active community on tree species is almost unknown.
Statistical analysis of the predicted functional profiles showed that several KEGG orthologs had significantly higher abundances in either aspen deme G2 or G8 at RNA but not at DNA level, indicating that the influence of different aspen demes is earlier detectable at metabolic active bacterial community.

Sampling time effect is earlier detectable in the active bacterial community

We found more sampling time effects in the active bacterial community composition compared to the total bacterial community, but the year effect was only visible at total bacterial community composition and their diversity. A study by Kuffner et al. (2012) observed in a warming experiment, established in a 130-years-old mountain forest, that seasonal community dynamics were subtle compared to the dynamics of soil respiration. Despite a pronounced respiration response to soil warming, they did not detect warming effects on community structure or composition. De Angelis et al. (2015) studied changes of soil bacterial community as response to soil warming in a long term forest ecological research site, where soil was warmed 5°C above ambient temperatures for 5, 8, and 20 years. They detected only a significant change on bacterial structure after 20 years of warming. Here, we could show, that sampling time is influencing the bacterial community and that this effect is earlier detectable in the active bacterial community. Also Maaløe and Kjeldgaard (1966) observed in shift experiments that ribosome synthesis was immediately affected by changing environmental conditions.

Until now, only few studies using large-scale sequencing of amplicons targeted the seasonal changes of total bacterial community composition in forest soils (Kuffner et al., 2012; Etto et al., 2014; Vasconcellos et al., 2014). Other studies using molecular fingerprint techniques (Prevost-Boure et al., 2011; Rasche et al., 2011) investigated the effect of seasonal changes on the total bacterial community, but not on the active bacterial community. Thus, it is difficult to compare the results by using different approaches. Consequently, it is possible that a stable microbial community existed, but biotic and abiotic factors might have an impact on shaping the bacterial community composition (Smit et al., 2001).

Conclusions

With the results of this study we want to get a comprehensive view about how aspen demes alter the bacterial community in soil. In this study, we evaluated a large bacterial 16S rRNA-based dataset from soil to understand the interaction between aspen demes and soil bacterial communities. Therefore, we analyzed both total and active bacterial community in soil of two aspen demes and performed functional predictions. In general, structure and diversity of the soil bacterial communities differed considerably between DNA-based and RNA-based assessed total and active bacterial communities, respectively. Because the composition of the active but not the total bacterial community was significantly different between the two aspen demes, the active bacterial community displayed a higher environmental sensitivity than the total community. Our results indicated that total and active bacterial communities are differently shaped by environmental factors. Furthermore, based on differential responses observed at DNA and RNA level, all studies targeting only the total bacterial community might overstate their results or oversee environmental impacts only visible at RNA level. Thus, further studies are needed to explain how the active bacterial community responds to biotic and abiotic parameters.

Author Contributions

SH, AP, and RD designed the experiment. SH performed the field and laboratory work. SH, BW, and FW analyzed the data and wrote the first draft of the manuscript. All authors contributed to interpretation of results and were involved in critical revision and approval of the final version. The authors declare no conflict of interest.

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References

Aßhauer, K.P., Wemheuer, B., Daniel, R., and Meinicke, P. (2015). Tax4Fun: predicting functional profiles from metagenomic 16S rRNA data. *Bioinformatics*. 31, 2882-2884.

Baldrian, P., Kolarik, M., Stursova, M., Kopecky, J., Valaskova, V., Vetrovsky, T., Zifcakova, L., Snajdr, J., Ridl, J., Vlcek, C., and Voriskova, J. (2012). Active and total microbial communities in forest soil are largely different and highly stratified during decomposition. *ISME J.* 6**,** 248-258.

Barnard, R.L., Osborne, C.A., and Firestone, M.K. (2015). Changing precipitation pattern alters soil microbial community response to wet-up under a Mediterranean-type climate. *ISME J.* 9**,** 946-957.

Baum, C., Eckhardt, K., Hahn, J., Weih, M., Dimitriou, I., and Leinweber, P. (2013). Impact of poplar on soil organic matter quality and microbial communities in arable soils. *Plant Soil Environ.* 59**,** 95-100.

Blazewicz, S.J., Barnard, R.L., Daly, R.A., and Firestone, M.K. (2013). Evaluating rRNA as an indicator of microbial activity in environmental communities: limitations and uses. *ISME J.* 7**,** 2061-2068.

Bradshaw, H., Ceulemans, R., Davis, J., and Stettler, R. (2000). Emerging model systems in plant biology: poplar (*Populus*) as a model forest tree. *Plant Growth Regul.* 19**,** 306-313.

Bragg, L., Stone, G., Imelfort, M., Hugenholtz, P., and Tyson, G. (2012). Fast, accurate error-correction of amplicon pyrosequences using Acacia. *Nature Methods.* 9**,** 425-426.

Caporaso, J., Kuczynski, J., Stombaugh, J., Bittinger, K., Bushman, F., Costello, E., Fierer, N., Pena, A., Goodrich, J., Gordon, J., Huttley, G., Kelley, S., Knights, D., Koenig, J., Ley, R., Lozupone, C., Mcdonald, D., Muegge, B., Pirrung, M., Reeder, J., Sevinsky, J., Tumbaugh, P., Walters, W., Widmann, J., Yatsunenko, T., Zaneveld, J., and Knight, R. (2010). QIIME allows analysis of high-throughput community sequencing data. *Nature Methods.* 7**,** 335-336.

Cavalcante, V.A., and Dobereiner, J. (1988). A new acid-tolerant nitrogen-fixing bacterium associated with sugarcane. *Plant Soil.* 108**,** 23-31.

Chirak, E.L., Pershina, E.V., Dol'nik, A.S., Kutovaya, O.V., Vasilenko, E.S., Kogut, B.M., Merzlyakova, Y.V., and Andronov, E.E. (2012). Taxonomic structure of microbial association in different soils investigated by high-throughput sequencing of 16S rRNA gene library. *Agricul. Biol.* 3**,** 100-109.

Deangelis, K.M., Pold, G., Topçuoğlu, B.D., Van Diepen, L.T.A., Varney, R.M., Blanchard, J.L., Melillo, J., and Frey, S.D. (2015). Long-term forest soil warming alters microbial communities in temperate forest soils. *Front. Microbiol.* 6. doi: 10.3389/fmicb.2015.00104

Dickmann, D.I., and Kuzovkina, J. (2008). "Poplars and willows in the world - 2. Poplars and willows of the world, with emphasis on silviculturally important species.". (Rome: FAO: Forestry Department, Working Paper IPC/9-2).

Edgar, R. (2010). Search and clustering orders of magnitude faster than BLAST. *Bioinformatics.* 26**,** 2460-2461.

El-Gendy, A.S., Svingos, S., Brice, D., Garretson, J.H., and Schnoor, J. (2009). Assessments of the efficacy of a long-term application of a phytoremediation system using hybrid poplar trees at former oil tank farm sites. *Water Environ. Res.* 81**,** 486-498.

Etto, R.M., Cruz, L.M., Da Conceição Jesus, E., Galvão, C.W., Galvão, F., De Souza, E.M., De Oliveira Pedrosa, F., and Reynaud Steffens, M.B. (2014). Seasonal changes in dominant bacterial taxa from acidic peatlands of the Atlantic Rain Forest. *Res. Microbiol.* 165**,** 517-525.

Fang, S., Liu, D., Tian, Y., Deng, S., and Shang, X. (2013). Tree species composition influences enzyme activities and microbial biomass in the rhizosphere: a rhizobox approach. *PloS One* 8**,** doi: 10.1371/journal.pone.0061461

Felsmann, K., Baudis, M., Gimbel, K., Kayler, Z.E., Ellerbrock, R., Bruehlheide, H., Bruckhoff, J., Welk, E., Puhlmann, H., and Weiler, M. (2015). Soil Bacterial Community Structure Responses to Precipitation Reduction and Forest Management in Forest Ecosystems across Germany. *PloS One* 10. doi: 10.1371/journal.pone.0122539

Fierer, N., Breitbart, M., Nulton, J., Salamon, P., Lozupone, C., Jones, R., Robeson, M., Edwards, R., Felts, B., Rayhawk, S., Knight, R., Rohwer, F., and Jackson, R. (2007). Metagenomic and small-subunit rRNA analyses reveal the genetic diversity of bacteria, archaea, fungi, and viruses in soil. *Appl. Environ. Microbiol.* 73**,** 7059-7066.

Gamalero, E., Cesaro, P., Cicatelli, A., Todeschini, V., Musso, C., Castiglione, S., Fabiani, A., and Lingua, G. (2012). Poplar clones of different sizes, grown on a heavy metal polluted site, are associated with microbial populations of varying composition. *Sci. Total Environ.* 425**,** 262-270.

Gerth, K., Pradella, S., Perlova, O., Beyer, S., and Müller, R. (2003). Myxobacteria: proficient producers of novel natural products with various biological activities - past and future biotechnological aspects with the focus on the genus *Sorangium*. *J. Biotech.*106**,** 233-253.

Gilmour, J.S., and Heslop-Harrison, J. (1955). The deme terminology and the units of micro-evolutionary change. *Genetica* 27**,** 147-161.

Gilmour, J.S.L., Gilmour, J.W., and Gregor (1939). Demes: A Suggested New Terminology. *Nature.* 144**,** 333-333.

Hajek, P., Hertel, D., and Leuschner, C. (2013). Intraspecific variation in root and leaf traits and leaf-root trait linkages in eight aspen demes (*Populus tremula* and *P. tremuloides*). *Front. Plant Sci.* 4. doi: 10.3389/fpls.2013.00415

Hajek, P., Leuschner, C., Hertel, D., Delzon, S., and Schuldt, B. (2014). Trade-offs between xylem hydraulic properties, wood anatomy and yield in *Populus*. *Tree Physiol.* 34**,** 744-756.

Hultén, E., and Fries, M. (1988). Atlas of North European Vascular Plant, North of the Tropic of Cancer by Hulten, E. & Fries, M. *Nord. J. Bot.* 8**,** 18-18.

Hur, M., Kim, Y., Song, H.R., Kim, J.M., Choi, Y.I., and Yi, H. (2011). Effect of genetically modified poplars on soil microbial communities during the phytoremediation of waste mine tailings. *Appl. Environ. Microbiol.* 77**,** 7611-7619.

Kaiser, D. (2008). *Myxococcus* - from Single-Cell Polarity to Complex Multicellular Patterns. *Annu. Rev. Genet.* 42**,** 109-130.

Keuter, A., Hoeft, I., Veldkamp, E., and Corre, M. (2013). Nitrogen response efficiency of a managed and phytodiverse temperate grassland. *Plant Soil.* 364**,** 193-206.

Khdhiri, M., Hesse, L., Popa, M.E., Quiza, L., Lalonde, I., Meredith, L.K., Röckmann, T., and Constant, P. (2015). Soil carbon content and relative abundance of high affinity H_2-oxidizing bacteria predict atmospheric H_2 soil uptake activity better than soil microbial community composition. *Soil Biol. Biochem.* 85**,** 1-9.

Kleemann, F., Von Fragstein, M., Vornam, B., Müller, A., Leuschner, C., Holzschuh, A., Tscharntke, T., Finkeldey, R., and Polle, A. (2011). Relating genetic variation of ecologically important tree traits to associated organisms in full-sib aspen families. *Eur. J. For. Res.* 130**,** 707-716.

Kuffner, M., Hai, B., Rattei, T., Melodelima, C., Schloter, M., Zechmeister-Boltenstern, S., Jandl, R., Schindlbacher, A., and Sessitsch, A. (2012). Effects of season and experimental warming on the bacterial community in a temperate mountain forest soil assessed by 16S rRNA gene pyrosequencing. *Fems Microbiol. Ecol.* 82**,** 551-562.

Kuramae, E., Yergeau, E., Wong, L., Pijl, A., Van Veen, J., and Kowalchuk, G. (2012). Soil characteristics more strongly influence soil bacterial communities than land-use type. *Fems Microbiol. Ecol.* 79**,** 12-24.

Lennon, J., and Jones, S. (2011). Microbial seed banks: the ecological and evolutionary implications of dormancy. *Nature Rev. Microbiol.* 9**,** 119-130.

Lu, Z., and Zhang, W. (2012). Comparative Phylogenies of Ribosomal Proteins and the 16S rRNA Gene at Higher Ranks of the Class Actinobacteria. *Curr. Microbiol.* 65**,** 1-6.

Maaløe, O., and Kjeldgaard, N.O. (1966). *Control of macromolecular synthesis; a study of DNA, RNA, and protein synthesis in bacteria.* New York: W. A. Benjamin.

Martin, M. (2011). Cutadapt removes adapter sequences from high-throughput sequencing reads. *EMBnet.journa.* 17, 10-12.

Mohagheghi, A., Grohmann, K., Himmel, M., Leighton, L., and Updegraff, D. (1986). Isolation and characterization of *Acidothermus cellulolyticus* gen. nov., sp. nov., a new genus of thermophilic, acisophilic, celluloytic bacteria. *Int. J. Syst. Bacteriol.* 36**,** 435-443.

Mukherjee, S., Sipila, T., Pulkkinen, P., and Yrjala, K. (2015). Secondary successional trajectories of structural and catabolic bacterial communities in oil-polluted soil planted with hybrid poplar. *Mol. Ecol.* 24**,** 628-642.

Müller, A., Horna, V., Kleemann, F., Vornam, B., and Leuschner, C. (2013). Physiological vs. morphological traits controlling the productivity of six aspen full-sib families. *Biomass Bioenerg.* 56**,** 274-283.

Nacke, H., Thurmer, A., Wollherr, A., Will, C., Hodac, L., Herold, N., Schoning, I., Schrumpf, M., and Daniel, R. (2011). Pyrosequencing-Based Assessment of Bacterial Community Structure Along Different Management Types in German Forest and Grassland Soils. *Plos One* 6. doi: 10.1371/journal.pone.0017000

Oksanen, J., Blanchet, F.G., Kindt, R., Legendre, P., Minchin, P.R., O'hara, R.B., Simpson, G.L., Solymos, P., Stevens, M.H.H., and Wagner, H. (2013). *VEGAN:* Community Ecology Package. R package version 2.0-10: Availible: URL http://cran.r-project.org, http://vegan.r-forge.r-project.org/.

Popham, D.L., Senguta, S., and Seltow, P. (1995). Heat, hydrogen-peroxide, and UV resistance of *Bacillis subtilis* spores with increased core water-content and with or without major DNA-binding proteins. *Appl. Environ. Microbiol.* 61**,** 3633-3638.

Prevost-Boure, N., Maron, P., Ranjard, L., Nowak, V., Dufrene, E., Damesin, C., Soudani, K., and Lata, J. (2011). Seasonal dynamics of the bacterial community in forest soils under different quantities of leaf litter. *Appl. Soil Ecol.* 47**,** 14-23.

Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P., Peplies, J., and Glockner, F. (2013). The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. *Nucleic Acids Res.* 41**,** D590-D596.

Rasche, F., Knapp, D., Kaiser, C., Koranda, M., Kitzler, B., Zechmeister-Boltenstern, S., Richter, A., and Sessitsch, A. (2011). Seasonality and resource availability control bacterial and archaeal communities in soils of a temperate beech forest. *ISME J.* 5**,** 389-402.

Scheibe, A., Steffens, C., Seven, J., Jacob, A., Hertel, D., Leuschner, C., and Gleixner, G. (2015). Effects of tree identity dominate over tree diversity on the soil microbial community structure. *Soil Biol. Biochem.* 81**,** 219-227.

Schmalenberger, A., Schwieger, F., and Tebbe, C. (2001). Effect of primers hybridizing to Different evolutionarily conserved regions of the small-subunit rRNA gene in PCR-based microbial community analyses and genetic profiling. *Appl. Environ. Microbiol.* 67**,** 3557-3563.

Schneider, D., Arp, G., Reimer, A., Reitner, J., and Daniel, R. (2013). Phylogenetic Analysis of a Microbialite-Forming Microbial Mat from a Hypersaline Lake of the Kiritimati Atoll, Central Pacific. *PLoS One.* 8, doi: 10.1371/journal.pone.0066662

Smit, E., Leeflang, P., Gommans, S., Van Den Broek, J., Van Mil, S., and Wernars, K. (2001). Diversity and seasonal fluctuations of the dominant members of the bacterial soil community in a wheat field as determined by cultivation and molecular methods. *Appl. Environ. Microbiol.* 67**,** 2284-2291.

Sun, H., Terhonen, E., Koskinen, K., Paulin, L., Kasanen, R., and Asiegbu, F.O. (2014). Bacterial diversity and community structure along different peat soils in boreal forest. *Appl. Soil Ecol.* 74**,** 37-45.

Taylor, G. (2002). Populus: arabidopsis for forestry. Do we need a model tree? *Ann. Bot.* 90**,** 681-689.

Team, R.D.C. (2011). "R: A Language and Environment for Statistical Computing". (Vienna, Austria: R Foundation for Statistical Computing).

Thurengia, G.M.O.P.A.T.S.O. (1910). Geological map of Prussia and the states of Thurengia 1:25.000/Topographic inventory of the royal Prussian general staff; Sievershausen. General Staff Prussia, Berlin.

Torsvik, V., Øvreås, L., and Thingstad, T.F. (2002). Prokaryotic diversity--magnitude, dynamics, and controlling factors. *Science.* 296**,** 1064-1066.

Tuskan, G., Difazio, S., Jansson, S., Bohlmann, J., Grigoriev, I., Hellsten, U, et al. (2006). The genome of black cottonwood, Populus trichocarpa (Torr. & Gray). *Science.* 313**,** 1596-1604.

Urbanova, M., Snajdr, J., and Baldrian, P. (2015). Composition of fungal and bacterial communities in forest litter and soil is largely determined by dominant trees. *Soil Biol. Biochem.* 84**,** 53-64.

Vasconcellos, R.F., Zucchi, T., Taketani, R., Andreote, F., and Cardoso, E.B.N. (2014). Bacterial community characterization in the soils of native and restored rainforest fragments. *Anton. van Leeuw. J. Microb.* 106, 947-957.

Wemheuer, B., Güllert, S., Billerbeck, S., Giebel, H.-A., Voget, S., Simon, M., and Daniel, R. (2013). Impact of a phytoplankton bloom on the diversity of the active bacterial community in the southern North Sea as revealed by metatranscriptomic approaches. *Fems Microbiol. Ecol.* 87**,** 378-389.

Wemheuer, B., Wemheuer, F., and Daniel, R. (2012). RNA-Based Assessment of Diversity and Composition of Active Archaeal Communities in the German Bight. *Archaea.* 2012**,** 1-8.

Whitman, W., Coleman, D., and Wiebe, W. (1998). Prokaryotes: The unseen majority. *Proc. Natil. Acad. Sci. U.S.A.* 95**,** 6578-6583.

Wietz, M., Wemheuer, B., Simon, H., Giebel, H.-A., Seibt, M.A., Daniel, R., Brinkhoff, T., and Simon, M. (2015). Bacterial community dynamics during polysaccharide degradation at contrasting sites in the Southern and Atlantic Oceans. *Environ. Microbiol.* doi: 10.1111/1462-2920.12842

Winder, R.S., Lamarche, J., Constabel, C.P., and Hamelin, R.C. (2013). The effects of high-tannin leaf litter from transgenic poplars on microbial communities in microcosm soils. *Front. Microbiol.* 4, 1-12.

Yoshimi, Y., Hiraishi, A., and Nakamura, K. (1996). Isolation and characterization of Microsphaera multipartita gen nov, sp nov, a polysaccharide-accumulating gram-positive bacterium from activated sludge. *Int. J. Syst.c Bacteriol.* 46**,** 519-525.

Zhang, C., Vornam, B., Volmer, K., Prinz, K., Kleemann, F., Kohler, L., Polle, A., and Finkeldey, R. (2015). Genetic diversity in aspen and its relation to arthropod abundance. *Front. Plant Sci.* 5, 1-14

Zhou, X., Li, S., Li, W., Jiang, D., Han, K., Wu, Z., and Li, Y. (2014). Myxobacterial community is a predominant and highly diverse bacterial group in soil niches. *Environ. Microbiol. Rep.* 6**,** 45-56.

3.2. Supplemental information

Table S1. Number of 16S rRNA gene sequences derived from the analyzed poplar deme Geismar 2 soil samples

Sample/plot	Season	Year	Aspen deme	Type	No. of sequences ≥ 200 bp
G2.1.apr10.D	April	2010	Geismar 2	DNA	46616
G2.2.apr10.D	April	2010	Geismar 2	DNA	28664
G2.3.apr10.D	April	2010	Geismar 2	DNA	35983
G2.1.jul10.D	July	2010	Geismar 2	DNA	36462
G2.2.jul10.D	July	2010	Geismar 2	DNA	44937
G2.3.jul10.D	July	2010	Geismar 2	DNA	37626
G2.1.sep10.D	September	2010	Geismar 2	DNA	35573
G2.2.sep10.D	September	2010	Geismar 2	DNA	30202
G2.3.sep10.D	September	2010	Geismar 2	DNA	45526
G2.1.apr11.D	April	2011	Geismar 2	DNA	21717
G2.2.apr11.D	April	2011	Geismar 2	DNA	16975
G2.3.apr11.D	April	2011	Geismar 2	DNA	28913
G2.1.jul11.D	July	2011	Geismar 2	DNA	18852
G2.2.jul11.D	July	2011	Geismar 2	DNA	12139
G2.3.jul11.D	July	2011	Geismar 2	DNA	29383
G2.1.sep11.D	September	2011	Geismar 2	DNA	27976
G2.2.sep11.D	September	2011	Geismar 2	DNA	20161
G2.3.sep11.D	September	2011	Geismar 2	DNA	26043
G2.1.apr10.R	April	2010	Geismar 2	RNA	43334
G2.2.apr10.R	April	2010	Geismar 2	RNA	32480
G2.3.apr10.R	April	2010	Geismar 2	RNA	26697
G2.1.jul10.R	July	2010	Geismar 2	RNA	20796
G2.2.jul10.R	July	2010	Geismar 2	RNA	18658
G2.3.jul10.R	July	2010	Geismar 2	RNA	27489
G2.1.sep10.R	September	2010	Geismar 2	RNA	24791
G2.2.sep10.R	September	2010	Geismar 2	RNA	26448
G2.3.sep10.R	September	2010	Geismar 2	RNA	41105
G2.1.apr11.R	April	2011	Geismar 2	RNA	32451
G2.2.apr11.R	April	2011	Geismar 2	RNA	31862
G2.3.apr11.R	April	2011	Geismar 2	RNA	27410
G2.1.jul11.R	July	2011	Geismar 2	RNA	54205
G2.2.jul11.R	July	2011	Geismar 2	RNA	33732
G2.3.jul11.R	July	2011	Geismar 2	RNA	24928
G2.1.sep11.R	September	2011	Geismar 2	RNA	25187
G2.2.sep11.R	September	2011	Geismar 2	RNA	30416
G2.3.sep11.R	September	2011	Geismar 2	RNA	34331

Table S1 continued. Number of 16S rRNA gene sequences derived from the analyzed poplar deme Geismar 2 soil samples

Sample/plot	Season	Year	Aspen deme	Type	No. of sequences ≥ 200 bp
G8.1.apr10.D	April	2010	Geismar 8	Type	40801
G8.2.apr10.D	April	2010	Geismar 8	DNA	30388
G8.3.apr10.D	April	2010	Geismar 8	DNA	41546
G8.1.jul10.D	July	2010	Geismar 8	DNA	43922
G8.2.jul10.D	July	2010	Geismar 8	DNA	36427
G8.3.jul10.D	July	2010	Geismar 8	DNA	68325
G8.1.sep10.D	September	2010	Geismar 8	DNA	29083
G8.2.sep10.D	September	2010	Geismar 8	DNA	23792
G8.3.sep10.D	September	2010	Geismar 8	DNA	56665
G8.1.apr11.D	April	2011	Geismar 8	DNA	23596
G8.2.apr11.D	April	2011	Geismar 8	DNA	37098
G8.3.apr11.D	April	2011	Geismar 8	DNA	24702
G8.1.jul11.D	July	2011	Geismar 8	DNA	23260
G8.2.jul11.D	July	2011	Geismar 8	DNA	24382
G8.3.jul11.D	July	2011	Geismar 8	DNA	25956
G8.1.sep11.D	September	2011	Geismar 8	DNA	26563
G8.2.sep11.D	September	2011	Geismar 8	DNA	30680
G8.3.sep11.D	September	2011	Geismar 8	DNA	24831
G8.1.apr10.R	April	2010	Geismar 8	DNA	56159
G8.2.apr10.R	April	2010	Geismar 8	RNA	46137
G8.3.apr10.R	April	2010	Geismar 8	RNA	36574
G8.1.jul10.R	July	2010	Geismar 8	RNA	45785
G8.2.jul10.R	July	2010	Geismar 8	RNA	44140
G8.3.jul10.R	July	2010	Geismar 8	RNA	37333
G8.1.sep10.R	September	2010	Geismar 8	RNA	38890
G8.2.sep10.R	September	2010	Geismar 8	RNA	34146
G8.3.sep10.R	September	2010	Geismar 8	RNA	20350
G8.1.apr11.R	April	2011	Geismar 8	RNA	48232
G8.2.apr11.R	April	2011	Geismar 8	RNA	26086
G8.3.apr11.R	April	2011	Geismar 8	RNA	35078
G8.1.jul11.R	July	2011	Geismar 8	RNA	30660
G8.2.jul11.R	July	2011	Geismar 8	RNA	25582
G8.3.jul11.R	July	2011	Geismar 8	RNA	47698
G8.1.sep11.R	September	2011	Geismar 8	RNA	31236
G8.2.sep11.R	September	2011	Geismar 8	RNA	25834
G8.3.sep11.R	September	2011	Geismar 8	RNA	28231

Table S2. Chao1, michaelis-menten-fit (MMF), observed OTUs, Shannon-, Simpson-indices, and the coverage of chao1 and MMF in % at 3% genetic distance calculated for poplar deme Geismar2

Sample	Obs. OTUs	MMF	coverage MMF %	Chao1	coverage Chao1 %	Shannon corr.	Simpson
G2.1.apr10.D	2,878	6,287	46	7,621	38	5.25	0.98
G2.2.apr10.D	3,188	6,525	49	7,937	40	5.569	0.99
G2.3.apr10.D	2,945	6,163	48	7,772	38	5.305	0.98
G2.1.jul10.D	2,941	6,408	46	7,491	39	5.312	0.98
G2.2.jul10.D	3,265	6,559	50	7,752	42	5.254	0.99
G2.3.jul10.D	2,774	5,955	47	7,405	37	5.884	0.98
G2.1.sep10.D	3,112	7,023	44	8,530	36	5.569	0.98
G2.2.sep10.D	3,309	7,231	46	8,570	39	5.646	0.98
G2.3.sep10.D	2,942	6,381	46	7,943	37	5.366	0.98
G2.1.apr11.D	3,267	7,571	43	8,842	37	5.366	0.97
G2.2.apr11.D	3,707	8,934	41	9,961	37	4.344	0.98
G2.3.apr11.D	3,498	8,056	43	9,573	37	4.521	0.97
G2.1.jul11.D	3,013	7,465	40	8,651	35	5.019	0.93
G2.2.jul11.D	3,864	8,982	43	10,694	36	4.549	0.99
G2.3.jul11.D	3,277	7,503	44	8,689	38	4.523	0.96
G2.1.sep11.D	2,773	6,323	44	7,581	37	5.267	0.95
G2.2.sep11.D	3,947	9,838	40	10,629	37	4.706	0.97
G2.3.sep11.D	3,323	7,808	43	9,239	36	4.927	0.97
G2.1.apr10.R	2,959	7,109	42	8,217	36	3.467	0.96
G2.2.apr10.R	2,898	6,259	46	7,250	40	3.398	0.97
G2.3.apr10.R	2,228	4,948	45	5,897	38	3.75	0.94
G2.1.jul10.R	2,840	6,344	45	7,277	39	3.628	0.97
G2.2.jul10.R	2,785	6,155	45	7,246	38	4.071	0.97
G2.3.jul10.R	2,537	5,810	44	7,049	36	3.99	0.96
G2.1.sep10.R	2,269	4,807	47	5,765	39	3.9	0.96
G2.2.sep10.R	2,803	6,470	43	7,496	37	3.681	0.96
G2.3.sep10.R	2,825	6,413	44	7,562	37	3.995	0.96
G2.1.apr11.R	2,380	5,635	42	6,648	36	3.902	0.93
G2.2.apr11.R	2,977	6,711	44	7,921	38	3.582	0.97
G2.3.apr11.R	3,043	7,127	43	7,958	38	3.418	0.96
G2.1.jul11.R	2,198	5,205	42	6,247	35	3.838	0.90
G2.2.jul11.R	2,804	6,122	46	7,371	38	3.328	0.98
G2.3.jul11.R	2,548	6,093	42	7,196	35	3.481	0.94
G2.1.sep11.R	2,580	5,941	43	7,139	36	3.811	0.95
G2.2.sep11.R	3,143	7,344	43	8,201	38	3.561	0.96
G2.3.sep11.R	2,756	6,163	45	7,166	38	3.336	0.95

Table S2 continued. Chao1, michaelis-menten-fit (MMF), observed OTUs, Shannon-, Simpson-indices, and the coverage of chao1 and MMF in % at 3% genetic distance calculated for poplar deme Geismar8 (G8)

Sample	Obs. OTUs	MMF	coverage MMF %	Chao1	coverage Chao1 %	Shannon corr.	Simpson
G8.1.apr10.D	2,857	6,119	47	7,311	39	6.09	0.98
G8.2.apr10.D	3,505	7,804	45	9,352	37	6.62	0.99
G8.3.apr10.D	2,777	5,718	49	6,883	40	6.07	0.98
G8.1.jul10.D	3,082	6,537	47	7,914	39	6.26	0.98
G8.2.jul10.D	2,816	6,022	47	7,528	37	6.15	0.99
G8.3.jul10.D	3,536	7,462	47	8,947	40	6.80	0.99
G8.1.sep10.D	3,737	8,499	44	9,815	38	6.72	0.99
G8.2.sep10.D	3,380	7,155	47	8,726	39	6.66	0.99
G8.3.sep10.D	2,949	6,215	47	7,478	39	6.20	0.99
G8.1.apr11.D	3,560	8,215	43	9,290	38	6.42	0.98
G8.2.apr11.D	2,808	6,594	43	7,912	35	5.55	0.94
G8.3.apr11.D	3,173	7,668	41	9,003	35	5.90	0.95
G8.1.jul11.D	3,464	8,199	42	9,693	36	6.21	0.96
G8.2.jul11.D	2,964	6,891	43	8,199	36	5.77	0.96
G8.3.jul11.D	3,246	8,001	41	9,104	36	5.83	0.95
G8.1.sep11.D	3,437	7,910	43	9,257	37	6.36	0.98
G8.2.sep11.D	3,096	7,113	44	8,482	36	5.90	0.95
G8.3.sep11.D	3,305	7,689	43	8,901	37	6.12	0.96
G8.1.apr10.R	2,395	5,446	44	6,769	35	5.10	0.93
G8.2.apr10.R	2,386	5,599	43	6,842	35	5.03	0.94
G8.3.apr10.R	2,576	5,868	44	6,885	37	5.39	0.96
G8.1.jul10.R	2,612	6,295	41	7,436	35	5.10	0.92
G8.2.jul10.R	2,710	6,379	42	7,682	35	5.48	0.96
G8.3.jul10.R	2,783	6,518	43	7,740	36	5.56	0.96
G8.1.sep10.R	2,794	6,580	42	7,999	35	5.48	0.94
G8.2.sep10.R	2,649	6,209	43	7,108	37	5.33	0.95
G8.3.sep10.R	2,950	7,017	42	8,419	35	5.85	0.98
G8.1.apr11.R	2,699	6,291	43	7,396	36	5.33	0.94
G8.2.apr11.R	2,537	5,936	43	6,987	36	5.16	0.95
G8.3.apr11.R	2,499	5,888	42	6,779	37	5.10	0.94
G8.1.jul11.R	2,597	6,199	42	7,456	35	5.20	0.94
G8.2.jul11.R	2,619	6,423	41	7,244	36	5.07	0.93
G8.3.jul11.R	2,514	5,879	43	6,948	36	5.12	0.94
G8.1.sep11.R	2,592	6,310	41	7,422	35	5.18	0.94
G8.2.sep11.R	2,603	6,359	41	7,431	35	4.92	0.91
G8.3.sep11.R	2,550	6,227	41	7,201	35	4.96	0.92

Table S3. Chao1, michaelis-menten-fit (MMF), observed OTUs, Shannon-, Simpson-indices, and the coverage of chao1 and MMF in % at 20% genetic distance calculated for poplar deme Geismar2 (G2)

Sample	Obs. OTUs	MMF	coverage MMF %	Chao1	coverage Chao1 %	Shannon corr.
G2.1.apr10.D	343.2	403.338	85	442.799	78	3.34
G2.2.apr10.D	388.1	455.005	85	492.688	79	3.93
G2.3.apr10.D	355.6	423.764	84	467.157	76	3.59
G2.1.jul10.D	342.4	401.409	85	462.037	74	3.56
G2.2.jul10.D	379.3	442.641	86	500.611	76	3.99
G2.3.jul10.D	335.6	398.085	84	438.867	76	3.61
G2.1.sep10.D	350.1	409.949	85	470.636	74	3.49
G2.2.sep10.D	370.9	440.854	84	503.955	74	3.65
G2.3.sep10.D	357.8	424.53	84	467.492	77	3.54
G2.1.apr11.D	336.6	387.532	87	443.886	76	3.43
G2.2.apr11.D	382.4	442.004	87	469.952	81	3.70
G2.3.apr11.D	385.5	450.396	86	519.319	74	3.62
G2.1.jul11.D	343.1	411.387	83	465.013	74	2.86
G2.2.jul11.D	377	437.164	86	466.267	81	3.77
G2.3.jul11.D	352.7	409.85	86	457.995	77	3.42
G2.1.sep11.D	303.9	356.452	85	393.488	77	3.06
G2.2.sep11.D	399	463.01	86	503.178	79	3.58
G2.3.sep11.D	358.7	422.503	85	461.705	78	3.40
G2.1.apr10.R	288.2	356.649	81	401.215	72	2.55
G2.2.apr10.R	323.7	386.813	84	440.235	74	3.00
G2.3.apr10.R	262.9	317.073	83	363.774	72	2.51
G2.1.jul10.R	314	367.585	85	386.164	81	3.09
G2.2.jul10.R	300.6	351.122	86	419.617	72	3.03
G2.3.jul10.R	289	341.977	85	396.938	73	2.73
G2.1.sep10.R	258.8	310.146	83	363.348	71	2.60
G2.2.sep10.R	307.3	359.195	86	394.04	78	2.93
G2.3.sep10.R	309	367.778	84	430.793	72	2.95
G2.1.apr11.R	285.3	347.401	82	373.95	76	2.40
G2.2.apr11.R	322	375.065	86	412.38	78	3.11
G2.3.apr11.R	320.6	376.013	85	419.065	77	2.98
G2.1.jul11.R	268.2	330.141	81	389.559	69	2.15
G2.2.jul11.R	293	336.794	87	384.761	76	3.22
G2.3.jul11.R	282	340.07	83	379.269	74	2.58
G2.1.sep11.R	282.8	341.395	83	372.172	76	2.65
G2.2.sep11.R	332.6	391.912	85	435.637	76	3.03
G2.3.sep11.R	279.3	331.817	84	365.548	76	2.65

Table S3 continued. Chao1, michaelis-menten-fit (MMF), observed OTUs, Shannon-, Simpson-indices, and the coverage of chao1 and MMF in % at 20% genetic distance calculated for poplar deme Geismar8 (G8)

Sample	Obs. OTUs	MMF	coverage MMF %	Chao1	coverage Chao1 %	Shannon corr.
G8.1.apr10.D	362.8	434.409	84	490.669	74	3.64
G8.2.apr10.D	389.3	454.717	86	484.435	80	3.86
G8.3.apr10.D	322.6	374.543	86	438.807	74	3.68
G8.1.jul10.D	375	444.569	84	503.693	74	3.68
G8.2.jul10.D	339	396.177	86	449.418	75	3.64
G8.3.jul10.D	388.7	450.829	86	518.158	75	4.08
G8.1.sep10.D	394.4	457.731	86	518.651	76	3.86
G8.2.sep10.D	372.8	430.987	86	485.19	77	3.91
G8.3.sep10.D	341.7	399.497	86	454.682	75	3.72
G8.1.apr11.D	387.3	448.29	86	490.603	79	3.72
G8.2.apr11.D	305.2	362.055	84	406.177	75	3.01
G8.3.apr11.D	329.4	388.922	85	423.48	78	3.13
G8.1.jul11.D	376.2	441.695	85	499.066	75	3.48
G8.2.jul11.D	332	391.748	85	423.264	78	3.15
G8.3.jul11.D	332.3	388.387	86	418.132	79	3.14
G8.1.sep11.D	373.9	439.097	85	475.302	79	3.65
G8.2.sep11.D	354.6	419.262	85	457.3	78	3.26
G8.3.sep11.D	363.8	425.598	85	473.76	77	3.42
G8.1.apr10.R	290.5	354.818	82	399.506	73	2.40
G8.2.apr10.R	271	336.734	80	366.998	74	2.36
G8.3.apr10.R	266.4	321.033	83	370.854	72	2.60
G8.1.jul10.R	284.6	335.366	85	376.212	76	2.51
G8.2.jul10.R	290	349.008	83	399.945	73	2.82
G8.3.jul10.R	281.9	335.294	84	373.459	75	2.77
G8.1.sep10.R	312.4	375.661	83	412.562	76	2.70
G8.2.sep10.R	272.3	329.577	83	361.23	75	2.55
G8.3.sep10.R	296.3	359.654	82	382.983	77	2.77
G8.1.apr11.R	316.7	379.687	83	429.127	74	2.70
G8.2.apr11.R	271.9	334.201	81	354.232	77	2.48
G8.3.apr11.R	258.1	309.932	83	337.191	77	2.37
G8.1.jul11.R	310.4	373.034	83	403.517	77	2.66
G8.2.jul11.R	266.6	327.763	81	351.614	76	2.31
G8.3.jul11.R	269.7	326.248	83	359.553	75	2.41
G8.1.sep11.R	295.5	355.796	83	409.363	72	2.64
G8.2.sep11.R	282.5	339.279	83	376.5	75	2.47
G8.3.sep11.R	269.2	321.31	84	364.812	74	2.31

Table S4: Welch two sample test of main phyla and proteobacterial classes with year, aspen deme, and nucleic acid type (DNA and RNA). The complete dataset (RNA+DNA), the total (DNA), and active bacterial community have been tested.

Phyla/ proteobacterial classes	**Year (2010 and 2011)**	**Aspen deme (G8 and G2)**	**Nucleic acid type (DNA and RNA)**
Complete dataset (DNA+RNA)	**P-value**		
Acidobacteria	0.567	0.8222	**$< 2.2e^{-16}$**
Actinobacteria	0.2584	0.5202	**$1.407e^{-07}$**
Chloroflexi	**0.02913**	0.2335	**$3.848e^{-09}$**
Firmicutes	**0.001183**	0.1478	**0.0001225**
Alphaproteobacteria	0.8678	0.7267	**$< 2.2e^{-16}$**
Betaproteobacteria	0.05733	0.4641	**$1.545e^{-10}$**
Deltaproteobacteria	0.7292	0.07488	**0.03607**
Gammaproteobacteria	**0.001933**	0.9081	**$1.223e^{-08}$**
Other *Proteobacteria*	0.1464	0.3277	**$3.165e^{-08}$**
Proteobacteria	**0.04635**	0.403	**$5.765e^{-10}$**
Total bacterial community (DNA)			
Acidobacteria	0.2496	0.3655	
Actinobacteria	**0.0272**	0.6912	
Chloroflexi	**0.01052**	0.8533	
Firmicutes	**$1.738e^{-08}$**	0.986	
Alphaproteobacteria	0.08278	0.2469	
Betaproteobacteria	**0.01641**	0.4415	
Deltaproteobacteria	0.8449	0.8992	
Gammaproteobacteria	**$1.388e^{-07}$**	0.7305	
Other *Proteobacteria*	0.2016	0.8127	
Proteobacteria	**$1.178e^{-08}$**	0.7238	
Active bacterial community (RNA)			
Acidobacteria	0.7926	0.3595	
Actinobacteria	0.5785	0.06854	
Chloroflexi	0.2258	**0.02293**	
Firmicutes	0.6965	**0.00407**	
Alphaproteobacteria	0.283	0.6611	
Betaproteobacteria	0.1582	0.3333	
Deltaproteobacteria	0.7705	**0.03957**	
Gammaproteobacteria	0.1729	0.5588	
Other *Proteobacteria*	0.1864	**0.009263**	
Proteobacteria	0.1763	0.2111	

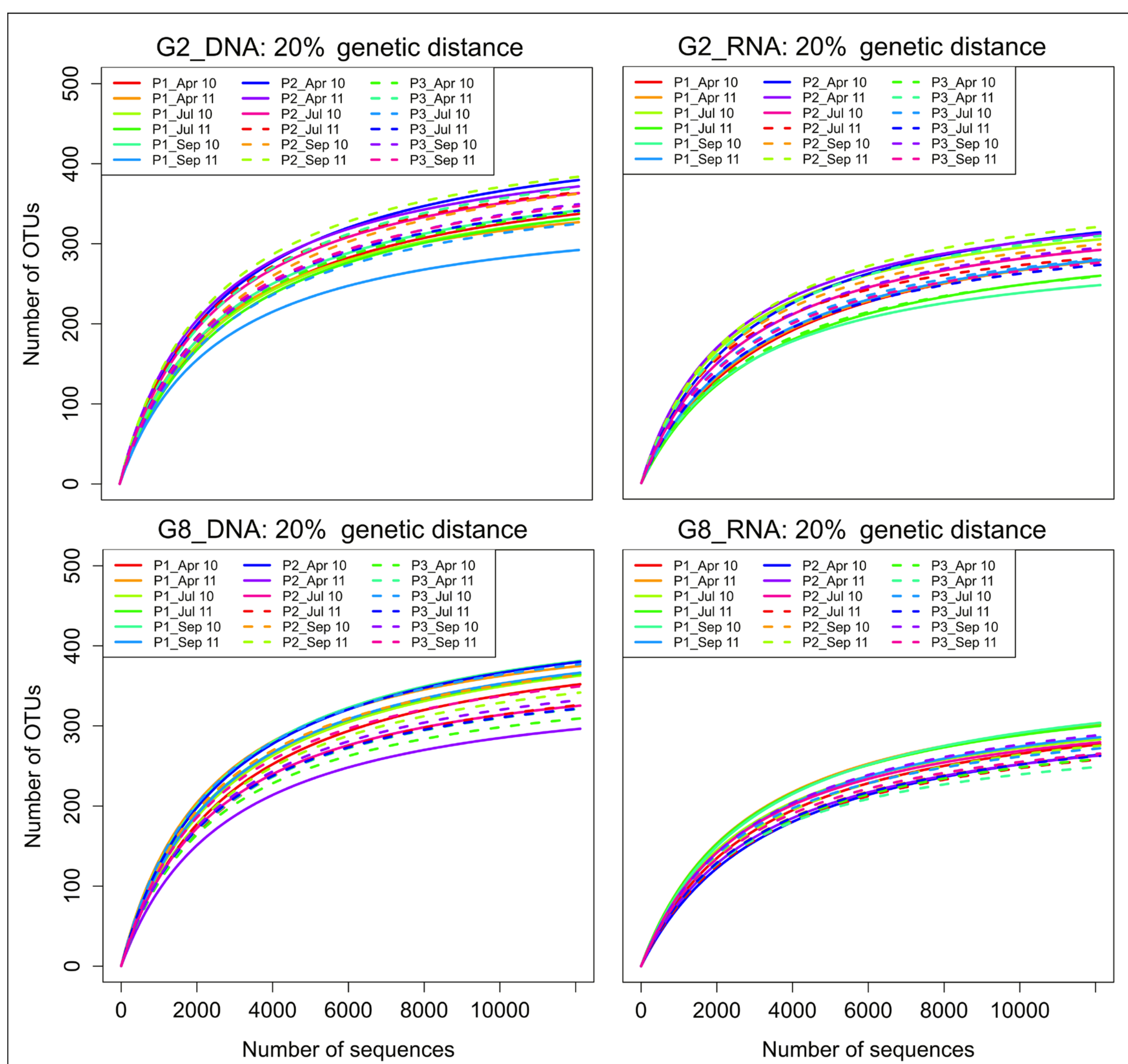

Figure S1: Rarefaction curves at 20% genetic distance calculated for poplar deme Geismar2 DNA (G2_DNA:20%), Geismar2 RNA (G2_RNA:20%), Geismar8 DNA (G8_DNA:20%), and Geismar8 RNA (G8_RNA:20%)

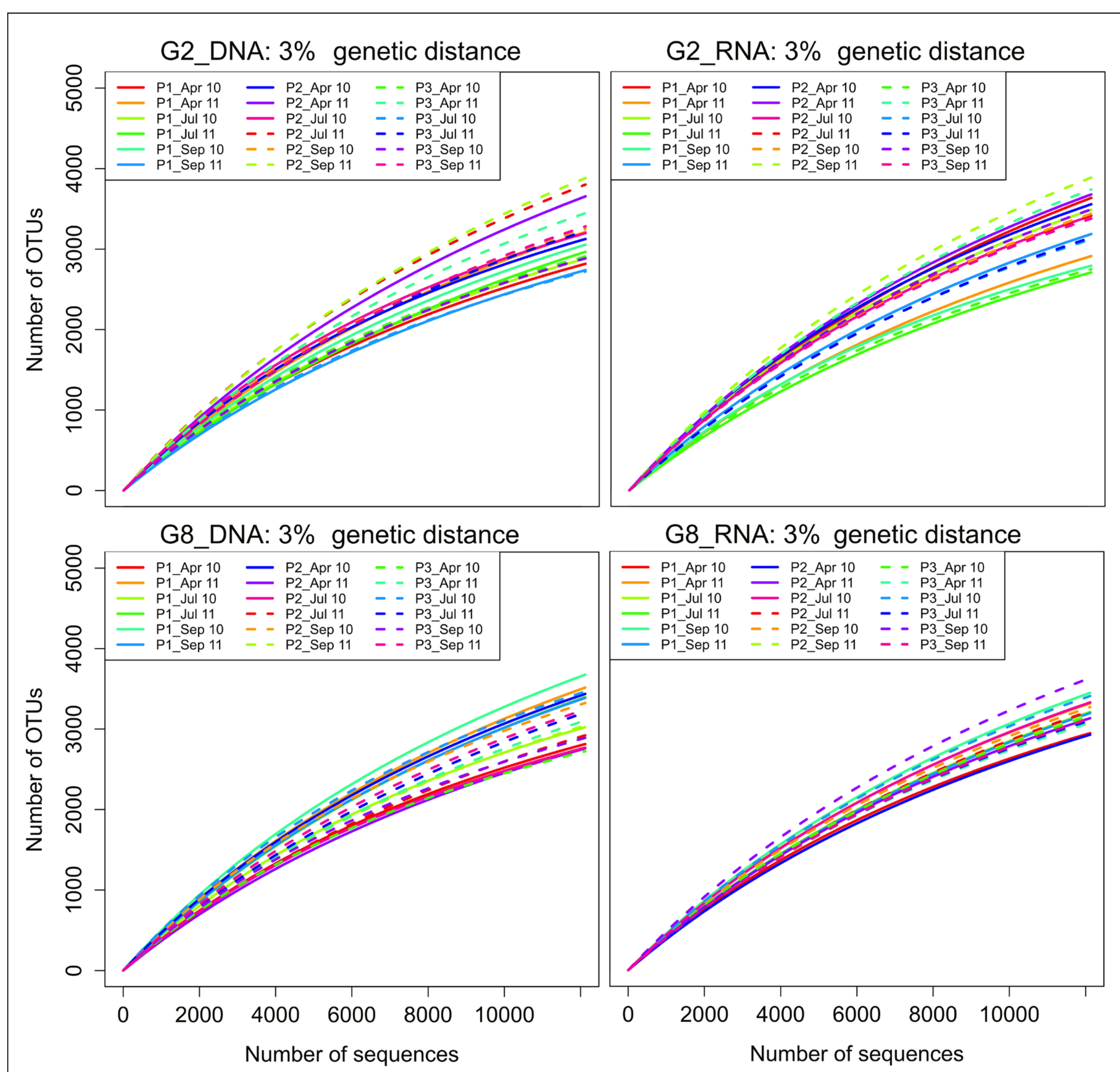

Figure S2: Rarefaction curves at 3% genetic distance calculated for poplar deme Geismar2 DNA (G2_DNA:20%), Geismar2 RNA (G2_RNA:20%), Geismar8 DNA (G8_DNA:20%), and Geismar8 RNA (G8_RNA:20%)

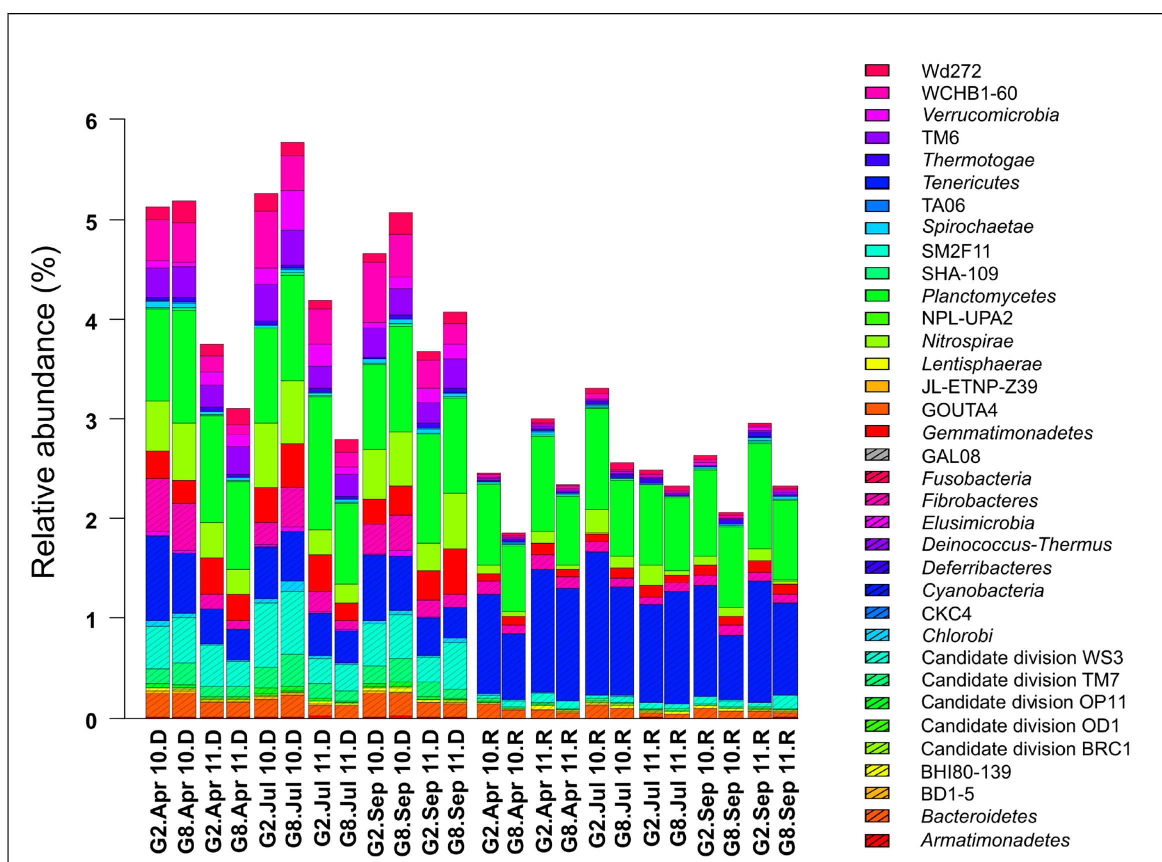

Figure S3: Relative abundances of the rare bacterial phyla (< 1% abundance) derived from the analyzed soil samples. Poplar deme Geismar2 (G2) and poplar deme Geismar8 (G8) samples are shown in this figure. Samples were taken in April (Apr), July (Jul), and September (Sep) in 2010 (10) and 2011 (11) and the total (D) and active (R) bacterial community were analyzed.

Chapter IV

4.1. Mixed effects of management regimes and herbivory on bacterial community composition in the rhizosphere of permanent grassland

Mixed effects of management regimes and herbivory on bacterial community composition in the rhizosphere of permanent grassland

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Performed the experiments: FW, DK

Analyzed data: FW, SH, BW

Wrote the publication: FW, RD, SV

Conceived and designed the experiments: FW, SV

Abstract

The plant rhizosphere is regarded as a dynamic environment in which several parameters influence the diversity, activity, and composition of bacterial communities. Despite their importance for soil and plant health, the response of these communities to different grassland management regimes and to above-ground herbivory is still poorly understood. This study aimed at assessing and exploiting the bacterial diversity in the plant rhizosphere with regard to sward composition, different fertilization and mowing regimes, as well as above-ground herbivory. For this purpose, a lysimeter experiment was conducted on a semi-natural, moderately species-rich grassland site. Following a two-week exposure to herbivory, soil samples were taken from the plant rhizosphere. Community structures were assessed by DGGE as well as large-scale pyrosequencing-based analysis of 16S rRNA gene sequences. More than 450,000 sequences were used to assess diversity and composition of bacterial communities. We recorded significant differences in bacterial diversity and richness with respect to the investigated parameters. Further analysis revealed that not only the parameters solely but also the combinations influenced the abundances of several bacterial taxa. Such combined effects led to either an enhanced, reduced, or, in rare cases, opposite bacterial response. These unique combinations of parameters studied and the high phylogenetic resolution provides exceptional insights into the diversity and ecology of bacterial communities in the plant rhizosphere. Moreover, the results of this study enable us to better validate the impact of different management regimes and herbivory on these communities and to predict potential ecological implications.

Introduction

The plant rhizosphere, defined as the soil layer surrounding the plant roots (Sørensen et al., 1997), is a complex and dynamic environment. Microbial communities colonizing these habitats play a major role for plant growth and health (Berg and Smalla, 2009; Compant et al., 2010) as well as for functioning of fundamental processes such as nutrient cycling (Berg and Smalla, 2009; Marschner et al., 2004) or denitrification processes (Pastorelli et al., 2011). Rhizospheric bacteria may form close mutualistic relationships with plants, which are important for the structure and dynamics of plant communities in almost all terrestrial ecosystems (van der Heijden et al., 2008a). Moreover, they may promote higher resistance to plant pathogens and parasites such as nematodes or insects (Kloepper et al., 1992; Lugtenberg and Kamilova, 2009; Ramamoorthy et al., 2001) and help plants to tolerate abiotic stress including salt, drought or nutrient deficiency (Dimkpa et al., 2009; Yang et al., 2009).

The development of culture-independent molecular approaches has significantly enhanced our understanding of bacterial communities in different environments such as rhizosphere bacteria in grassland soils (Nunan et al., 2005; Singh et al., 2007). One of the most frequently used techniques to explore bacterial communities in soil or rhizosphere is denaturing gradient gel electrophoresis (DGGE) (Costa et al., 2006; Duineveld et al., 2001; Nunan et al., 2005; Smalla et al., 2001; Yang and Crowley, 2000). Recently, high-throughput pyrosequencing of 16S rRNA gene fragments has been applied for in-depth analysis of these communities (Acosta-Martinez et al., 2008; Gottel et al., 2011; Nacke et al., 2011).

The diversity, activity, and structure of bacterial communities in the rhizosphere are shaped by several parameters. Soil type or plant species are regarded as the most dominant factors (Berg and Smalla, 2009; Duineveld et al., 2001; Garbeva et al., 2008; Gottel et al., 2011; Grayston et al., 1998; Kowalchuk et al., 2002). Additional important factors shaping bacterial communities in the rhizosphere are plant root exudates(Garbeva et al., 2008; Haichar et al., 2008), the soil pH (Marschner et al., 2004), and fertilizer application (Doi et al., 2011; Marschner et al., 2004). A few recent studies have also examined the influence of land use and management regime on rhizosphere bacterial communities (Costa et al., 2006; Garbeva et al., 2008).

In addition to the parameters mentioned above, below-ground herbivory also affects bacterial communities in the rhizosphere (Dematheis et al., 2012; Denton et al., 1999; Poll

et al., 2007; Treonis et al., 2005). For example, soil dwelling pests such as the western corn rootworm larvae (*Diabrotica virgifera virgifera*) or the leather jacket larvae (*Tipula paludosa*) have been shown to change the rhizosphere bacterial community composition by feeding on the roots (Dematheis et al., 2012; Treonis et al., 2005). The authors suggest that these changes are linked to shifts in root exudates patterns. However, studies investigating the influence of above-ground herbivory on bacterial communities in the rhizosphere are still missing.

In this study, we investigated the bacterial community composition in the rhizosphere with regard to sward composition (monocot-reduced, dicot-reduced, and species-rich as control), different grassland management regimes (with vs. without fertilization; mown once vs. thrice per year), and above-ground herbivory.

More specifically, we wanted to evaluate the impact of these four parameters on rhizospheric bacterial communities separately and in combination. Therefore, a lysimeter experiment was established on a semi-natural, moderately species-rich grassland site near Silberborn (Solling; Germany). Soil samples were collected from the lysimeters after two-weeks herbivory and further investigated employing different metagenomic approaches. To gain insights into the bacterial community composition, total DNA was extracted from the samples and subjected to 16S rRNA gene analyses. The community composition was either studied by DGGE analysis or pyrosequencing-based sequencing of 16S rRNA genes.

To our knowledge, this is the first study using two metagenomic approaches to analyze the impact of (1) sward composition, (2) fertilization, (3) different mowing frequencies, (4) above-ground herbivory on the bacterial community in the rhizosphere in one single field experiment on a permanent semi-natural grassland site.

Materials and methods

Study site

The full-factorial design of this study included two mowing frequencies (once per year in July vs. thrice per year in May, July, and September, respectively) and two fertilization treatments (no vs. NPK fertilization). The N fertilizer was applied as calcium ammonium nitrate N27 in two equal doses (180 kg N ha^{-1} yr^{-1}) in April and end of May. In addition, 30

kg P ha^{-1} yr^{-1} plus 105 kg K ha^{-1} yr^{-1} as Thomaskali® (8% P_2O_5, 15% K_2O, 20% CaO) were applied at the end of May. All plots were cut to a height of 7 cm with a Haldrup® harvester. The third factor established in this experiment was a manipulation of the sward composition (monocot-reduced, dicot-reduced, species-rich), established by selective herbicide applications to decrease either dicots (Starane® and Duplosan KV; active ingredients: Mecoprop-P® and Fluroxypyr/ Triclopyr; 3 l ha^{-1} each) or monocots (Select 240 EC® by Stähler Int., Stade, Germany; active ingredients: Clethodim; 0.5 l ha^{-1}). One third of the plots were maintained as species-rich controls. The application of herbicides took place on 31st of July 2008 resulting in significant changes in species richness and in functional group abundances(Petersen et al., 2012). Each treatment was replicated six times, resulting in 72 plots of 15 x 15 m size arranged in a Latin rectangle (for further details see Petersen *et al*. 2012).

Additionally, a lysimeter experiment was established with two lysimeters per plot in August and September 2010. The lysimeters consisted of a transparent plexiglass tube (diameter 14.4 cm, length 30 cm), which contained the original and intact soil core. The tubes were installed without damaging the vegetation and the soil core; they were slowly pushed downwards into the soil by applying hydraulic pressure. Drainage water was collected in a PE bottle that was placed underneath all lysimeters. One lysimeter per plot was used as herbivory lysimeter; the other one was left as control lysimeter. Four adult female grasshoppers
(*Chorthippus* spec.) and two Roman snails (*Helix pomatia* L.) per herbivory lysimeter and plot were applied. Cages for the herbivores were built of gauze of 1.5 mm mash size and were fixed on the top of the lysimeter. The experiments were started in August 2011, and were run for two weeks.

Sample collection, pH measurement, and DNA extraction

After two-weeks herbivory, soil samples were taken in autumn 2011. For this purpose, the lysimeter core was harvested, the above-ground vegetation was removed, and the top 5 cm of the soil core were homogenized. Coarse roots and stones (>5 mm) were subsequently removed. Soil samples were immediately cooled down (below 4°C), transported to the laboratory and kept frozen at -80°C until further use.

To measure the soil pH, 2 g of soil per lysimeter were mixed with 5 ml 1 M KCl. The pH was determined after 12 h incubation time (Supplemental Tab. S1). As soil pH can

influence the bacterial community structures in rhizosphere (Marschner et al., 2004) and measured pH values were inhomogeneous over the research area, we initially tested for correlation between pH and the four studied parameters (sward composition, fertilization, mowing frequency, and above-ground herbivory). No significant correlation was found.
Environmental DNA was extracted employing the MoBio PowerSoil DNA isolation kit (MoBio Laboratories, Carlsbad, CA, USA) according to the manufacturer's instructions. The lysimeter samples of at least three plots (DGGE 4, pyrosequencing 3 samples) per treatment were used for DNA extraction and further analysis. The samples were analyzed by DGGE as well as large-scale pyrosequencing-based analysis of 16S rRNA gene sequences.

Amplification of the 16S rRNA genes for DGGE analysis

PCR amplification targeting the V6-V8 region of the 16S rRNA gene was performed with the primers F968-GC (5'-AACGCGAAGAACCTTAC-3') and R1401 (5'-CGG TGTGTACAAGACCC-3') (Nübel et al., 1996, Zoetendal et al., 2002). In order to prevent complete denaturation of the fragment, a GC-rich sequence (5'-CGCCCGCCGCGCCCCG CGCCCGTCCCGCCGCCCCCGCCCG-3') was attached at the 5'-end of the primer F968-GC (Muyzer et al., 1993).
The PCR reaction mixture (25 µl) for amplification of the target gene contained 2.5 µl of 10-fold Mg-free Taq polymerase buffer (Fermentas, St. Leon-Rot, Germany), 200 µM of each of the four deoxynucleoside triphosphates, 1.75 mM $MgCl_2$, 0.4 µM of each primer, 5% DMSO, 1 U of Taq DNA polymerase (Fermentas), and approximately 25 ng of the DNA sample as template. Negative controls were performed by using the reaction mixture without template. Three independent PCR reactions were performed and obtained PCR products were pooled in equal amounts. The following thermal cycling scheme was used: initial denaturation at 94°C for 5 min, 11 cycles of: 1 min at 94°C, 1 min at 60°C (minus 1°C per cycle) and 2 min at 72°C, followed by 17 cycles of: 1 min at 94°C, 1 min at 53°C and 2 min at 72°C. The final extension was carried out at 72°C for 10 min. The resulting PCR products were checked for appropriate size by agarose gel electrophoresis.

Denaturing Gradient Gel Electrophoresis (DGGE)

The DGGE analyses of the bacterial communities were performed by using a PhorU2 apparatus (Ingeny, Goes, the Netherlands) with a double gradient. The first gradient ranged from 55 to 68% denaturant with an additional gradient of 6.2 to 9% acrylamide. This enhances the bands' sharpness and resolution (Cremonesi *et al.*, 1997). The denaturant (100%) contained 7M urea and 40% formamide. Approximately 100ng of the pooled PCR product were loaded on the gel. For each treatment, at least three independent DGGE were performed. The run was performed in Tris-acetate-EDTA buffer (40mM Tris, 20mM NaAcetate, 1mM Na_2EDTA, pH 7.4) at 60°C. After electrophoresis for 16h at 100V, the gels were stained for 45min with SYBRGold (Invitrogen, Carlsbad, USA). The stained DGGE gels were immediately photographed on a UV trans-illumination table.

DGGE data analysis and statistical testing

Analysis of DGGE profiles was performed using the software package GELCOMPAR II, version 5.1 (Applied Math, Ghent, Belgium). Cluster analyses (UPGMA) based on Pearson correlation were performed to evaluate the percentage of similarity shared among the samples from the different treatments.

Community analysis using pyrosequencing

To analyze the bacterial diversity, the V3-V5 region of the bacterial 16S rRNA was amplified by PCR. The PCR reaction (25µl) contained 5 µl of 5-fold Phusion GC buffer (Finnzymes, Vantaa, Finland), 200µM of each of the four desoxynucleoside triphosphates, 1.5mM $MgCl_2$, 4µM of each primer (see below), 2.5% DMSO, 1U of Phusion High Fidelity Hot Start DNA polymerase (Finnzymes), and approximately 25 ng of extracted DNA. The following thermal cycling scheme was used: initial denaturation at 98°C for 5 min, 25 cycles of denaturation at 98°C for 45s, annealing at 68°C for 45s, followed by extension at 72°C for 30s. The final extension was carried out at 72°C for 5min. Negative controls were performed by using the reaction mixture without template.
The V3-V5 region was amplified with the following set of primers according to Muyzer et al. (1995) containing the Roche 454-pyrosequencing adaptors, keys, and one unique MID per sample (underlined): V3for (341f) 5′- CCATCTCATCCCTGCGTGTCTCCGACT

CAG-$(dN)_{10}$-CCTACGGGAGGCAG CAG-3′ and V5rev (907r) 5′- CCTATCCCCTGT GTGCCTTGGCAGTCTCAG-CCGTCAATTCCTTTRAGTTT-3′. The resulting PCR products were checked for appropriate size and purified employing the peqGOLD Gel Extraction Kit (Peqlab) as recommended by the manufacturer.

Quantification of the PCR products was performed using the Quant-iTdsDNAHS assay kit and a Qubit fluorometer (Invitrogen) as recommended by the manufacturer. Three independent PCR reactions were performed per sample and the obtained PCR products were pooled in equal amounts. The Göttingen Genomics Laboratory determined the sequences of the 16S rRNA by using a Roche GS-FLX+ 454 pyrosequencer with Titanium chemistry (Roche, Mannheim, Germany).

Generated 16S rRNA datasets were processed and analyzed according to Wemheuer et al. (2014). In summary: after raw data extraction, pyrosequencing reads shorter than 250bp, with an average quality value below 25, or possessing long homopolymer stretches (> 8bp) were removed. Afterwards, the sequences were denoised. Chimeric sequences were subsequently removed using UCHIME (Edgar et al., 2011) and the most recent Greengenes CoreSet (DeSantis et al., 2006) as reference dataset. Processed sequences of all samples were joined, sorted by decreasing length, and clustered employing the UCLUST algorithm (Edgar, 2010) implemented in the QIIME software package.

Sequences were clustered in operational taxonomic units (OTUs) at 1%, 3%, and 20% genetic dissimilarity. Phylogenetic composition was determined using the QIIME assign_taxonmy.py script. A BLAST alignment against the Silva SSURef 111 NR database (Pruesse et al., 2007) was thereby performed. Sequences were classified with respect to the silva taxonomy of their best hit. Rarefaction curves, Shannon indices, ACE indices, and Chao1 indices were calculated employing QIIME. In addition, the maximal number of OTUs (n_{max}) was estimated for each sample using the Michaelis-Menten-fit alpha diversity metrics included in the QIIME software package. To compare bacterial community structures across all samples based on phylogenetic or count-based distance metrics, Principal Coordinate Analyses (PCoA) were generated using QIIME. A phylogenetic tree was calculated prior to PCoA generation. For this purpose, sequences were aligned using the PyNAST algorithm implemented in the QIIME software package. The phylogenetic tree and the respective OTU table were subsequently used to calculate PCoAs.

Statistical analyses

Statistical analyses were performed employing R (RDevelopmentCoreTeam, 2012; Version 2.15.0). To validate the impact of the different management regimes and herbivory on the measured soil pH as well as on the diversity indices, an Analysis of Variance (ANOVA) was performed. The effects of the different treatments on relative abundances of predominant bacterial groups were tested by Dirichelet regression in R using the DirichletReg package. Either the most abundant bacterial phyla and proteobacterial classes or the abundant (> 0.1%) OTUs at 3% genetic divergence were used for in this analysis.

Results

General analyses of the pyrosequencing-derived dataset

To fully assess the bacterial community structures, we applied amplicon-based pyrosequencing. A total of 468,538 high-quality bacterial 16S rRNA gene sequences with an average read length of 504bp were used for the community analyses. The number of sequences per sample ranged from 2,291 to 12,795. All sequences could be classified below phylum level. Rarefaction curves, richness, and alpha diversity indices were calculated at 1, 3, 20% genetic distance using 2,280 randomly selected sequences per sample. At 20% genetic divergence, most rarefaction curves reached saturation, indicating that the surveying effort covered almost the full extent of taxonomic diversity at this genetic distance (Supplemental Fig. S3C). The calculated coverage varied between 71.81 and 87.63% (Supplemental Tab. S2). At 3 and 1% genetic distance, the rarefaction curves were not saturated (Supplemental Fig. 3A and B). The calculated coverage was between 30.40 and 72.59% (3% genetic distance) and between 25.50 and 71.74% (1% genetic distance) (Supplemental Tab. S2). For all samples, the Shannon index of diversity (H') was determined (Supplemental Tab. S2). The Shannon index ranged from 2.65 to 3.51, from 4.94 to 6.1, and from 5.29 to 6.34 at a genetic distance of 20, 3, and 1%, respectively.

Characterization of bacterial community structure in the rhizosphere

Sequences were mainly affiliated to 7 phyla and 4 proteobacterial classes (Fig. 1 and 2, and Supplemental Tab. S3). The dominant phyla and proteobacterial classes across all samples were *Acidobacteria*, *Alphaproteobacteria*, *Actinobacteria*, *Betaproteobacteria*, *Bacteroidetes*, *Deltaproteobacteria*, *Gammaproteobacteria*, *Firmicutes*, *Gemmatimonadetes*, and *Chloroflexi*, representing 24.63, 21.77, 16.16, 7.27, 6.18, 5.59, 4.72, 3.59, 2.98, and 2.97%, respectively. These phylogenetic groups were present in all samples. The three dominant phyla *Proteobacteria*, *Acidobacteria*, and *Actinobacteria* represented approximately 84% of all classified sequences. Other bacterial phyla were less abundant (<1% of all classified sequences) (Fig. 2, Supplemental Tab. S4). The members of these rare phyla included, i.e., *Chlorobi*, *Nitrospirae*, *Fibrobacteres*, *Verrucomicrobia*, *Cyanobacteria*, *Spirochaetes*, *Planctomyces*, *Fusobacteria*, and *Deinococcus-Thermus*.

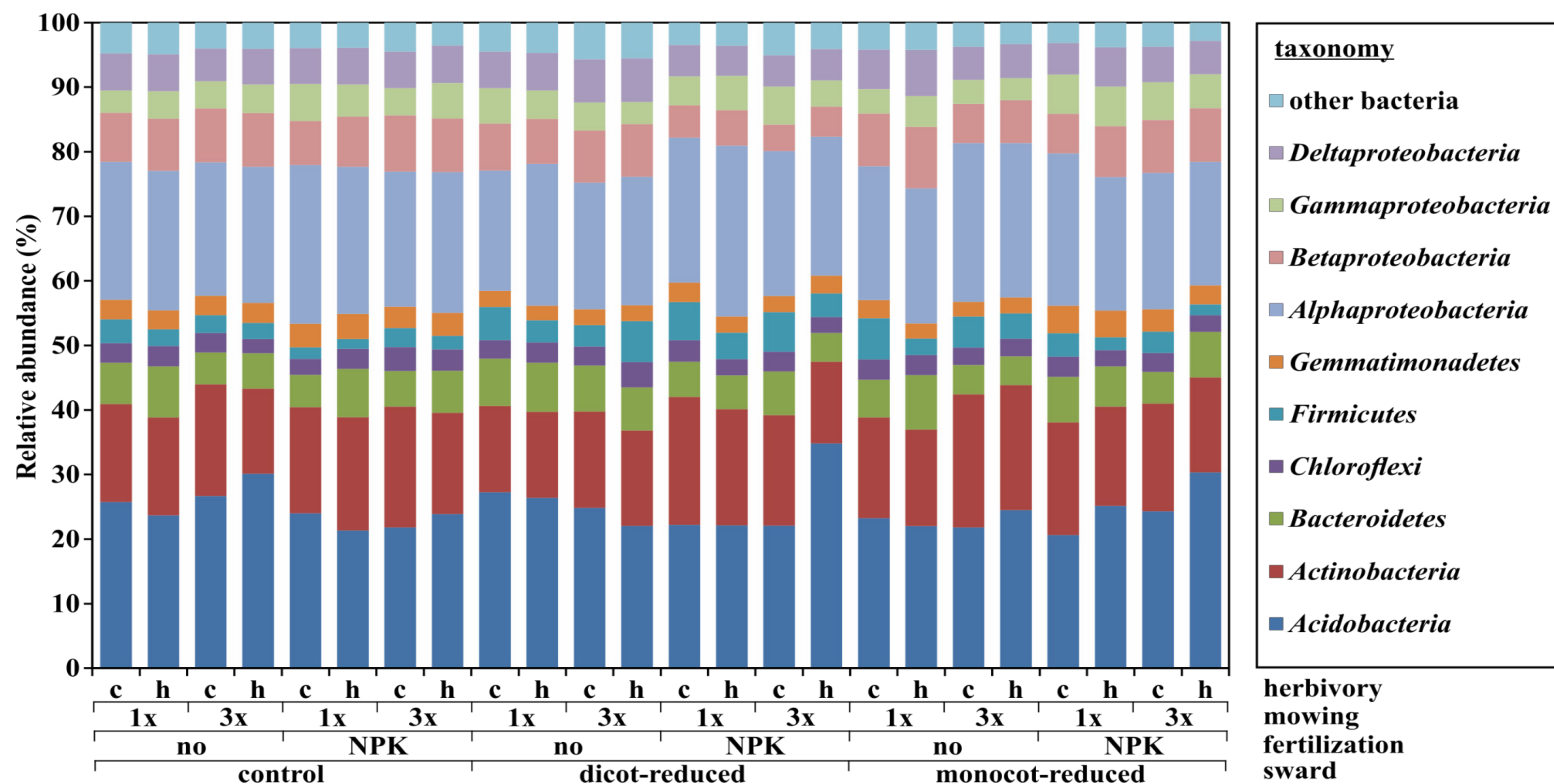

Fig. 1. Relative abundances of different predominant bacterial phyla and proteobacterial classes as revealed by pyrosequencing-based analysis of generated 16S rRNA gene amplicons. Abundances are mean values of the three replications per treatment. Only phyla and proteobacterial classes with more than 1% mean abundance are shown.

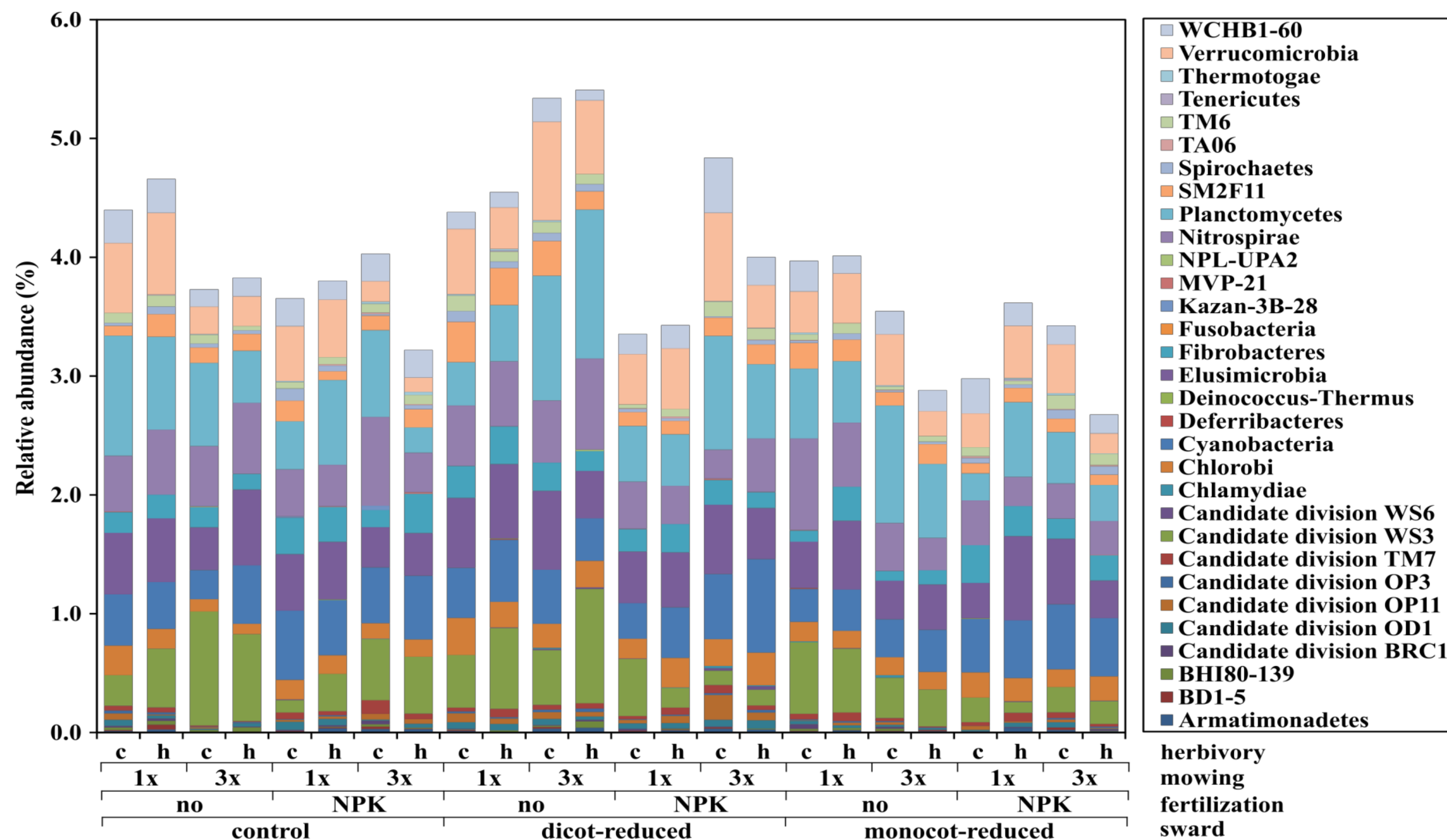

Fig. 2. Relative abundances of rare bacterial phyla as revealed by pyrosequencing-based analysis of generated 16S rRNA gene amplicons. Abundances are mean values of the three replications per treatment. Bacterial phyla with less than 1% mean abundance are shown.

In this study, 44,452 OTUs at 3% genetic divergence were detected in all samples. We identified 148 abundant bacterial OTUs at 3% genetic divergence (>0.1% of all classified sequences) (relative abundances of the 25 most abundant OTUs are shown in Supplemental Tab. S5).

Together, these OTUs contributed for approximately 54.78% of the total bacterial community. The most abundant phylotype at a genetic distance of 3% across all samples was a *Bradyrhizobium*, belonging to the order *Rhizobiales*, representing 4.8% of all sequences. The second and third most abundant phylotypes at the same genetic distance were an uncultured *Acidobacterium* (unknown order) and the bacterium Ellin6561 (order *Rhizobiales*), representing 1.95 or 1.90% of the sequences, respectively.

In addition, sequences were related to several uncultured bacteria of the *Bacillaceae* (unknown order), *Nitrosomonadaceae* (order *Nitrosomonadales*), *Rhodospirillaceae* (order *Rhodospirales*), as well as an uncultured *Acidobacteria* bacterium (order Incertae Sedis, and *Catellatospora* sp., belonging to the order *Micromonosporales*. The 25 most abundant phylotypes and their taxonomic affiliations are shown in Tab. 3.

Sward composition-dependent bacterial communities

To investigate the impact of sward composition on bacterial richness, rarefaction curves and alpha diversity indices were calculated with regard to the three sward types. The rarefaction analysis revealed a significant decrease in bacterial richness at 20, 3 and, 1% genetic distance in the herbicide-treated plots compared to the species-rich control plots (Fig. 3).

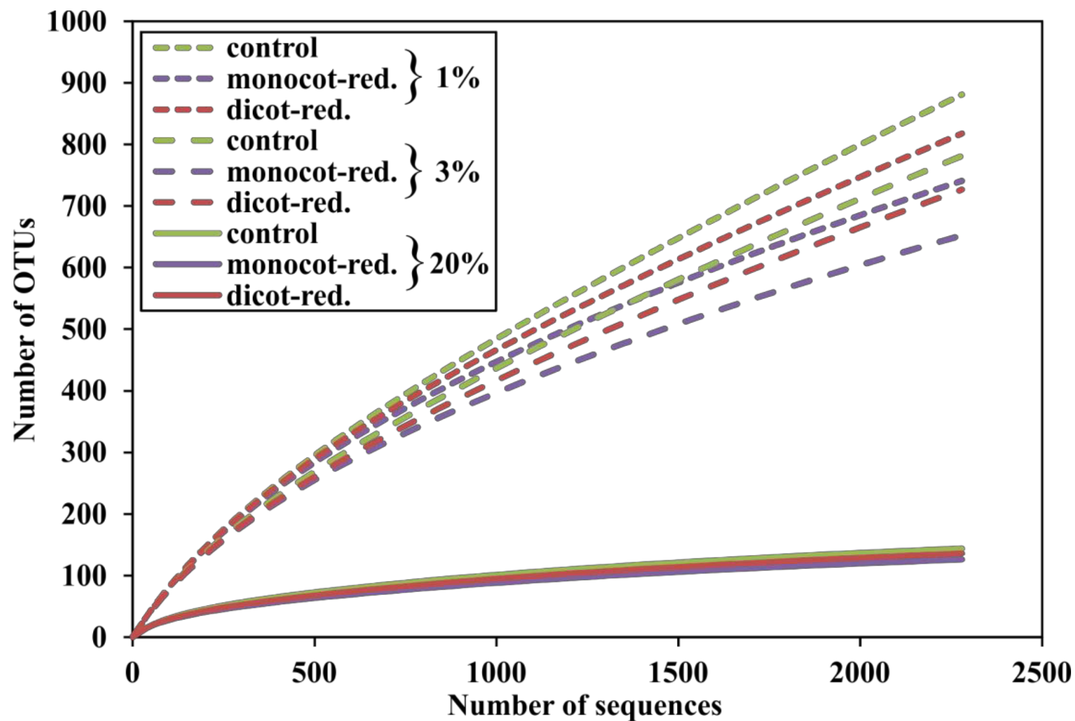

Fig. 3. Rarefaction curves at 1%, 3%, and 20% genetic distance with respect to sward diversity. Curves were calculated with QIIME (Caporaso *et al*., 2010).

The lowest richness was recorded on the dicot-reduced plots at all three genetic distance levels (Tab. 1). The observed number of OTUs varied between 143.8±9.8 (control plots), 126.5±17.8 (dicot-reduced plots) and between 135.4±8.2 (monocot-reduced plots). The lower diversity in herbicide-treated plots was supported by the calculated alpha diversity indices. The Shannon index showed higher values at control plots (3.28 ±0.15) compared to dicot-reduced plots (3.14 ±0.19) and monocot-reduced plots (3.18 ±0.14) at genetic distances of 20%. The same results were obtained for genetic distances at 3 and 1%. The observed number of OTUs as well as the diversity indices at all three genetic distance levels were significantly (p value < 0.05) reduced in herbicide-treated plots. As a consequence, the decrease of plant species diversity also led to a reduction of bacterial diversity in the rhizosphere.

Tab. 1: Impact of sward diversity, fertilization, different mowing frequencies, and above-ground herbivory on bacterial richness at 99%, 97%, and 80% genetic similarity. Alpha diversity indices were calculated with QIIME (Caporaso et al., 2010).

Sward type	***Observed number of OTUs***			***Maximal number of OTUs***			***ACE***			***Chao1***			***Shannon (H')***		
	80%	***97%***	***99%***	***80%***	***97%***	***99%***	***80%***	***97%***	***99%***	***80%***	***97%***	***99%***	***80%***	***97%***	***99%***
Control	143.8± 9.8	779.6± 97.2	883.4± 114.4	188.2 ± 12.2	1747.7 ± 515.3	2146.0 ± 761.7	223.8 ± 16.0	2985 ± 1042	3980 ± 2005	221.6 ± 19.6	3159 ± 806.2	5044 ± 2030	3.28 ± 0.15	5.77 ± 0.15	6.00 ± 0.15
Dicot-reduced	126.5± 17.8	650.9± 111.3	743.8± 133.3	164.7 ± 26.5	1179.0 ± 402.9	1404.6 ± 524.7	211.8 ± 10.8	2457 ± 897.1	2998 ± 1667	211.2 ± 13.9	2762 ± 830.2	4031 ± 2137	3.14 ± 0.19	5.63 ± 0.21	5.87 ± 0.22
Monocot-reduced	135.4± 8.2	726.7± 76.5	821.0± 90.9	177.7 ± 11.1	1493.7 ± 384.6	1765.2 ± 581.0	203.4 ± 31.2	1641 ± 795.4	1880 ± 1045	197.6 ± 29.8	1812 ± 852.8	2256 ± 1359	3.18 ± 0.14	5.70 ± 0.15	5.95 ± 0.15
Fertilization	***80%***	***97%***	***99%***	***80%***	***97%***	***99%***	***80%***	***97%***	***99%***	***80%***	***97%***	***99%***	***80%***	***97%***	***99%***
Control	135.6 ± 15.8	708.7 ± 108.1	799.6 ± 133.7	176.8 ± 22.9	1420.9 ± 443.8	1693.8 ± 624.1	214 ± 25	2243 ± 946	2740 ± 1546	211 ± 26	2491 ± 932	3522 ± 1948	3.23 ± 0.16	5.69 ± 0.15	5.91 ± 0.17
Fertilized	134.9 ± 13.1	729.5 ± 110.2	832.5 ± 119.0	176.9 ± 17.6	1525.9 ± 538.2	1850.0 ± 759.5	212 ± 20	2479 ± 1171	3165 ± 2063	209 ± 23	2665 ± 1064	4032 ± 2398	3.17 ± 0.17	5.71 ± 0.21	5.97 ± 0.19
Mowing frequency	***80%***	***97%***	***99%***	***80%***	***97%***	***99%***	***80%***	***97%***	***99%***	***80%***	***97%***	***99%***	***80%***	***97%***	***99%***
One	131.9 ± 13.6	697.0 ± 94.5	790.2 ± 119.3	172.0 ± 19.9	1364.1 ± 389.5	1626.7 ± 540.0	208 ± 21	2143 ± 905	2614 ± 1446	205 ± 21.8	2354 ± 889	3440 ± 1990	3.18 ± 0.15	5.69 ± 0.14	5.91 ± 0.17
Thrice	138.6 ± 14.6	741.2 ± 118.9	841.9 ± 130.4	181.7 ± 19.8	1582.8 ± 562.6	1917.1 ± 803.1	218 ± 23	2579 ± 1175	3291 ± 2102	216 ± 25.1	2802 ± 1061	4113 ± 2343	3.22 ± 0.19	5.71 ± 0.21	5.97 ± 0.20
Herbivory	***80%***	***97%***	***99%***	***80%***	***97%***	***99%***	***80%***	***97%***	***99%***	***80%***	***97%***	***99%***	***80%***	***97%***	***99%***
Control	135.3 ± 12.2	719.7 ± 93.9	814.9 ± 110.7	177.4 ± 17.6	1458.7 ± 417.5	1728.9 ± 567.9	215.1 ± 19.1	2331.8 ± 988.4	2842.4 ± 1586.4	212.4 ± 21.5	2557.9 ± 966.1	3733.9 ± 2194.8	3.20 ± 0.14	5.71 ± 0.14	5.95 ± 0.15
Herbivory	135.1 ± 16.4	718.5 ± 123.4	817.2 ± 142.5	176.3 ± 22.9	1488.2 ± 563.4	1815.0 ± 807.7	210.8 ± 25.8	2390.2 ± 1146.9	3062.6 ± 2048.0	207.8 ± 26.4	2598.0 ± 1040.3	3819.7 ± 2203.4	3.20 ± 0.20	5.69 ± 0.22	5.93 ± 0.21

The PCoA analysis revealed that species-rich control plots shared a more similar community structure followed by monocot-reduced plots. Dicot-reduced plots exhibited a more dissimilar community structure when compared to the other sward types (Fig. 4).

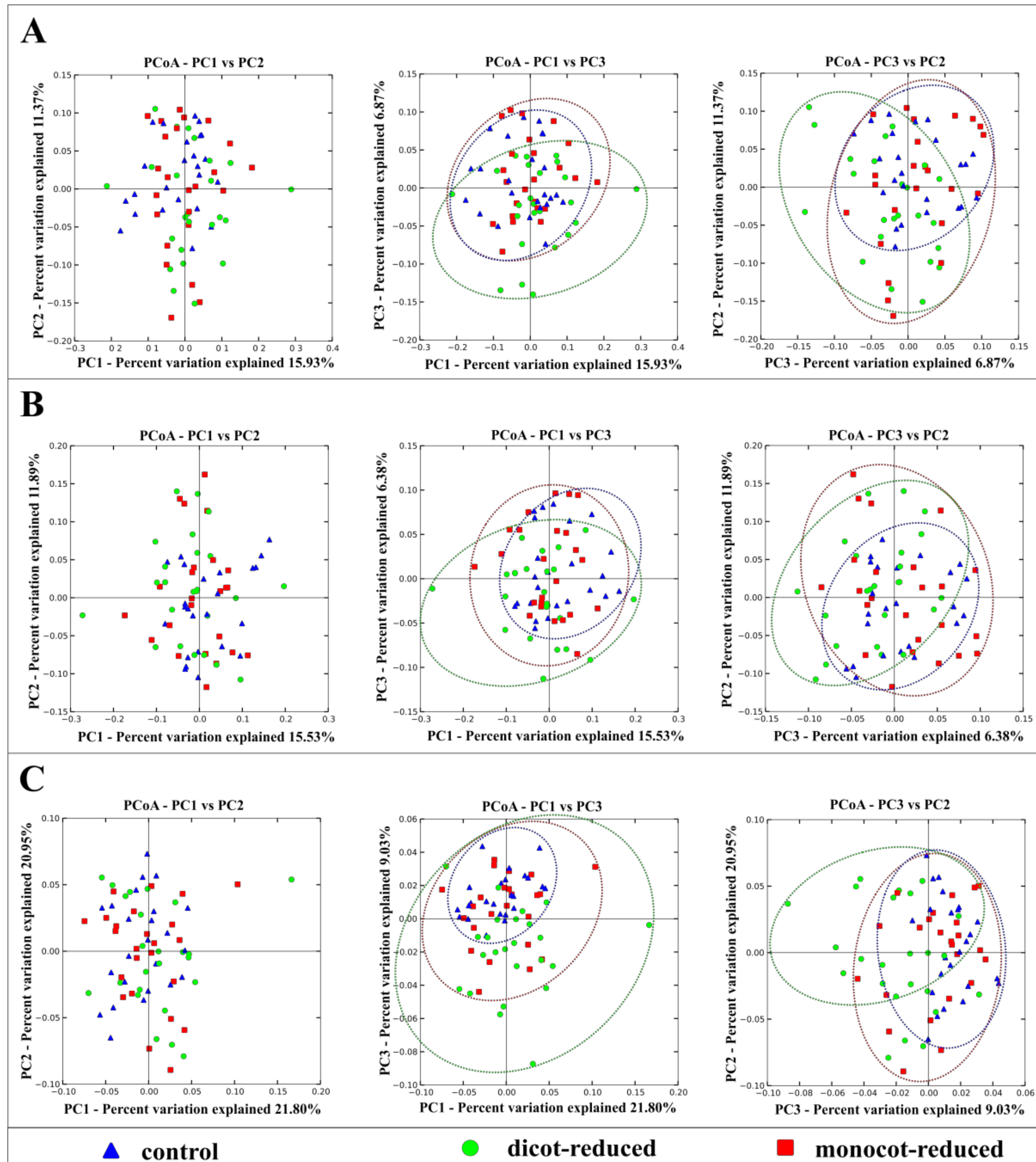

Fig. 4: Impact of sward composition on bacterial community structures in the rhizosphere at 99% (A), 97% (B), and 80% (C) genetic similarity. PCoA plots were calculated with QIIME (Caporaso *et al.*, 2010). Circles are drawn to highlight differences between the different sward types.

Bacterial community composition is affected by fertilization and different mowing frequencies

Bacterial community composition in regard to different management regimes was initially assessed by DGGE analysis of partial 16S rRNA gene sequences. DGGE of species-rich plots revealed complex patterns with approximately 20 bands for each treatment (Fig. 5). The same results were obtained for monocot-reduced plots (Supplemental Fig. S2), while DGGE of dicot-reduced plots revealed complex patterns with more than 30 bands (Supplemental Fig. S3).

Cluster analysis of DGGE was performed with regard to different grassland management regimes (with vs. without NPK fertilization; mowing once vs. thrice per year), and above-ground herbivory for the three different sward compositions. UPGMA dendrograms of bacterial communities in the plant rhizosphere showed that the different management regimes and herbivory influenced the composition of bacterial communities. For example, cluster analysis of the DGGE patterns of the rhizosphere bacterial community of species-rich plots revealed a strong impact of fertilizer application on community composition (Fig. 6A).

The effect of mowing frequency was influenced by the fertilization regime. Samples derived from unfertilized plots exhibited distinct cluster formation for the two mowing frequencies, indicating a more similar community composition in the once and thrice mown plots, respectively. However, some samples collected from the fertilized plots mown thrice as well as from the fertilized plots mown once grouped also in distinct clusters. In contrast to these findings, the above-ground herbivory did not strongly impact the bacterial community in the rhizosphere, although some samples exhibited distinct clusters. Similar results were observed for the bacterial community composition in the plant rhizosphere in samples collected from dicot-reduced (Fig. 6B) as well as from monocot-reduced plots (Fig. 6C).

To gain a more detailed picture about the changes of bacterial community in the rhizosphere in response to management regimes and above-ground herbivory, we analyzed the samples with next generation sequencing.

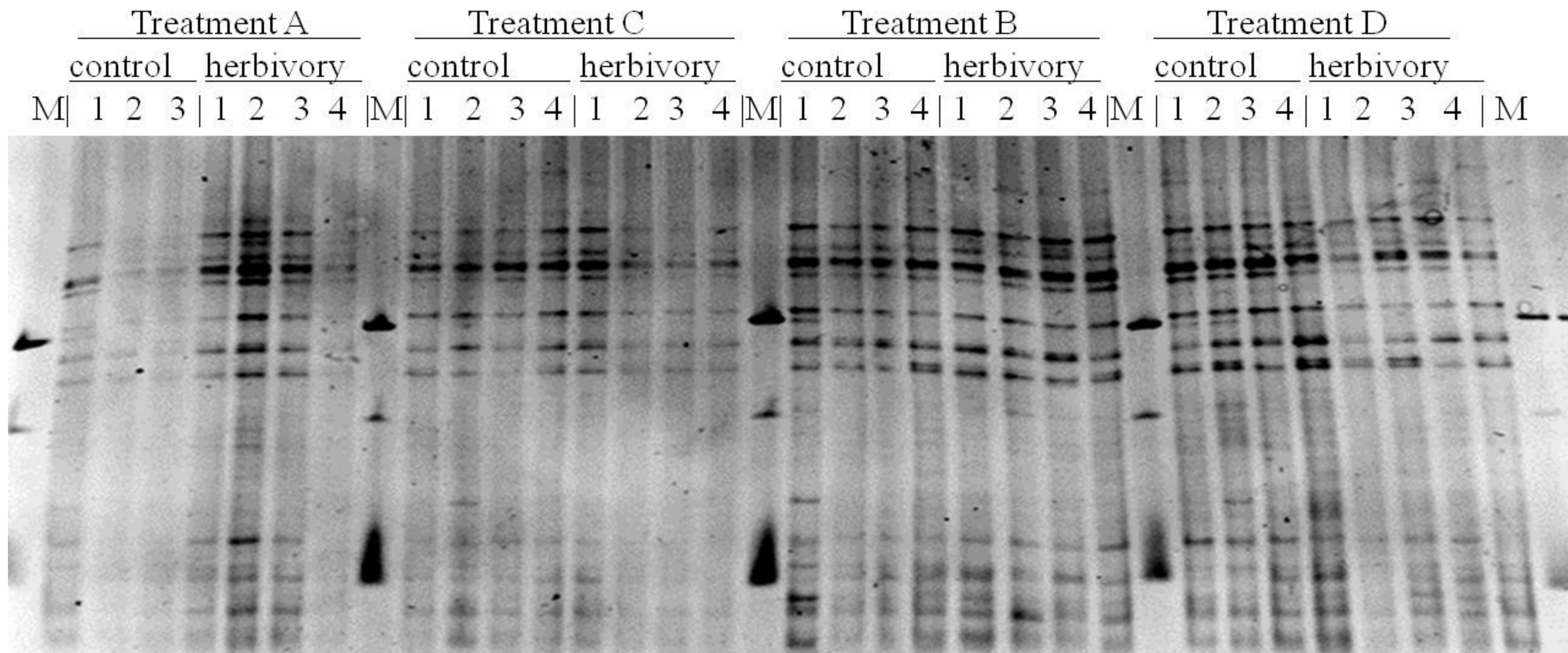

Fig. 5. DGGE profile of species-rich plots showing the influence of different fertilization and mowing regimes as well as above-ground herbivory on bacterial communities in the rhizosphere. Soil samples were taken in summer 2011. Independent replicates are indicated with numbers from 1 to 4. Treatment A: 1 x mowing/ year, no NPK; treatment B: 3 x mowing/ year, no NPK; treatment C: 1 x mowing/ year, NPK; treatment D: 3 x mowing/ year, NPK. M: GeneRuler 1 kb DNA Ladder (Fermentas, St. Leon-Rot, Germany).

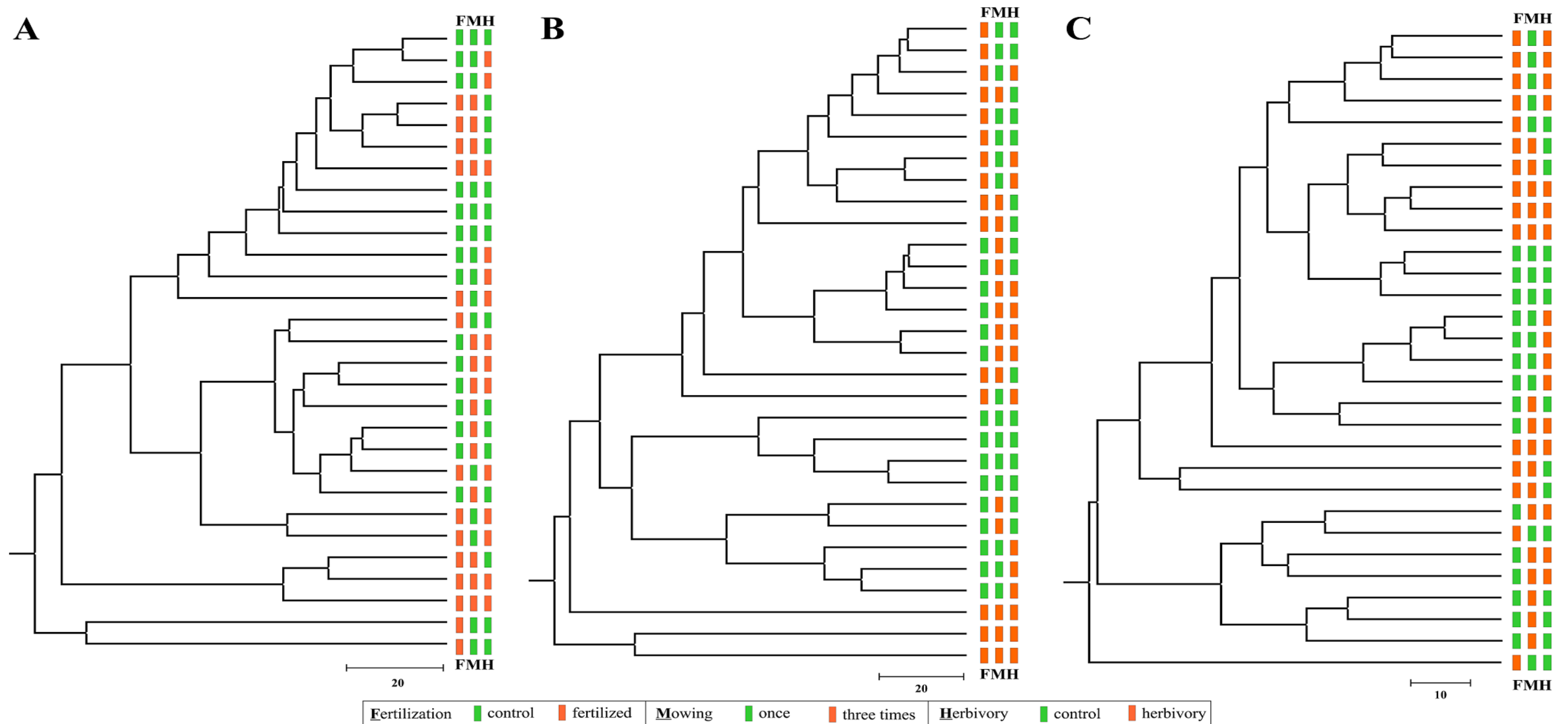

Fig. 6. UPGMA dendrogram generated by cluster analysis of DGGE fingerprints on the influence of different management regimes and above-ground herbivory on the bacterial community in the rhizosphere for (A) species-rich plots, (B) dicot-reduced plots, and (C) monocot-reduced plots. Soil samples were taken in summer 2011. The dendrogram was constructed using the Pearson correlation coefficient. The scale shows similarity values.

To investigate the impact of fertilization application and mowing frequencies on bacterial richness, rarefaction curves and alpha diversity indices were calculated with regard to these regimes. The rarefaction analysis revealed an increase in bacterial richness at 97% and 99% genetic similarity in the fertilized plots compared to the control plots (Fig. 7A). Despite the recorded change, this increase in richness was not supported by the calculated alpha diversity indices (Tab. 1). The observed number of OTUs as well as the diversity indices at all three genetic distance levels did not significantly (p value < 0.05) differ in the fertilized and unfertilized plots. As a consequence, the fertilizer application did not significantly affect the bacterial richness in the rhizosphere.

A comparison of rarefaction curves with regard to the two mowing frequencies revealed a higher bacterial richness at all three genetic distance levels in the plot mown three times compared to the plots mown only once (Fig. 7B). The observed number of OTUs at all three genetic distance levels were significantly (p value < 0.05) higher in the plots mown three times (138.6±14.6, 741.2±118.9, 841.9±130.4 compared to 131.9±13.6, 697.0±94.5, 790.2±119.3 in once mown plots at a genetic distance of 20, 3, and 1%, respectively). The same was recorded for the maximal number of OTUs. Thus, an increasing number of mowing events led to an increase of bacterial richness in the rhizosphere.

This higher richness was supported by the calculated alpha diversity indices (Tab. 1). ACE and Chao1 indices were significantly higher at 97% and 80% genetic similarity only. In contrast to this, no differences was recorded for the calculated Shannon indices (3.18±0.15, 5.69±0.14, 5.91±0.17 in thrice mown plots compared to 3.22±0.19, 5.71±0.21, 5.97±0.20 in once mown plots at a genetic distance of 20, 3, and 1%, respectively).

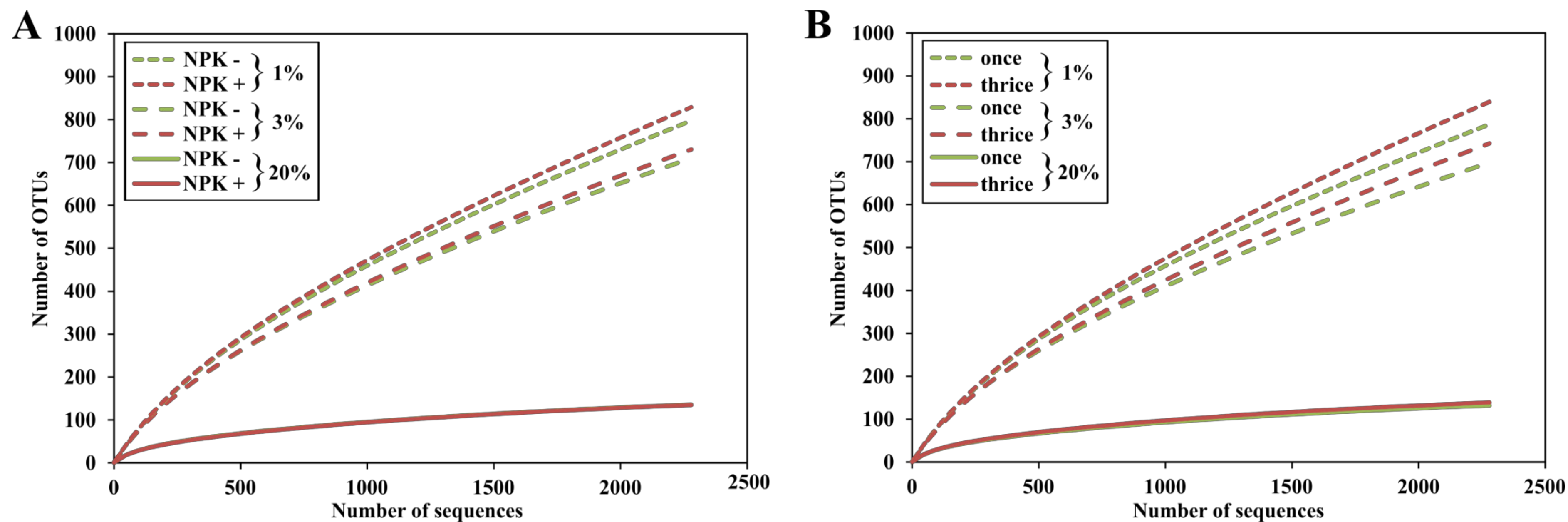

Fig. 7. Rarefaction curves at 1%, 3%, and 20% genetic distance with respect to fertilizer application (A) and mowing frequencies (B). Curves were calculated with QIIME (Caporaso *et al.*, 2010).

We further validated the impact of fertilization as well as mowing frequency on bacterial community structures by Principal Coordinate Analysis (PCoA).Whereas no difference in the generated PCoA plots was found at 80% genetic similarity, plots exhibited a clear separation between fertilized and control plots at 97% and 99% genetic similarity indicating a strong influence of fertilizer application on bacterial community structures (Fig. 8). As calculated plots did not show separation or cluster formation of differently treated plots, mowing frequencies (Fig. 9) did not impact bacterial community structure.

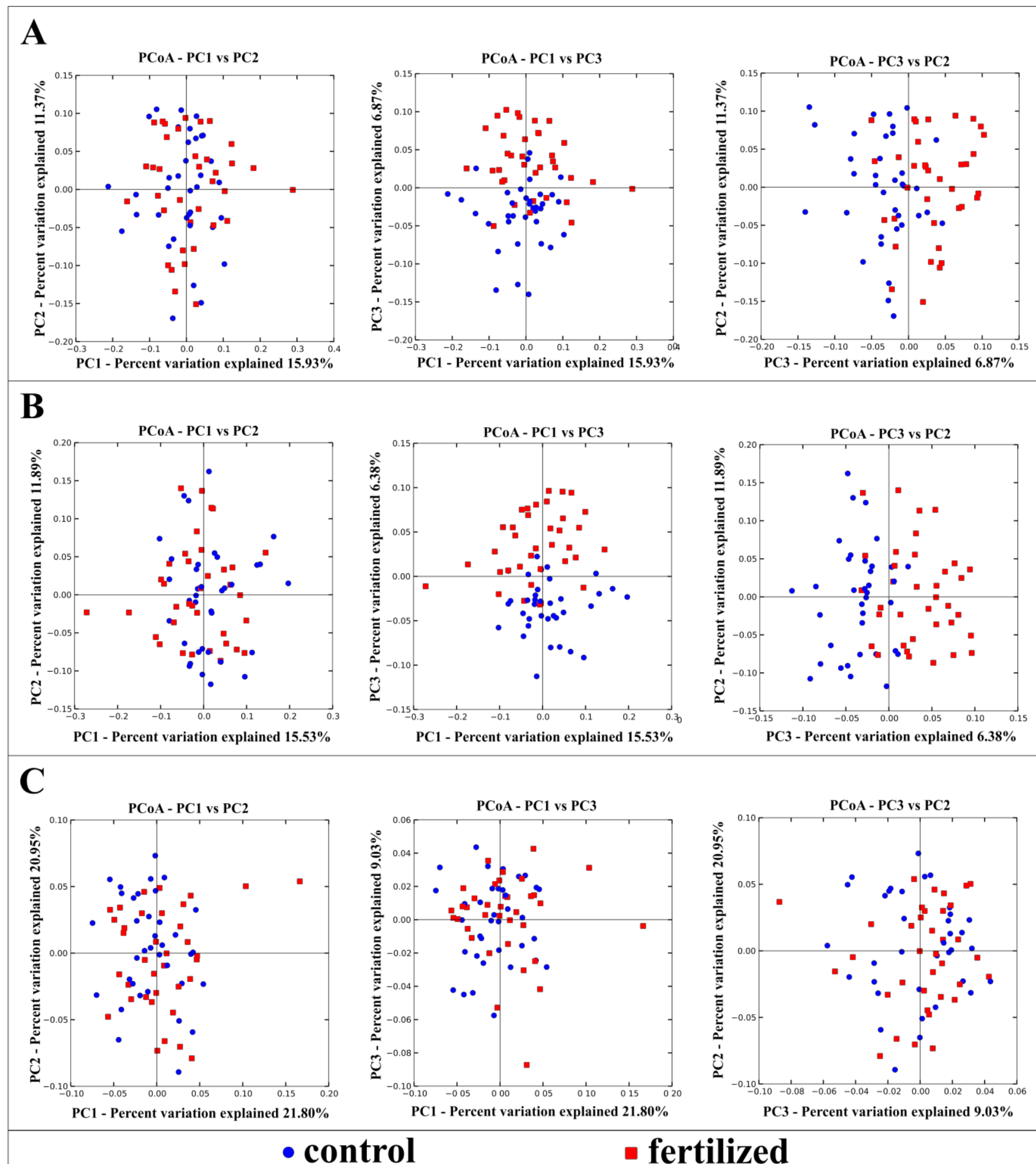

Fig. 8. Impact of fertilization on bacterial community structures at 99% (A), 97% (B), and 80% (C) genetic similarity. PCoA plots were calculated with QIIME (Caporaso et al., 2010).

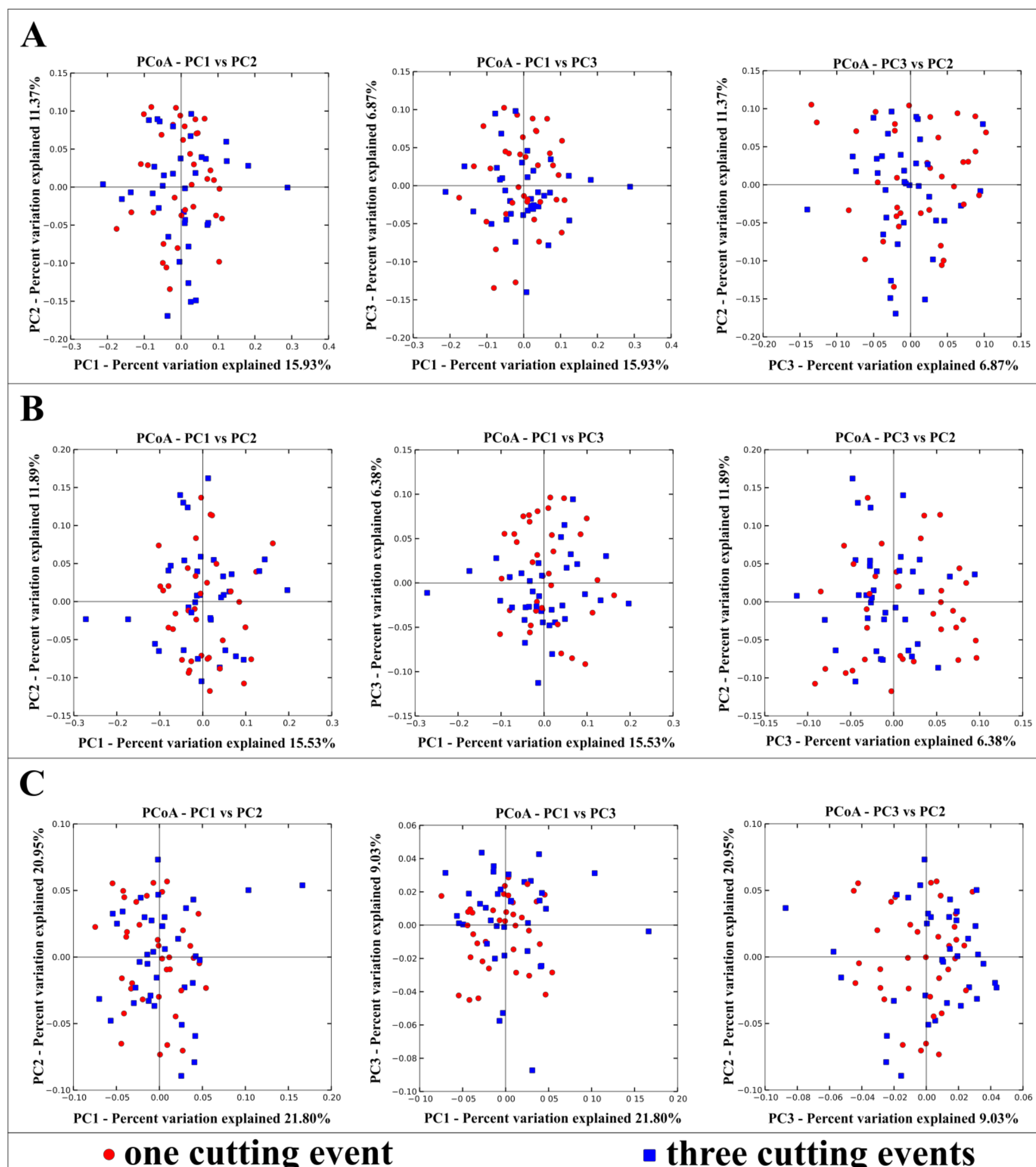

Fig. 9. Impact of mowing frequencies on bacterial community structures at 99% (A), 97% (B), and 80% (C) genetic similarity. PCoA plots were calculated with QIIME (Caporaso *et al.*, 2010).

Changes in bacterial community composition with regard to herbivory

A comparison of rarefaction curves and alpha diversity indices with regard to the herbivory treatments did not reveal any differences between control and herbivory plots (Fig. 10). The observed number of OTUs as well as the diversity indices at all three genetic distance levels did not significantly (p value < 0.05) differ in the control and herbivory plots (Tab. 1).

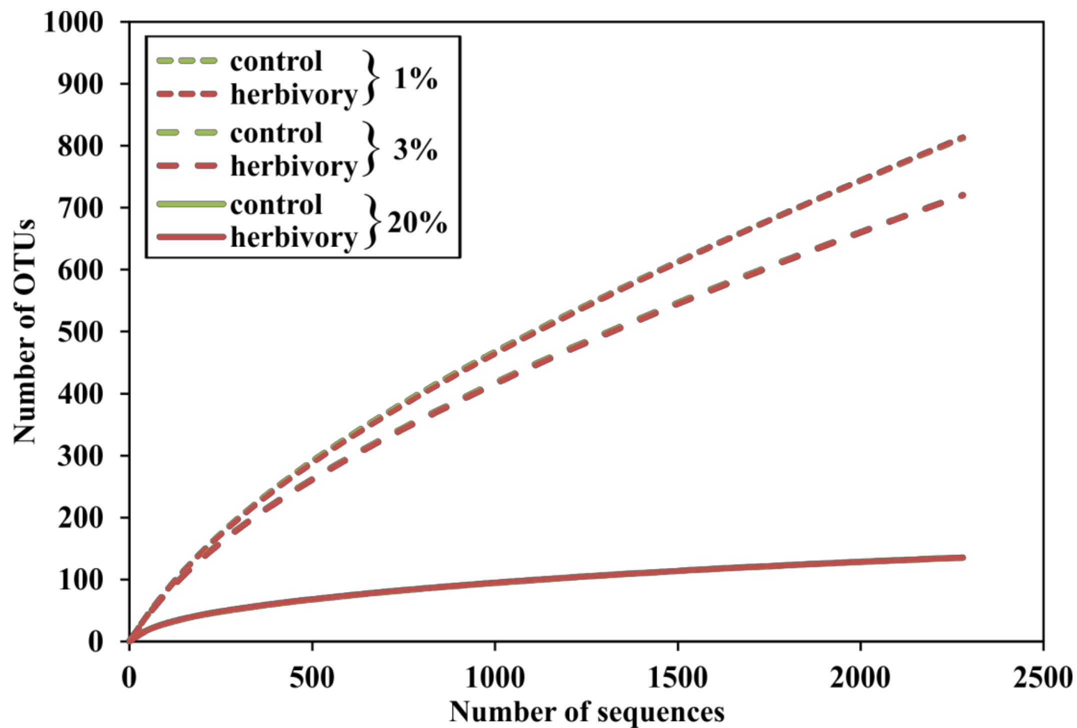

Fig. 10. Rarefaction curves at 1%, 3%, and 20% genetic distance with respect to herbivory. Curves were calculated with QIIME (Caporaso *et al.*, 2010).

We further validated the impact of the above-ground herbivory on bacterial community structures by Principal Coordinate Analysis (PCoA) (Fig. 11). No differences in the generated PCoA plots were found at the three genetic distances. Thus, herbivory had no impact on the bacterial community structure.

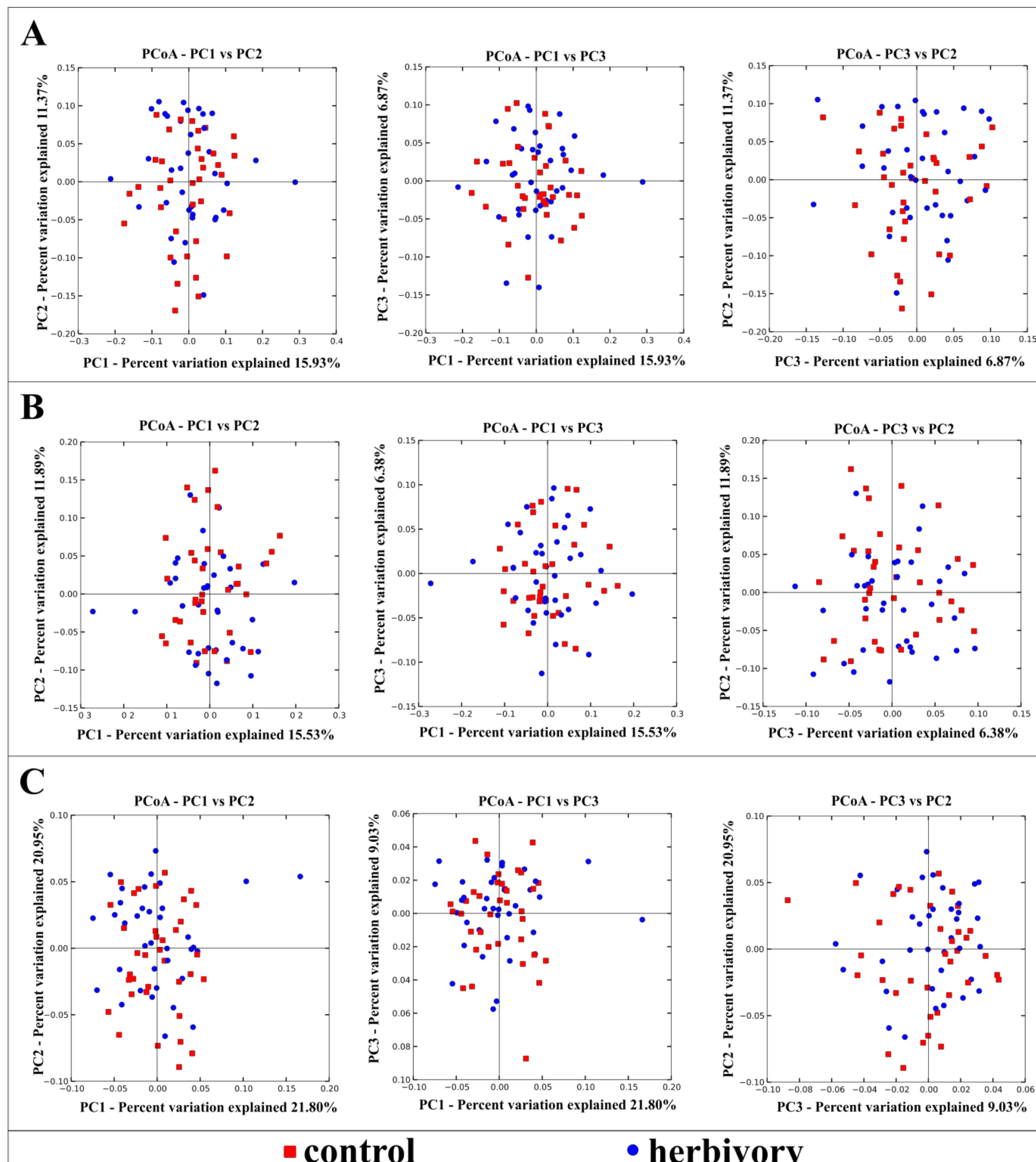

Fig. 11. Impact of above-ground herbivory on bacterial community structures at 99% (A), 97% (B), and 80% (C) genetic similarity. PCoA plots were calculated with QIIME (Caporaso *et al.*, 2010).

Sward diversity, management regimes, and above-ground herbivory alter the bacterial community in the rhizosphere

We analyzed the effect of management regimes, sward composition, and above-ground herbivory on the relative abundance of predominant bacterial groups and species by statistical modeling using Dirichlet regression. The sward composition had a significant influence (p value < 0.05) on the Firmicutes and the *Gammaproteobacteria* (Tab. 2).

Tab. 2: Effect of different fertilization regimes, mowing frequencies, herbicide application, above-ground herbivory, and the combination of these treatments on bacterial phyla and proteobacterial classes.

phylum or proteobacterial class	dicot-reduced	monocot-reduced	fertilization	mowing	herbivory	dicot-reduced:fertilization	monocot-reduced:fertilization	dicot-reduced:mowing	monocot-reduced:mowing	fertilization:mowing	dicot-reduced:herbivory	monocot-reduced:herbivory	fertilization:herbivory	Mowing:herbivory	dicot-reduced:fertilization:mowing	monocot-reduced:fertilization:mowing	dicot-reduced:fertilization:herbivory	monocot-reduced:fertilization:herbivory	dicot-reduced:mowing:herbivory	monocot-reduced:mowing:herbivory	fertilization:mowing:herbivory	dicot-reduced:fertilization:mowing:herbivory	monocot-reduced:fertilization:mowing:herbivory
Acidobacteria			***	***		***	***	**	*	***			*		***	***	*	**			***	**	***
Actinobacteria			***	***		*	**	*	.	***			.	**	*	***	.	*			***	***	***
Bacteroidetes			**	**		**	.		.	***				*	**	**	.				**	***	*
Chloroflexi			**	***		.	*	.	.	***				*	.	**					**	**	**
Cyanobacteria			*			*				*						.							
Elusimicrobia			*	.		.	.			**					.	**							*
Fibrobacteres			*			.				**						.					*		
Firmicutes	*	*	*	***			*	**	*	***		*			**	**		*	.		*	***	***
Gemmatimonadetes			***	***		*	*	.	*	***					*	***		*			**	*	***
Nitrospirae			.	*			*			*													
Planctomycetes										.													
Alphaproteobacteria			***	***		**	**	*		***			*	.	**	***	*	*			***	***	***
Betaproteobacteria			***	***		**	***	*	**	***			.	*	*	***	*	*			**	**	***
Gammaproteobacteria	*		***	***		***	**	**	*	***			*	*	***	***	**	*			***	***	***
Deltaproteobacteria			***	***		**	**		*	***			.		*	***	.	*			**	**	***
Verrucomicrobia			.							**						.						.	
WS3				***				*	**	*						*							

*** p-value < 0.001; ** p-value < 0.01; * p-value < 0.05; . p-value < 0.1

The abundance of *Firmicutes* was significantly enhanced on all herbicide-treated plots (Fig. 12A), while the *Gammaproteobacteria* did only respond to the herbicide application targeting dicots (Tab. 2). In combination with the other investigated parameters, sward composition affected almost all bacterial phyla and proteobacterial classes such as the *Nitrospirae*. This phylum was significantly influenced by fertilization on the monocot-reduced plots (Tab. 2).

We further analyzed the impact of sward composition on the relative abundance of predominant bacterial phylotypes (Supplemental Tab. S5). The results for the top 25 OTUs (3% genetic distances) are shown in Tab. 3. The abundance of several of the analyzed OTUs was affected by at least one parameter. Sward composition, fertilizer application and mowing frequency had the highest impact on bacterial abundance. Many bacterial phylotypes were influenced by herbicide treatment against dicots and/or monocots. Whereas the bacterium Ellin6561 (order *Rhizobiales*) and some uncultured bacteria of the orders *Acidobacteriales*, *Rhodospirillales*, and *Rhizobiales* were significantly affected by herbicide application against dicots, some uncultured bacterium of the *Bacillaceae* (unknown order) and of the order *Frankiales*, as well as an uncultured *Acidobacterium* (unknown order) were influenced by herbicide application against monocots.

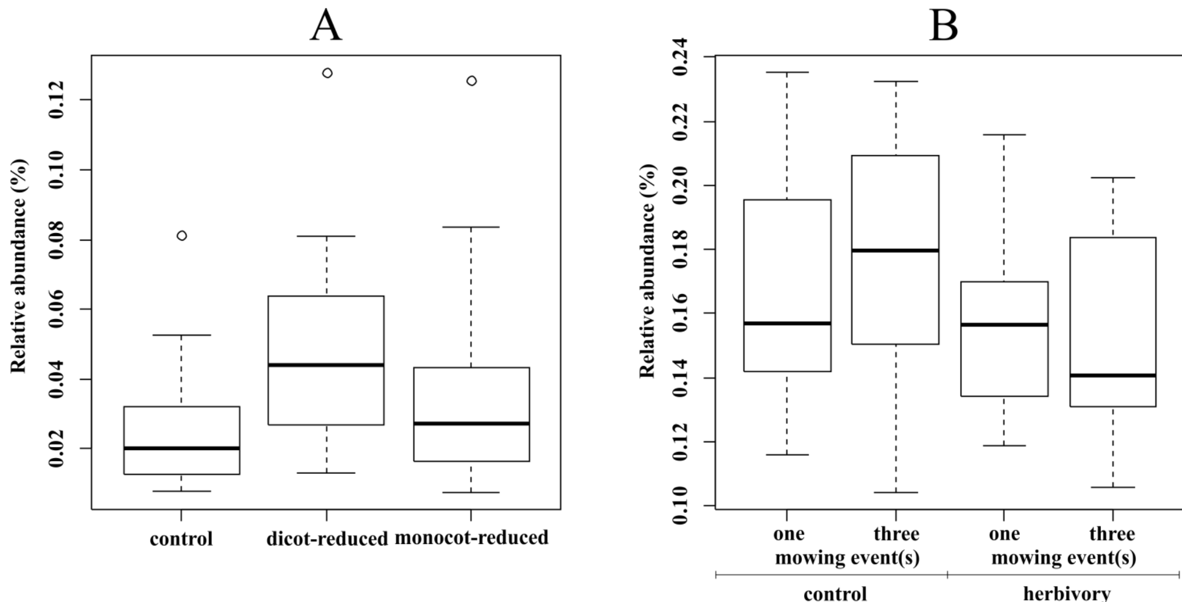

Fig. 12. Effect of sward composition on the abundance of Firmicutes (A) and of mowing frequency in combination with herbivory on the abundance of Actinobacteria (B).

Tab. 3: Effect of different fertilization regimes, mowing frequencies, herbicide application, above-ground herbivory, and the combination of these treatments on the 25 most abundant bacterial OTUs (3% genetic divergence).

OTU ID	*dicote-reduced*	*monocots-reduced*	*fertilization*	*mowing*	*herbivory*	*dicote-reduced:fertilization*	*monocots-reduced:fertilization*	*dicote-reduced:mowing*	*monocots-reduced:mowing*	*fertilization:mowing*	*dicote-reduced:herbivory*	*monocots-reduced:herbivory*	*fertilization:herbivory*	*Mowing:herbivory*	*dicote-reduced:fertilization:mowing*	*monocots-reduced:fertilization:mowing*	*dicote-reduced:fertilization:herbivory*	*monocots-reduced:fertilization:herbivory*	*dicote-reduced:mowing:herbivory*	*monocots-reduced:mowing:herbivory*	*fertilization:mowing:herbivory*	*dicote-reduced:fertilization:mowing:herbivory*	*monocots-reduced:fertilization:mowing:herbivory*	*taxonomic affiliation*
15254							*			*							**							Bacteria.Proteobacteria.Alphaproteobacteria.Rhizobiales.Bradyrhizobiaceae.Bradyrhizobium.Bradyrhizobium.sp.
3020	**	***	**	*		*	***		*	**						**		*	**					Bacteria.Acidobacteria.Acidobacteria.Candidatus.Solibacter.uncultured.Acidobacteria.bacterium
15334	***			*	*			***		*		**					*	*					**	Bacteria.Proteobacteria.Alphaproteobacteria.Rhizobiales.Xanthobacteraceae.uncultured.bacterium.Ellin6561
430			**			*				*							**		**					Bacteria.Acidobacteria.Acidobacteria.Acidobacteriales.Acidobacteriaceae.Candidatus.Koribacter.uncultured.bacterium
31887		**				*			*		*	***					*		***	*		**		Bacteria.Firmicutes.Bacilli.4.15.uncultured.Bacillaceae.bacterium
10498				*			*	**	*								*	*	*					Bacteria.Proteobacteria.Alphaproteobacteria.Rhizobiales.alphaI.cluster.uncultured.bacterium
33544																			*					Bacteria.Acidobacteria.Acidobacteria.DA052.uncultured.bacterium
30880			***			***	*			**			**		*		**				*			Bacteria.Acidobacteria.Acidobacteria.Order.Incertae.Sedis.Family.Incertae.Sedis.Bryobacter.uncultured.Acidobacteria.bacterium
26950	***	**	***			***	***								**		**	*	**					Bacteria.Acidobacteria.Acidobacteria.Acidobacteriales.Acidobacteriaceae.uncultured.uncultured.bacterium
15204			***		*	***	***			***			**	*	***	***	**	***			***	*	***	Bacteria.Proteobacteria.Alphaproteobacteria.Rhizobiales.Family.Incertae.Sedis.Rhizomicrobium.uncultured.bacterium
35896	*	*		***				*	**							**								Bacteria.Proteobacteria.Betaproteobacteria.Burkholderiales.Comamonadaceae.uncultured.uncultured.bacterium
1434			*				*	*				*	*				*	**	**					Bacteria.Bacteroidetes.Sphingobacteriia.Sphingobacteriales.Chitinophagaceae.uncultured.uncultured.bacterium
43557	**	**	***	***		**	**	***	***	***			**	***	***	***	**			***	**	*	***	Bacteria.Acidobacteria.Acidobacteria.Acidobacteriales.Acidobacteriaceae.uncultured.uncultured.bacterium
10655	**	*							*		*													Bacteria.Bacteroidetes.Cytophagia.Cytophagales.Cytophagaceae.Flexibacter.uncultured.bacterium
42418		*			**		*	*	***		**	***		*	*	***		***	***	***			***	Bacteria.Actinobacteria.Actinobacteria.Frankiales.Acidothermaceae.Acidothermus.uncultured.bacterium
10041	***		*			***		***			**				***		*		*					Bacteria.Proteobacteria.Alphaproteobacteria.Rhizobiales.Beijerinckiaceae.uncultured.uncultured.proteobacterium
23893			**				*			***			*			**	*	*			***	**	***	Bacteria.Proteobacteria.Alphaproteobacteria.Rhodospirillales.Acetobacteraceae.uncultured.uncultured.bacterium
24136	*	**		***	*		*	*	*	*					*									Bacteria.Proteobacteria.Alphaproteobacteria.Rhizobiales.Xanthobacteraceae.uncultured.uncultured.bacterium
17761	*					**	*							*	**		*		**					Bacteria.Acidobacteria.Acidobacteria.Acidobacteriales.Acidobacteriaceae.uncultured.uncultured.bacterium
10097		**		*			*	***			*		*				***				*	*	*	Bacteria.Acidobacteria.Acidobacteria.Candidatus.Solibacter.uncultured.Acidobacteria.bacterium
1655	*								*					*		*	*		*	*			*	Bacteria.Proteobacteria.Alphaproteobacteria.Rhodospirillales.DA111.uncultured.bacterium
30883			**	*		**				*	**		*				***				*	**		Bacteria.Proteobacteria.Betaproteobacteria.Nitrosomonadales.Nitrosomonadaceae.uncultured.uncultured.beta.proteobacterium
41226	*		*	**						*			*				*					*	*	Bacteria.Proteobacteria.Alphaproteobacteria.Rhodospirillales.DA111.uncultured.bacterium
22924										**											*			Bacteria.Proteobacteria.Alphaproteobacteria.Rhodospirillales.JG37.AG.20.uncultured.Rhodospirillaceae.bacterium
38931								**							**				**		*	***		Bacteria.Acidobacteria.Acidobacteria.Candidatus.Solibacter.uncultured.Acidobacteria.bacterium

*** p-value < 0.001; ** p-value < 0.01; * p-value < 0.05; . p-value < 0.1

In combination with the other investigated parameters, sward composition had a significant effect on almost all bacterial phylotypes of the top 25 OTUs. These impacts were stronger for the more abundant phylotypes than for rare phylotypes (Tab. 3, Supplemental Tab. S5). This is also true for fertilization, mowing frequency, and above-ground herbivory separately or in combination with each other (Supplemental Tab. S5).

The majority of the abundant bacterial phyla and proteobacterial classes was significantly affected by fertilizer application and different mowing frequencies (Tab. 2). *Acidobacteria* were significantly less abundant on fertilized plots (Fig. 13A). The opposite was recorded for *Actinobacteria* (Fig. 13B). When analyzing the effect of the parameters fertilization or mowing separately or in combination, we found synergistic effects. The abundance of the phylum *Bacteroidetes* was significantly affected to a higher extend by fertilization and mowing frequency as by fertilization or mowing separately (Fig. 13C). On the other hand, the relative abundance of this phylum was reduced by fertilization on plots mown once, but it increased by fertilization on plots mown thrice. The same effect was recorded for *Chloroflexi* (data not shown). Moreover, the abundance of *Verrucomicrobia* was significantly affected by fertilization and mowing but not by fertilization or mowing only (Tab. 2).

In addition, fertilization as well as mowing frequency and the combination of both treatments had a significant impact on most phylotypes of the top 25 OTUs (Tab. 3). The most abundant phylotype was affiliated to *Bradyrhizobium.* The abundance of this OTU was reduced by fertilization (Fig. 14A), but only significantly on plots mown thrice per year (Fig. 14B). In addition, the abundance was decreased by fertilization on monocot-reduced plots (Fig. 14C).

We did not find direct correlations between above-ground herbivory and the abundance of predominant bacterial groups. However, significant changes in combination with other treatments were detectable (Tab. 3). For example, the abundance of *Actinobacteria* was significantly reduced by herbivory, but only on plots mown three times per year (Fig. 12B). As only few OTUs reacted towards herbivory, its influence on community structure must be considered to be weaker as originally expected.

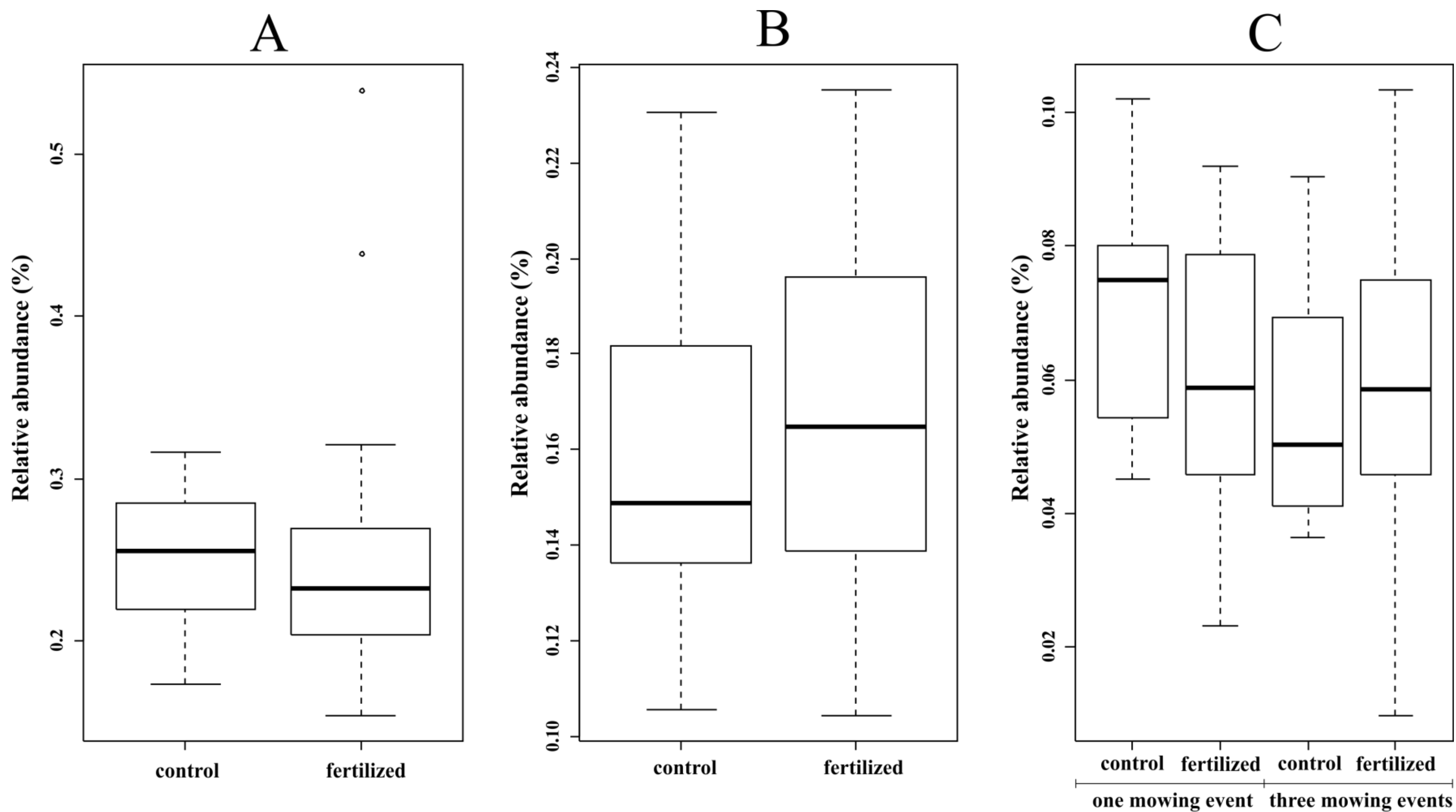

Fig. 13. Effect of fertilization on the Acidobacteria (A) and Actinobacteria (B) as well as the effect of fertilization and mowing regimes on Bacteroidetes (C).

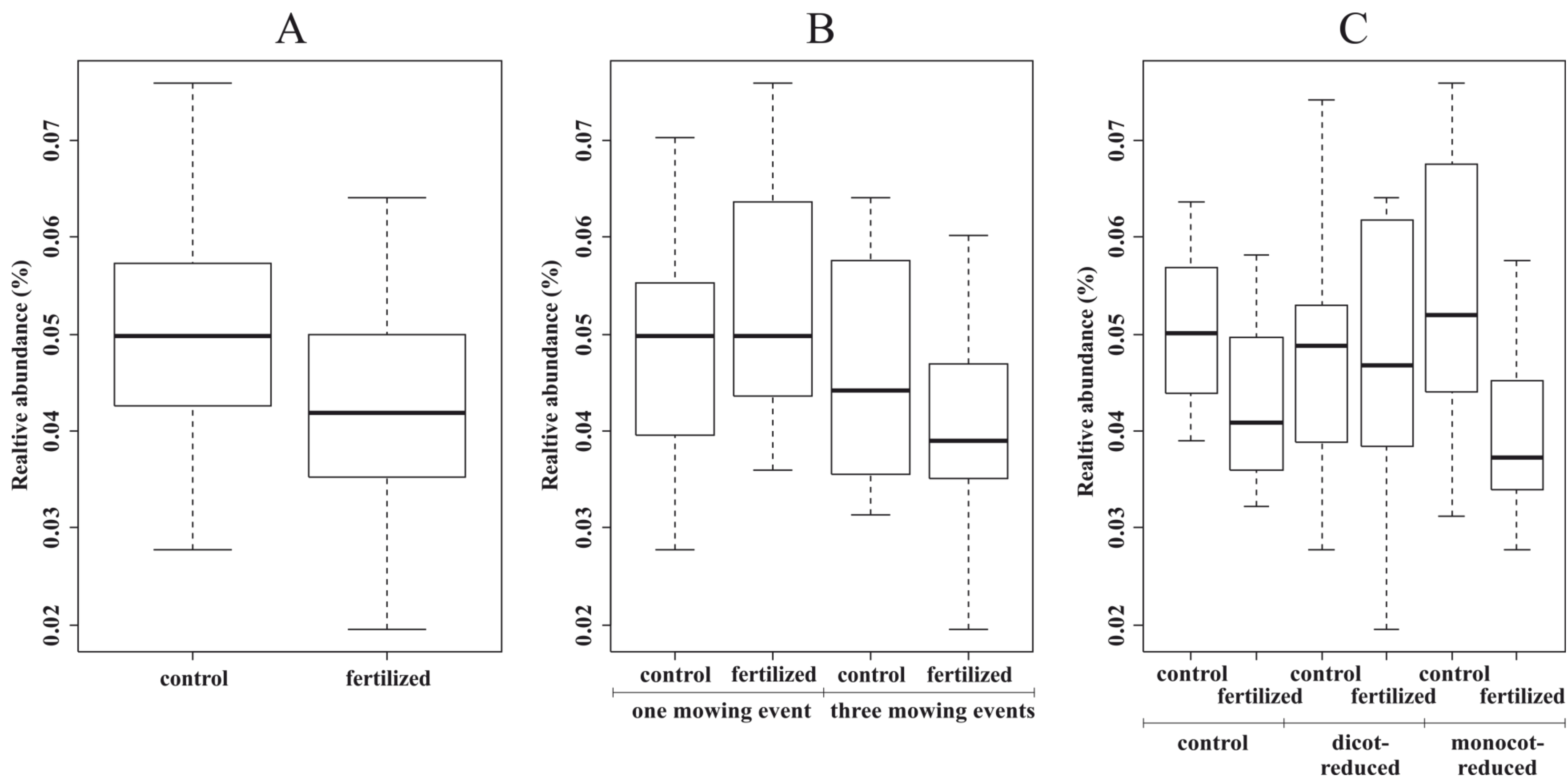

Fig. 14. Effect of fertilization (A), fertilization and mowing (B), and sward composition and fertilization (C) on the abundance of an OTU affiliated to *Bradyrhizobium* sp.

Discussion

Characterization of bacterial community structure in the rhizosphere

To gain insights into the bacterial community structures in the rhizosphere in the grassland system, we applied 454 pyrosequencing. The seven predominant bacterial phyla and the 4 proteobacterial classes observed in this study agreed with other studies (Gardner et al., 2011; Nacke et al., 2011). In this study, 44,452 OTUs at 3% genetic divergence were detected in all samples (Supplemental Tab. S5). Some of them are known as typical soil or rhizosphere bacteria such as *Bradyrhizobium* (order *Rhizobiales*), *Bacillus* (order *Bacillales*) or *Rhizomicrobium* (order *Rhizobiales*). These findings are consistent with the results of Duineveld *et al*. (2001).The authors investigated the bacterial community in the rhizosphere of chrysanthemum and found that most species were closely related to those of previously described soil bacteria such as *Pseudomonas*, *Acetobacter*, *Bacillus*, and *Arthrobacter*.

The bacterial genera *Rhizobium* and *Bradyrhizobium* are the most important dinitrogen fixers; they form symbiotic associations with specific legumes and some nonlegumes (Beauchamp et al., 1997). Furthermore, there are huge numbers of free-living nitrogen-fixing diazotrophs such as *Bacillus*. Nitrogen-fixing bacteria can promote plant growth and can reduce susceptibility to diseases caused by plant pathogenic bacteria, fungi, viruses and nematodes (Kloepper et al., 2004). Therefore, they are known as Plant Growth-Promoting Rhizobacteria (PGPR) (Kloepper et al., 1999).

Influence of sward composition on the bacterial community structure in the rhizosphere

In the present study, the bacterial richness (number of OTUs) was negatively affected by herbicide application against dicots and monocots. In species-rich plots, higher numbers of OTUs were detected (Tab. 1). This is consistent with a study from El Fantroussi *et al.* (1999). The authors showed that different phenylurea herbicides significantly decreased the number of cultivable heterotrophic bacteria in soil. In addition, Benizri and Amiaud (2005) found that the diversity of soil bacteria in fertilized grasslands increased significantly with increasing plant diversity. The application of herbicides against dicots and/or monocots had a significant impact on many phylotypes and on

Gammaproteobacteria and *Firmicutes* (Tab. 3, Supplemental Tab. S5). Many bacterial phylotypes were influenced by herbicide treatment against monocots and/or dicots (Tab. 1). Whereas some bacteria were significantly affected by herbicide application against dicots, other bacteria were influenced by herbicide application against monocots.
However, the sole effect of sward composition was weaker compared with the effect of sward composition in combination with mowing frequency and/or fertilization (Figs. 12A, 14C). These observations support the results of previous studies which showed that the selective effect of a certain plant species on the bacterial community in the soil or in the rhizosphere of grasslands varies with soil fertility or soil type (Bardgett et al., 1999a; Harrison and Bardgett, 2010; Innes et al., 2004). According to Marschner *et al.* (2004), the bacterial community structure in the rhizosphere was influenced by a complex interaction between plant factors such as genotype and by different soil factors including the soil type.
The herbicide application against both dicots and monocots resulted in significant changes in plant species richness and in functional group abundances in the GrassMan experimental field (Petersen et al., 2012). Plant species have been previously reported to affect specific bacterial groups in the rhizosphere (Costa et al., 2006; Garbeva et al., 2008; Grayston et al., 1998; Singh et al., 2007). Kowalchuk et al. (2002) found a clear plant-induced influence on bacterial community structure in the rhizosphere of non-agricultural plant species. The authors assumed that the rhizosphere selects for specific soil-borne microbial populations, resulting in a lower diversity of rhizosphere bacterial communities. In contrast to the previously reported studies, Singh et al. (2007) showed that the rhizosphere bacterial community composition from different plant species in grassland soils was mainly determined by soil type. The authors conclude that the influence of plant species is only weak and that there is no evidence for the selection of bacteria by plants in the rhizosphere.

Fertilization and mowing shape the bacterial community composition in the rhizosphere

We investigated the impact of different grassland management regimes on bacterial community composition in the rhizosphere by 454 pyrosequencing and by DGGE. Both methods showed that mowing frequency as well as fertilization had a strong influence on the bacterial community composition. When analyzing the effect of the parameters

fertilization or mowing in combination, we found synergistic effects (Figs. 13C and 14B, Supplemental Fig. S2).

Pyrosequencing-based analyses of 16S rRNA genes revealed no significant effects of fertilization on bacterial richness in the rhizosphere (Tab. 1), but significant effects on community composition (Tab. 2, Figs. 13 and 14). These findings are in line with a study of Fierer et al. (2011) who observed no significant effects of N fertilization on soil bacterial diversity, but significant effects on community composition. Beauregard et al. (2010) found that fertilizer application led to shifts in the composition of bacterial communities without affecting their richness. In a study of soil microbial community composition and land use history in cultivated and grassland ecosystems, fertilizer and herbicide application were associated with a distinctive microbial community composition (Steenwerth et al., 2002). In contrast to this, long-term fertilization regimes resulted in changes of soil bacterial community structure and diversity in northern China (Ge et al., 2008).

In our study, fertilizer application had a significant impact on several bacterial phyla in the rhizosphere, for instance *Chloroflexi*, *Bacteroidetes*, *Acidobacteria*, *Actinobacteria*, and *Proteobacteria* (Tab. 2). The abundance of *Acidobacteria* was significantly lower in fertilized plots. This finding corresponds to Kielak et al. (2009) who showed that this phylum appeared significantly lower in nutrient rich rhizosphere than in the surrounding bulk soil. In another study, the *Acidobacteria* were negative correlated with the nitrogen input level (Fierer et al., 2012). This group is often considered to be oligotrophic (Kielak et al., 2009).

Interestingly, high proportions of OTUs belonging to the Bacteroidetes were more abundant in fertilized plots which were mown thrice a year compared to the unfertilized plots (Fig. 13B). In addition, *Actinobacteria* were positive correlated with the fertilization. These results are in line with a study of Fierer *et al.* (2012). The authors showed that copiotrophic taxa including members of the *Bacteroidetes*, *Proteobacteria*, and *Actinobacteria* typically increased in relative abundance in the high N plots.

Furthermore, the relative abundance of *Chloroflexi* was lower in plots with high levels of nitrogen input. This finding is in line with our study. The abundance of *Chloroflexi* decreased by fertilizer application, but only on plots mown once a year (data not shown). As mentioned before, the phylum *Nitrospirae* was significantly influenced by fertilization on the monocot-reduced plots (Tab. 2). Members of this phylum belong to the nitrite-oxidizing bacterial group. In our study, an uncultured bacterium affiliated to the

Nitrosomonadaceae was influenced by mowing frequency and fertilization (data not shown). This is of ecological importance because the genus *Nitrosomonas* is a key player in the N cycling of soil (Acosta-Martinez et al., 2008).

In addition, fertilization as well as mowing frequency had a significant impact on most phylotypes of the top 25 OTUs (Tab. 3). The combination of both treatments led to interesting results. The abundance of *Bradyrhizobium* was reduced by fertilization (Fig. 14A). However, this effect was only significant on plots mown thrice per year (Fig. 14B). In addition, the abundance was decreased by fertilization on monocot-reduced plots (Fig. 14C). As mentioned before, the bacterial genera *Bradyrhizobium* belongs to the most important dinitrogen fixers. In soils with high level of N, nodule formation is decreased (Beauchamp et al., 1997) which might be explained the lower abundance of nitrogen-fixing bacteria in the rhizosphere. Furthermore, the relative abundances of soil microbial taxa associated with specific components of the soil N cycle such as nitrifiers often changes when soils are fertilized with N (Fierer et al., 2012).

Effects of mowing on N fluxes and N retention in grasslands have been reported previously (Maron and Jeffries, 2001). Grazing and mowing can also affect the size and composition of key microbial functional groups driving N dynamics (Patra et al., 2006). According to Denef et al. (2009) mowing intensity did not affect the relative abundance or activity of microbial communities in the rhizosphere of temperate grassland. This result is not consistent with the results of our study in which different mowing frequencies strongly influenced the bacteria in the rhizosphere. The reason for these differences could be that different methodologies were used which strongly varied in phylogentic resolution.

Impact of above-ground herbivory on rhizosphere bacterial community structures

Whereas herbivory did not seem to affect the bacterial richness, although slight changes in the relative abundances of members of the *Rhizobiales*, *Frankiales*, and *Acidimicrobiales* were recorded. These findings are in line with the results of Techau et al. (2004) who showed that above-ground herbivory had no influence on the number of rhizosphere bacteria in pea plants.

In the present study, there was a significant interaction of the herbivory effect with fertilization and mowing (Tabs. 2 and 3). In combination with these regimes, above-ground herbivory had a significant influence on most abundant phyla such as *Actinobacteria*, *Bacteroidetes*, *Acidobacteri*a, *Proteobacteria*, *Chloroflexi*, and *Firmicutes.*

In addition, the abundance of the *Actinobacteria* was significantly reduced by herbivory, but only on plots mown three times per year (Fig. 12B).

It is well-known that below-ground herbivory influences bacterial communities in the rhizosphere (Dematheis et al., 2012; Denton et al., 1999; Poll et al., 2007; Treonis et al., 2005). Denton et al. (1998) showed that low amounts of root herbivory (below the damage threshold) positively influence the rhizosphere microbial community in a grassland soil. According to Holland et al. (1995), above-ground herbivory stimulate soil bacteria at least at moderate levels of herbivory in no-tillage fields. Furthermore, grazing induces changes in the size and in the structure of bacterial communities in the soil (Northup et al., 1999; Patra et al., 2005). Northup et al. (1999) showed that grazing pressure had a stronger effect on microbial biomass than other soil or vegetative characteristics. The long-term removal of sheep grazing resulted in significant reductions in microbial biomass and activity in the surface soil while the abundance of active soil bacteria were unaffected by the removal of sheep grazing (Bardgett et al., 1997).

So far, previous studies often used either cultivation-dependent approaches (Dawson et al., 2004; Grayston et al., 2001), microbial respiration measurements (Bardgett et al., 1997; Bardgett et al., 1999a; Holland, 1995) or cultivation-independent approaches such as DGGE (Dematheis et al., 2012; Patra et al., 2005) to study the effect of herbivory or grazing on the bacteria in the soil or in the rhizosphere. To our knowledge, above-ground herbivory and its influence on the bacteria in the rhizosphere have never been investigated by 454 pyrosequencing below phylum level.

Ecological significance

The effects on bacterial diversity of the studied parameters have been addressed frequently in many studies over the past years. For example, it was shown that fertilizer application influenced certain bacterial groups being involved in important nutrient cycles, e.g., the soil nitrogen cycle. Therefore, herbicide and fertilizer application as well as different mowing frequencies and above-ground herbivory are of ecological and economic importance as soil fertility is strongly affected. However, most previous studies investigated the effect of just a single biotic or abiotic factor.

The analysis conducted in this study aimed at evaluating the combined impact of different management regimes and above-ground herbivory on bacterial community structures in the rhizosphere. Although we were able to confirm the results of former studies, we also

recorded discrepancies as not only a single factor but also different combinations of the studied factors influenced the abundances of several bacterial taxa in the soil.
Consequently, we have to restrict the results of former studies and their interpretation as mixed effects led to either an enhanced, reduced, or, in rare cases, opposite bacterial response. One prominent ecological example is the effect of fertilization on soil nitrogen fixation. We were able to demonstrate that fertilization does lead to a reduction of bacterial taxa capable of nitrogen fixation. However, this effect was only significant in combination with higher mowing frequencies. Therefore, fertilization does affect nitrogen fixation but only under certain circumstances.
Based on the high recorded number of mixed effects of management regimes and herbivory, versatile changes in the bacterial community composition and, correspondingly, versatile ecological outcomes can occur.

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References

Acosta-Martinez V, Dowd S, Sun Y, Allen V (2008). Tag-encoded pyrosequencing analysis of bacterial diversity in a single soil type as affected by management and land use. *Soil Biology and Biochemistry* 40: 2762-2770.

Bardgett RD, Mawdsley JL, Edwards S, Hobbs PJ, Rodwell JS, et al. (1999). Plant species and nitrogen effects on soil biological properties of temperate upland grasslands. *Functional Ecology* 13: 650-660.

Bardgett, D. R, Leemans, K. D, Cook, et al. (1997). Seasonality of the soil biota of grazed and ungrazed hill grasslands. *Soil Biology and Biochemistry* 29: 1285-1294.

Beauchamp E, Hume D, Elsas Jv, Trevors J, Wellington E (1997). Agricultural soil manipulation: the use of bacteria, manuring, and plowing. *Modern soil microbiology*: 643-664.

Beauregard MS, Hamel C, Atul N, St-Arnaud M (2010). Long-Term Phosphorus Fertilization Impacts Soil Fungal and Bacterial Diversity but not AM Fungal Community in Alfalfa. *Microbial Ecology* 59: 379-389.

Benizri E, Amiaud B (2005). Relationship between plants and soil microbial communities in fertilized grasslands. *Soil Biology and Biochemistry* 37: 2055-2064.

Berg G, Smalla K (2009). Plant species and soil type cooperatively shape the structure and function of microbial communities in the rhizosphere. *FEMS Microbiology Ecology* 68: 1-13.

Bissett A, Richardson AE, Baker G, Thrall PH (2011). Long-term land use effects on soil microbial community structure and function. *Applied Soil Ecology* 51: 66-78.

Caporaso JG, Kuczynski J, Stombaugh J, Bittinger K, Bushman FD, et al. (2010). QIIME allows analysis of high-throughput community sequencing data. *Nature Methods* 7: 335-336.

Compant S, Clément C, Sessitsch A (2010). Plant growth-promoting bacteria in the rhizo- and endosphere of plants: their role, colonization, mechanisms involved and prospects for utilization. *Soil Biology and Biochemistry* 42: 669-678.

Costa R, Gotz M, Mrotzek N, Lottmann J, Berg G, et al. (2006). Effects of site and plant species on rhizosphere community structure as revealed by molecular analysis of microbial guilds. *FEMS Microbiology Ecology* 56: 236-249.

Cremonesi L, Firpo S, Ferrari M, Righetti PG, Gelfi C (1997). Double-gradient DGGE for optimized detection of DNA point mutations. *Biotechniques* 22: 326-330.

Dawson LA, Grayston SJ, Murray PJ, Ross JM, Reid EJ, et al. (2004). Impact of *Tipula paludosa* larvae on plant growth and the soil microbial community. *Applied Soil Ecology* 25: 51-61.

Dematheis F, Zimmerling U, Flocco C, Kurtz B, Vidal S, et al. (2012). Multitrophic

interaction in the rhizosphere of maize: root feeding of Western corn rootworm larvae alters the microbial community composition. *PLoS One* 7: e37288-e37288.

Denef K, Roobroeck D, Manimel Wadu MC, Lootens P, Boeckx P (2009). Microbial community composition and rhizodeposit-carbon assimilation in differently managed temperate grassland soils. *Soil Biology and Biochemistry* 41: 144-153.

Denton CS, Bardgett RD, Cook R, Hobbs PJ (1998). Low amounts of root herbivory positively influence the rhizosphere microbial community in a temperate grassland soil. *Soil Biology and Biochemistry* 31: 155-165.

DeSantis TZ, Hugenholtz P, Larsen N, Rojas M, Brodie EL, et al. (2006). Greengenes, a chimera-checked 16S rRNA gene database and workbench compatible with ARB. *Applied and Environmental Microbiology* 72: 5069-5072.

Dimkpa C, Weinand T, Asch F (2009). Plant-rhizobacteria interactions alleviate abiotic stress conditions. *Plant, Cell and Environment* 32: 1682-1694.

Doi T, Abe J, Shiotsu F, Morita S (2011). Study on rhizosphere bacterial community in lowland rice grown with organic fertilizers by using PCR- denaturing gradient gel electrophoresis. *Plant Root* 5: 5-16.

Duineveld BM, Kowalchuk GA, Keijzer A, van Elsas JD, van Veen JA (2001). Analysis of bacterial communities in the rhizosphere of chrysanthemum via denaturing gradient gel electrophoresis of PCR-amplified 16S rRNA as well as DNA fragments coding for 16S rRNA. *Applied and Environmental Microbiology* 67: 172-178.

Edgar RC (2010). Search and clustering orders of magnitude faster than BLAST. *Bioinformatics* 26: 2460-2461.

Edgar RC, Haas BJ, Clemente JC, Quince C, Knight R (2011). UCHIME improves sensitivity and speed of chimera detection. *Bioinformatics* 27: 2194-2200.

El Fantroussi S, Verschuere L, Verstraete W, Top EM (1999). Effect of phenylurea herbicides on soil microbial communities estimated by analysis of 16S rRNA gene fingerprints and community-level physiological profiles. *Applied and Environmental Microbiology* 65: 982-988.

Fierer N, Bradford MA, Jackson RB (2007). Toward an ecological classification of soil bacteria. *Ecology* 88: 1354-1364.

Fierer N, Lauber CL, Ramirez KS, Zaneveld J, Bradford MA, et al. (2011). Comparative metagenomic, phylogenetic and physiological analyses of soil microbial communities across nitrogen gradients. *The ISME Journal* 6: 1007-1017.

Garbeva P, Elsas JD, Veen JA (2008). Rhizosphere microbial community and its response to plant species and soil history. *Plant and Soil* 302: 19-32.

Gardner T, Acosta-Martinez V, Senwo Z, Dowd SE (2011). Soil rhizosphere microbial communities and enzyme activities under organic farming in Alabama. *Diversity* 3: 308-328.

Ge Y, Zhang J-b, Zhang L-m, Yang M, He J-z (2008). Long-term fertilization regimes affect bacterial community structure and diversity of an agricultural soil in northern China. *Journal of Soils and Sediments* 8: 43- 50.

Gottel NR, Castro HF, Kerley M, Yang Z, Pelletier DA, et al. (2011). Distinct microbial communities within the endosphere and rhizosphere of *Populus deltoides* roots across contrasting soil types. *Applied and Environmental Microbiology* 77: 5934-5944.

Grayston SJ, Dawson LA, Treonis AM, Murray PJ, Ross J, et al. (2001). Impact of root herbivory by insect larvae on soil microbial communities. *European Journal of Soil Biology* 37: 277-280.

Grayston SJ, Wang S, Campbell CD, Edwards AC (1998). Selective influence of plant species on microbial diversity in the rhizosphere. *Soil Biology and Biochemistry* 30: 369-378.

Haichar FeZ, Marol C, Berge O, Rangel-Castro JI, Prosser JI, et al. (2008). Plant host habitat and root exudates shape soil bacterial community structure. *The ISME Journal* 2: 1221-1230.

Harrison KA, Bardgett RD (2010). Influence of plant species and soil conditions on plant–soil feedback in mixed grassland communities. *Journal of Ecology* 98: 384-395.

Heuer H, Krsek M, Baker P, Smalla K, Wellington EM (1997). Analysis of actinomycete communities by specific amplification of genes encoding 16S rRNA and gel-electrophoretic separation in denaturing gradients. *Applied and Environmental Microbiology* 63: 3233-3241.

Holland J (1995). Effects of above-ground herbivory on soil microbial biomass in conventional and no-tillage agroecosystems. *Applied Soil Ecology* 2: 275- 279.

Innes L, Hobbs P, Bardgett R (2004). The impacts of individual plant species on rhizosphere microbial communities in soils of different fertility. *Biology and Fertility of Soils* 40: 7-13.

Keuter A, Hoeft I, Veldkamp E, Corre M (2013). Nitrogen response efficiency of a managed and phytodiverse temperate grassland. *Plant and Soil* 364: 193- 206.

Kielak A, Pijl AS, van Veen JA, Kowalchuk GA (2008). Phylogenetic diversity of Acidobacteria in a former agricultural soil. *The ISME Journal* 3: 378-382.

Kloepper J, Rodríguez-Kábana R, McInroy J, Young R (1992). Rhizosphere bacteria antagonistic to soybean cyst (*Heterodera glycines*) and root-knot (*Meloidogyne incognita*) nematodes: Identification by fatty acid analysis and frequency of biological control activity. *Plant and Soil* 139: 75-84.

Kloepper JW, Rodríguez-Kábana R, Zehnder AW, Murphy JF, Sikora E, et al. (1999). Plant root-bacterial interactions in biological control of soilborne diseases and potential extension to systemic and foliar diseases. *Australasian Plant Pathology* 28: 21-26.

Kloepper JW, Ryu C-M, Zhang S (2004). Induced systemic resistance and promotion of plant growth by *Bacillus* spp. *Phytopathology* 94: 1259- 1266.

Kowalchuk GA, Buma DS, de Boer W, Klinkhamer PG, van Veen JA (2002). Effects of above-ground plant species composition and diversity on the diversity of soil-borne microorganisms. *Antonie Van Leeuwenhoek* 81: 509-520.

Lugtenberg B, Kamilova F (2009). Plant-growth-promoting rhizobacteria. *Annual Review of Microbiology* 63: 541-556.

Maron JL, Jefferies RL (2001). Restoring enriched grasslands: effects of mowing on species richness, productivity, and nitrogen retention. *Ecological Applications* 11: 1088-1100.

Marschner P, Crowley D, Yang CH (2004). Development of specific rhizosphere bacterial communities in relation to plant species, nutrition and soil type. *Plant and Soil* 261: 199-208.

Muyzer G, de Waal EC, Uitterlinden AG (1993). Profiling of complex microbial populations by denaturing gradient gel electrophoresis analysis of polymerase chain reaction-amplified genes coding for 16S rRNA. *Applied and Environmental Microbiology* 59: 695-700.

Muyzer G, Teske A, Wirsen CO, Jannasch HW (1995). Phylogenetic relationships of *Thiomicrospira* species and their identification in deep-sea hydrothermal vent samples by denaturing gradient gel electrophoresis of 16S rDNA fragments. *Archives of Microbiology* 164: 165-172.

Nacke H, Thürmer A, Wollherr A, Will C, Hodac L, et al. (2011). Pyrosequencing-Based Assessment of Bacterial Community Structure Along Different Management Types in German Forest and Grassland Soils. *PLoS ONE* 6: e17000.

Northup BK, Brown JR, Holt JA (1999). Grazing impacts on the spatial distribution of soil microbial biomass around tussock grasses in a tropical grassland. *Applied Soil Ecology* 13: 259-270.

Nübel U, Engelen B, Felske A, Snaidr J, Wieshuber A, et al. (1996). Sequence heterogeneities of genes encoding 16S rRNAs in *Paenibacillus polymyxa* detected by temperature gradient gel electrophoresis. *Journal of Bacteriology* 178: 5636-5643.

Nunan N, Daniell TJ, Singh BK, Papert A, McNicol JW, et al. (2005). Links between plant and rhizoplane bacterial communities in grassland soils. *Applied and Environmental Microbiology* 71: 6784-6792.

Pastorelli R, Landi S, Trabelsi D, Piccolo R, Mengoni A, et al. (2011). Effects of soil management on structure and activity of denitrifying bacterial communities. *Applied Soil Ecology* 49: 46-58.

Patra AK, Abbadie L, Clays-Josserand A, Degrange V, Grayston SJ, et al. (2006). Effects of management regime and plant species on the enzyme activity and genetic. *Environmental Microbiology* 8: 1005-1016.

Patra AK, Abbadie L, Clays-Josserand A, Degrange V, Grayston SJ, et al. (2005). Effects of Grazing on Microbial Functional Groups Involved in Soil N Dynamics. *Ecological Monographs* 75: 65-80.

Petersen U, Wrage N, Köhler L, Leuschner C, Isselstein J (2012). Manipulating the species composition of permanent grasslands-A new approach to biodiversity experiments. *Basic and Applied Ecology* 13: 1-9.

Poll J, Marhan S, Haase S, Hallmann J, Kandeler E & Ruess L (2007). Low amounts of herbivory by root-knot nematodes affect microbial community dynamics and carbon allocation in the rhizosphere. *FEMS Microbiology Ecology* 62: 268-279.

Pruesse E, Quast C, Knittel K, Fuchs BM, Ludwig W, et al. (2007). SILVA: a comprehensive online resource for quality checked and aligned ribosomal RNA sequence data compatible with ARB. *Nucleic Acids Research* 35: 7188-7196.

Ramamoorthy V, Viswanathan R, Raguchander T, Prakasam V, Samiyappan R (2001). Induction of systemic resistance by plant growth promoting rhizobacteria in crop plants against pests and diseases. *Crop Protection* 20: 1-11.

R Development Core Team (2012). R: A Language and Environment for Statistical Computing. Vienna, Austria: *R Foundation for Statistical Computing*.

Singh BK, Munro S, Potts JM, Millard P (2007). Influence of grass species and soil type on rhizosphere microbial community structure in grassland soils. *Applied Soil Ecology* 36: 147-155.

Smalla K, Wieland G, Buchner A, Zock A, Parzy J, et al. (2001). Bulk and rhizosphere soil bacterial communities studied by denaturing gradient gel electrophoresis: plant-dependent enrichment and seasonal shifts revealed. *Applied and Environmental Microbiology* 67: 4742-4751.

Sørensen J (1997). The rhizosphere as a habitat for soil microorganisms. *Modern soil microbiology*: 21-45.

Steenwerth KL, Jackson LE, Calderón FJ, Stromberg MR, Scow KM (2002). Soil microbial community composition and land use history in cultivated and grassland ecosystems of coastal California. *Soil Biology and Biochemistry* 34: 1599-1611.

Techau M, Bjørnlund L, Christensen S (2004). Simulated herbivory effects on rhizosphere organisms in pea (*Pisum sativum*) depended on phosphate. *Plant and Soil* 264: 185-194.

Treonis AM, Grayston SJ, Murray PJ, Dawson LA (2005). Effects of root feeding, cranefly larvae on soil microorganisms and the composition of rhizosphere solutions collected from grassland plants. *Applied Soil Ecology* 28: 203- 215.

van der Heijden MG, Bardgett RD, van Straalen NM (2008). The unseen majority: soil microbes as drivers of plant diversity and productivity. *Ecology Letters* 11: 296-310.

Wemheuer B, Güllert S, Billerbeck S, Giebel H, Voget S, et al. (2014). Impact of a phytoplankton bloom on the diversity of the active bacterial community in the German Bight as revealed by metatranscriptomic approaches. *FEMS Microbiology Ecology* 87: 378-389.

Yang CH, Crowley DE (2000). Rhizosphere microbial community structure in

relation to root location and plant iron nutritional status. *Applied and Environmental Microbiology* 66: 345-351.

Yang J, Kloepper JW, Ryu CM (2009). Rhizosphere bacteria help plants tolerate abiotic stress. *Trends in Plant Science* 14: 1-4.

Zoetendal EG, von Wright A, Vilpponen-Salmela T, Ben-Amor K, Akkermans AD, et al. (2002). *Mucosa*-associated bacteria in the human gastrointestinal tract are uniformly distributed along the colon and differ from the community recovered from feces. *Applied and Environmental Microbiology* 68: 3401-3407.

4.2. Supplemental information

Tab. S1: pH values of grassland soils subjected to different fertilization and mowing regimes as well as above-ground herbivory at soil depths of 1-5 cm.

Treatment	Sward composition	Mowing	Fertilization	Herbivory	pH KCl	SE	n
1	species-rich	once	no	control	4.60	0.36	6
	species-rich	once	no	herbivory	4.63	0.19	6
2	species-rich	once	NPK	control	4.87	0.29	6
	species-rich	once	NPK	herbivory	4.63	0.28	6
3	species-rich	thrice	no	control	4.63	0.17	5
	species-rich	thrice	no	herbivory	4.57	0.10	6
4	species-rich	thrice	NPK	control	4.77	0.18	6
	species-rich	thrice	NPK	herbivory	4.75	0.27	6
5	dicot-reduced	once	no	control	4.54	0.25	6
	dicot-reduced	once	no	herbivory	4.65	0.40	5
6	dicot-reduced	once	NPK	control	4.59	0.20	6
	dicot-reduced	once	NPK	herbivory	4.58	0.10	6
7	dicot-reduced	thrice	no	control	4.80	0.41	5
	dicot-reduced	thrice	no	herbivory	4.62	0.22	6
8	dicot-reduced	thrice	NPK	control	4.56	0.23	6
	dicot-reduced	thrice	NPK	herbivory	4.47	0.21	6
9	monocot-reduced	once	no	control	4.50	0.20	4
	monocot-reduced	once	no	herbivory	4.59	0.19	5
10	monocot-reduced	once	NPK	control	4.60	0.16	6
	monocot-reduced	once	NPK	herbivory	4.63	0.11	6
11	monocot-reduced	thrice	no	control	4.42	0.19	6
	monocot-reduced	thrice	no	herbivory	4.50	0.21	6
12	monocot-reduced	thrice	NPK	control	4.63	0.20	6
	monocot-reduced	thrice	NPK	herbivory	4.76	0.28	6

Tab. S2: Observed Operational Taxonomic Units (OTUs) and alpha diversity indices at 1%, 3%, and 20% genetic distance. Number of observed clusters, ACE indices, Shannon indices, Chao1 indices, and the maximal OTU number (michaelis_menten_fit index) were calculated with QIIME [63]. Coverage was determined based on observed clusters and the maximal OTU number. To compare community structures, 2,280 randomly selected sequences from each sample were used for the calculations.

Sample	*Observed OTUs*			*Max. OTU number*			*Coverage (%)*			*ACE*			*Chao1*			*Shannon*		
	80%	*97%*	*99%*	*80%*	*97%*	*99%*	*80%*	*97%*	*99%*	*80%*	*97%*	*99%*	*80%*	*97%*	*99%*	*80%*	*97%*	*99%*
Lys02L	124.40	613.20	708.10	168.58	1001.99	1208.45	73.79	61.20	58.60	200.63	1364.01	1525.41	195.51	1604.65	1880.80	2.99	5.63	5.86
Lys02R	137.10	707.90	812.10	184.55	1419.44	1683.01	74.29	49.87	48.25	221.31	2230.31	2553.97	220.51	2531.96	3518.24	3.09	5.63	5.88
Lys04L	117.00	588.40	615.20	152.30	878.42	920.01	76.82	66.98	66.87	217.03	973.71	986.25	201.04	1108.66	1137.23	3.17	5.71	5.79
Lys04R	102.50	470.70	516.40	128.17	662.04	741.54	79.97	71.10	69.64	162.52	784.23	897.99	154.21	938.67	1196.40	2.96	5.34	5.48
Lys06L	137.20	714.50	773.20	182.57	1301.03	1422.44	75.15	54.92	54.36	222.91	1720.44	1809.08	218.79	1933.13	2128.75	3.13	5.75	5.89
Lys06R	152.80	888.40	1010.40	195.54	2196.83	2721.60	78.14	40.44	37.13	236.11	3946.04	5273.56	254.00	4064.21	6879.47	3.42	5.96	6.20
Lys07L	131.90	751.30	863.60	175.67	1307.26	1576.65	75.08	57.47	54.77	225.19	1539.07	1722.25	210.18	1639.08	1836.72	3.18	5.96	6.20
Lys07R	127.00	689.40	784.30	166.51	1267.56	1502.44	76.27	54.39	52.20	195.70	1736.50	2026.33	188.50	2018.92	2571.20	3.02	5.62	5.88
Lys08L	169.40	967.90	1081.50	224.07	2510.03	3120.77	75.60	38.56	34.65	272.53	4289.96	5557.27	264.58	4414.91	6756.57	3.45	6.10	6.27
Lys08R	135.90	675.20	760.70	178.50	1228.68	1381.90	76.13	54.95	55.05	215.70	1690.94	1815.09	213.74	1890.07	2214.62	3.26	5.68	5.93
Lys09L	137.50	679.30	754.50	182.86	1220.89	1389.77	75.20	55.64	54.29	216.25	1798.06	1954.09	211.41	2143.97	2344.01	3.20	5.71	5.90
Lys09R	133.60	684.90	761.30	181.44	1290.96	1413.66	73.64	53.05	53.85	209.39	1790.39	1878.50	205.21	2116.47	2353.94	3.04	5.61	5.88
Lys10L	149.20	755.60	834.00	194.84	1511.56	1729.33	76.58	49.99	48.23	222.03	2450.08	2697.58	221.45	2866.43	3511.94	3.40	5.82	5.98
Lys10R	139.20	704.60	775.30	184.14	1376.68	1531.70	75.59	51.18	50.62	227.23	2376.65	2622.32	242.36	2849.38	3870.21	3.21	5.69	5.88
Lys11L	124.00	635.40	701.60	169.72	1148.32	1256.79	73.06	55.33	55.82	205.66	1741.26	1753.94	195.38	1966.87	2121.43	2.88	5.50	5.73
Lys11R	133.20	713.80	812.30	173.16	1539.47	1844.10	76.92	46.37	44.05	217.33	2854.44	3470.23	221.85	3396.18	5561.57	3.16	5.58	5.82
Lys12L	150.50	818.10	924.30	194.55	1813.60	2286.28	77.36	45.11	40.43	234.17	3111.08	4177.66	230.48	3192.01	5302.55	3.40	5.86	6.04
Lys12R	122.90	610.10	675.80	163.87	1078.12	1198.98	75.00	56.59	56.36	213.29	1612.31	1659.61	213.66	1942.62	2113.82	2.97	5.46	5.65
Lys14L	138.60	705.00	779.30	181.58	1257.44	1417.55	76.33	56.07	54.98	206.06	1781.28	2037.97	211.69	2178.10	2736.29	3.24	5.82	6.02
Lys14R	134.60	786.50	898.60	171.70	1742.72	2191.38	78.39	45.13	41.01	206.91	3195.23	4555.72	214.06	3460.09	6024.78	3.31	5.82	6.03
Lys20L	134.90	675.60	794.20	177.55	1293.59	1685.77	75.98	52.23	47.11	207.92	2052.92	2879.40	204.58	2162.22	3823.17	3.13	5.61	5.82
Lys20R	152.40	815.40	917.40	203.73	1835.56	2195.07	74.81	44.42	41.79	253.06	3370.62	3999.23	251.56	3706.62	5322.22	3.24	5.84	6.04
Lys22L	158.50	1044.80	1186.30	205.73	3436.94	4706.66	77.04	30.40	25.20	240.38	5872.04	10226.30	240.26	4992.06	8827.88	3.48	6.09	6.34
Lys22R	163.00	866.00	947.30	216.33	2030.21	2453.99	75.35	42.66	38.60	264.53	3771.14	4993.91	275.45	3903.51	6051.90	3.51	5.97	6.09
Lys23L	110.50	490.90	506.20	133.87	676.31	705.56	82.54	72.59	71.74	176.83	746.41	800.34	171.59	906.67	998.79	3.24	5.52	5.55
Lys23R	92.80	531.50	546.50	105.90	741.98	767.93	87.63	71.63	71.17	128.47	780.93	800.94	128.36	908.19	925.09	3.22	5.67	5.70
Lys27L	132.40	719.20	815.90	174.47	1412.66	1642.76	75.89	50.91	49.67	216.75	2277.72	2642.58	221.07	2790.22	3763.79	3.18	5.73	5.97
Lys27R	135.30	709.90	817.00	174.05	1375.95	1632.01	77.74	51.59	50.06	196.09	2245.04	2494.40	188.35	2766.23	3490.92	3.29	5.73	6.00
Lys28L	153.00	887.60	1017.10	199.62	2169.49	2754.93	76.65	40.91	36.92	231.84	3788.49	5404.44	223.67	4048.60	7715.37	3.40	5.95	6.23
Lys28R	131.00	755.60	858.00	168.16	1344.81	1544.56	77.90	56.19	55.55	205.04	1657.87	1752.96	196.17	1795.84	2009.55	3.28	5.96	6.25
Lys29L	126.20	629.20	720.00	169.70	1130.64	1303.95	74.37	55.65	55.22	210.40	1698.94	1735.93	206.11	1923.23	2106.96	2.98	5.50	5.75
Lys29R	128.40	639.90	724.70	178.79	1120.06	1306.53	71.81	57.13	55.47	221.85	1528.13	1734.60	212.56	1718.57	2087.78	2.93	5.58	5.79
Lys30L	142.20	688.10	768.20	192.62	1249.57	1416.22	73.82	55.07	54.24	242.86	1817.90	1909.38	230.97	2103.55	2279.68	3.20	5.67	5.88

Tab. S2: continued.

Sample	*Observed OTUs*			*Max. OTU number*			*Coverage (%)*			*ACE*			*Chao1*			*Shannon*		
	80%	*97%*	*99%*	*80%*	*97%*	*99%*	*80%*	*97%*	*99%*	*80%*	*97%*	*99%*	*80%*	*97%*	*99%*	*80%*	*97%*	*99%*
Lys30R	159.90	822.30	957.50	211.67	1868.18	2380.07	75.54	44.02	40.23	247.76	2934.14	3649.31	247.42	3179.95	4523.74	3.48	5.80	6.07
Lys31L	133.70	662.90	755.60	175.30	1211.72	1441.56	76.27	54.71	52.42	210.89	1646.03	1861.35	218.73	1866.55	2207.82	3.22	5.53	5.74
Lys31R	132.70	648.90	764.20	171.16	1205.28	1483.60	77.53	53.84	51.51	207.86	1923.17	2111.38	203.92	2206.34	2692.24	3.15	5.50	5.79
Lys33L	101.40	457.30	553.50	133.58	748.86	933.45	75.91	61.07	59.30	152.87	1211.14	1444.46	151.82	1352.07	1852.43	2.65	4.94	5.29
Lys33R	120.10	617.90	712.40	157.86	1100.85	1288.07	76.08	56.13	55.31	181.78	1570.99	1716.97	176.89	1733.68	2053.00	2.94	5.47	5.76
Lys36L	121.40	587.60	655.60	168.20	1025.58	1128.25	72.18	57.29	58.11	206.25	1563.93	1589.38	200.20	1808.04	1996.19	2.88	5.41	5.63
Lys36R	135.80	699.90	786.60	183.16	1388.37	1535.81	74.14	50.41	51.22	217.95	2130.46	2269.74	217.68	2526.99	3225.60	2.99	5.59	5.86
Lys37L	135.00	756.80	839.30	175.67	1478.60	1670.54	76.85	51.18	50.24	198.00	2370.97	2616.99	197.81	3006.85	3754.14	3.15	5.84	6.08
Lys37R	126.10	724.30	854.10	163.10	1733.00	2165.42	77.32	41.79	39.44	200.23	3385.80	4839.11	199.01	3146.18	6147.46	3.04	5.49	5.86
Lys38L	126.90	663.00	743.00	165.25	1125.51	1286.48	76.79	58.91	57.75	213.20	1501.90	1584.54	216.94	1655.79	1789.88	3.20	5.75	5.96
Lys38R	139.00	721.70	802.00	184.79	1309.40	1474.78	75.22	55.12	54.38	211.09	1863.92	1938.11	201.15	2127.29	2312.06	3.25	5.84	6.07
Lys40L	125.10	712.00	833.60	156.18	1502.08	1823.71	80.10	47.40	45.71	185.73	2676.19	3399.58	188.27	2700.32	5013.58	3.18	5.63	5.95
Lys40R	127.60	723.30	831.80	164.26	1488.83	1778.35	77.68	48.58	46.77	199.59	2489.79	2989.29	201.00	2974.71	4460.57	3.15	5.68	5.94
Lys41L	125.40	615.90	703.80	159.45	1002.48	1185.80	78.65	61.44	59.35	205.52	1246.04	1402.96	201.36	1356.61	1530.74	3.24	5.63	5.86
Lys41R	156.60	813.80	925.70	202.90	1807.92	2166.17	77.18	45.01	42.73	240.51	3089.32	3659.52	236.19	3607.25	4952.03	3.51	5.85	6.10
Lys43L	115.70	628.70	717.80	150.10	1089.98	1234.26	77.08	57.68	58.16	193.00	1430.94	1492.82	191.49	1540.46	1695.69	3.02	5.56	5.86
Lys43R	118.00	658.20	793.30	153.20	1193.55	1524.10	77.02	55.15	52.05	186.36	1617.69	1832.38	182.10	1712.27	2019.81	3.05	5.59	5.91
Lys44L	140.90	709.00	798.90	190.02	1395.81	1609.61	74.15	50.79	49.63	240.00	2218.70	2405.03	233.06	2723.59	3292.70	3.22	5.67	5.88
Lys44R	136.80	784.90	921.00	170.16	1902.55	2645.66	80.39	41.26	34.81	204.75	3272.74	5810.00	199.75	3008.28	5959.91	3.42	5.62	5.87
Lys47L	134.40	690.50	792.50	174.07	1408.84	1679.25	77.21	49.01	47.19	198.52	2296.38	2663.35	197.20	2696.86	3651.99	3.14	5.54	5.80
Lys47R	122.20	655.40	793.70	154.22	1064.94	1361.81	79.24	61.54	58.28	204.11	1206.90	1420.85	202.18	1274.13	1493.25	3.27	5.77	6.13
Lys48L	149.90	921.60	1085.00	187.62	2496.16	3473.12	79.90	36.92	31.24	219.16	4450.74	7679.82	211.54	4037.66	8800.12	3.48	5.94	6.24
Lys48R	147.00	750.50	844.20	191.83	1523.18	1793.60	76.63	49.27	47.07	222.68	2558.71	3014.31	215.48	2977.90	4259.41	3.39	5.77	5.97
Lys55L	148.70	814.50	942.10	192.05	1761.81	2218.63	77.43	46.23	42.46	238.54	2975.24	3895.65	224.93	3372.36	5482.30	3.37	5.88	6.14
Lys55R	140.40	741.30	838.10	186.10	1760.34	2084.64	75.44	42.11	40.20	206.06	3492.35	4646.39	202.29	3289.92	6285.88	3.13	5.58	5.82
Lys56L	135.70	637.30	750.90	177.98	1052.85	1271.97	76.25	60.53	59.03	211.47	1328.26	1455.68	202.84	1438.20	1641.13	3.20	5.68	6.02
Lys56R	110.10	562.30	697.70	137.60	859.05	1119.90	80.02	65.46	62.30	174.74	1004.28	1197.72	173.05	1091.27	1320.93	3.10	5.51	5.93
Lys62L	141.90	782.70	876.00	184.88	1826.56	2066.45	76.75	42.85	42.39	227.65	3248.01	3849.42	237.84	3886.90	5951.37	3.26	5.69	5.96
Lys62R	140.90	774.70	871.60	185.74	1658.70	1912.94	75.86	46.71	45.56	215.88	2873.92	3211.71	208.47	3440.93	4916.07	3.16	5.77	6.02
Lys64L	125.70	667.20	787.60	167.86	1145.97	1426.57	74.88	58.22	55.21	213.54	1368.42	1582.89	205.35	1340.94	1571.61	3.04	5.73	6.00
Lys64R	126.10	672.00	778.60	166.15	1179.30	1395.59	75.90	56.98	55.79	202.40	1486.07	1614.79	191.90	1602.96	1731.96	3.12	5.69	5.98
Lys67L	149.60	873.40	992.00	189.61	2244.28	2736.72	78.90	38.92	36.25	212.07	4207.05	5790.60	205.10	3836.27	8024.21	3.39	5.87	6.12
Lys67R	150.40	808.50	927.80	192.67	1722.22	2139.62	78.06	46.95	43.36	216.99	2812.65	3704.16	211.75	3374.15	5217.90	3.47	5.89	6.13
Lys68L	152.70	961.00	1096.60	200.33	2885.49	3977.67	76.22	33.30	27.57	230.58	5538.82	9245.21	234.93	5112.44	10786.18	3.41	5.95	6.16
Lys68R	136.80	714.80	801.60	179.15	1406.60	1545.16	76.36	50.82	51.88	210.06	2322.89	2309.22	200.34	3011.73	3235.55	3.20	5.71	6.00
Lys70L	140.00	706.50	776.60	185.02	1346.22	1496.11	75.67	52.48	51.91	226.09	2112.45	2286.81	215.77	2612.04	3119.94	3.22	5.72	5.93
Lys70R	146.60	814.10	924.50	193.73	1889.15	2183.08	75.67	43.09	42.35	225.60	3336.21	3884.92	222.32	3623.32	5935.47	3.22	5.82	6.10

Tab. S2: continued.

Sample	*Observed OTUs*			*Max. OTU number*			*Coverage (%)*			*ACE*			*Chao1*			*Shannon*		
	80%	*97%*	*99%*	*80%*	*97%*	*99%*	*80%*	*97%*	*99%*	*80%*	*97%*	*99%*	*80%*	*97%*	*99%*	*80%*	*97%*	*99%*
Lys71L	147.80	761.40	821.50	197.19	1502.13	1657.54	74.95	50.69	49.56	221.85	2440.77	2625.47	223.65	3046.96	3523.22	3.18	5.84	5.97
Lys71R	152.30	842.00	948.00	198.32	2204.90	2629.08	76.80	38.19	36.06	229.33	4194.41	5540.61	238.22	4332.39	8038.56	3.29	5.70	5.99

Tab. S3: Relative abundances of abundant bacterial phyla and proteobacterial classes with respect to the different treatments (Supplemental Tab. S1) and the above-ground herbivory (c=control, h=herbivory).

Treatment	**1**		**3**		**2**		**4**		**6**		**7**		**5**		**8**		**9**		**11**		**10**		**12**		**Mean value (%)**
Phyla	c	h	c	h	c	h	c	h	c	h	c	h	c	h	c	h	c	h	c	h	c	h	c	h	
Acidobacteria	0.26	0.24	0.27	0.3	0.24	0.21	0.22	0.24	0.27	0.26	0.25	0.22	0.22	0.22	0.22	0.35	0.23	0.22	0.22	0.24	0.21	0.25	0.24	0.3	24.63
Actinobacteria	0.15	0.15	0.17	0.13	0.16	0.18	0.19	0.16	0.13	0.13	0.15	0.15	0.2	0.18	0.17	0.13	0.16	0.15	0.21	0.19	0.17	0.15	0.17	0.15	16.16
Bacteroidetes	0.06	0.08	0.05	0.05	0.05	0.07	0.06	0.07	0.07	0.08	0.07	0.07	0.05	0.05	0.07	0.04	0.06	0.08	0.05	0.04	0.07	0.06	0.05	0.07	6.18
Chloroflexi	0.03	0.03	0.03	0.02	0.02	0.03	0.04	0.03	0.03	0.03	0.03	0.04	0.03	0.03	0.03	0.02	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	2.97
Firmicutes	0.04	0.03	0.03	0.03	0.02	0.02	0.03	0.02	0.05	0.03	0.03	0.06	0.06	0.04	0.06	0.04	0.06	0.03	0.05	0.04	0.04	0.02	0.03	0.02	3.59
Gemmatimonadetes	0.03	0.03	0.03	0.03	0.04	0.04	0.03	0.04	0.02	0.02	0.02	0.02	0.03	0.03	0.02	0.03	0.03	0.02	0.02	0.02	0.04	0.04	0.03	0.03	2.98
Alphaproteobacteria	0.21	0.22	0.21	0.21	0.25	0.23	0.21	0.22	0.19	0.22	0.2	0.2	0.22	0.26	0.22	0.22	0.21	0.21	0.25	0.24	0.24	0.21	0.21	0.19	21.77
Betaproteobacteria	0.08	0.08	0.08	0.08	0.07	0.08	0.09	0.08	0.07	0.07	0.08	0.08	0.05	0.05	0.04	0.05	0.08	0.1	0.06	0.07	0.06	0.08	0.08	0.08	7.27
Gammaproteobacteria	0.03	0.04	0.04	0.04	0.06	0.05	0.04	0.05	0.05	0.04	0.04	0.03	0.04	0.05	0.06	0.04	0.04	0.05	0.04	0.03	0.06	0.06	0.06	0.05	4.72
Deltaproteobacteria	0.06	0.06	0.05	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.07	0.07	0.05	0.05	0.05	0.05	0.06	0.07	0.05	0.05	0.05	0.06	0.06	0.05	5.59
other bacteria	0.05	0.05	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.05	0.06	0.06	0.03	0.04	0.05	0.04	0.04	0.04	0.04	0.03	0.03	0.04	0.04	0.03	4.13

Tab. S4: Relative abundances of rare bacterial phyla with respect to the different treatments (Supplemental Tab. S1) and the above-ground herbivory (c=control, h=herbivory).

Treatment	1		3		2		4		6		7		5		8		9		11		10		12		Mean value (%)
Phyla	c	h	c	h	c	h	c	h	c	h	c	h	c	h	c	h	c	h	c	h	c	h	c	h	
Armatimonadetes	1E-04	3E-04	5E-05	0	6E-05	3E-04	3E-04	3E-04	1E-04	5E-05	3E-04	4E-04	9.2E-05	2E-04	3E-04	2E-04	8E-05	2E-04	9E-05	1E-04	0	4E-04	2E-04	1E-04	0.018
BD1-5	6E-05	4E-04	0	6E-05	6E-05	8E-05	2E-04	6E-05	1E-04	0	1E-04	0	7E-05	5E-05	1E-04	0	4E-05	3E-05	4E-05	1E-04	0	0	2E-04	0	0.008
BHI80-139	2E-04	3E-04	2E-04	4E-04	0	6E-05	2E-04	7E-05	3E-05	1E-04	1E-04	5E-04	0	7E-05	0	9E-05	2E-04	2E-04	2E-04	0	0	0	0	4E-05	0.012
Candidate division BRC1	1E-04	2E-04	0	6E-05	1E-04	1E-04	3E-04	0	5E-05	0	4E-05	1E-04	0.00014	4E-05	7E-05	0	4E-04	0	1E-04	0	7E-05	7E-05	0	1E-04	0.009
Candidate division OD1	5E-04	3E-04	1E-04	3E-04	7E-04	5E-04	1E-04	3E-04	6E-04	5E-04	5E-04	3E-04	0.00047	4E-04	6E-04	7E-04	3E-04	2E-04	2E-04	1E-04	1E-04	4E-04	4E-04	4E-05	0.036
Candidate division OP11	5E-04	7E-05	1E-04	0	2E-04	2E-04	5E-04	4E-04	8E-04	5E-04	6E-04	4E-04	0.0003	6E-04	0.002	7E-04	4E-05	2E-04	2E-04	0	3E-04	1E-04	2E-04	7E-05	0.038
Candidate division OP3	2E-04	2E-04	5E-05	1E-04	5E-05	1E-04	0	0	1E-04	1E-04	2E-04	3E-04	6.5E-05	9E-05	2E-04	2E-04	8E-05	1E-04	9E-05	6E-05	0	0	1E-04	1E-04	0.011
Candidate division TM7	4E-04	4E-04	1E-04	6E-05	6E-04	3E-04	0.001	5E-04	3E-04	7E-04	4E-04	4E-04	0.00026	6E-04	7E-04	4E-04	5E-04	7E-04	3E-04	9E-05	3E-04	7E-04	5E-04	2E-04	0.044
Candidate division WS3	0.003	0.005	0.01	0.007	0.001	0.003	0.005	0.005	0.004	0.007	0.005	0.01	0.0048	0.002	0.001	0.001	0.006	0.005	0.003	0.003	0.002	9E-04	0.002	0.002	0.408
Candidate division WS6	0	0	0	0	6E-05	0	0	0	0	3E-05	8E-05	1E-04	3.8E-05	0	2E-04	3E-04	0	3E-05	0	0	0	6E-05	0	4E-05	0.004
Chlamydiae	0	0	0	0	0	0	5E-05	0	0	0	1E-04	0	0	4E-05	2E-04	7E-05	8E-05	0	2E-04	0	0	0	0	0	0.003
Chlorobi	0.002	0.002	0.001	9E-04	0.002	0.002	0.001	0.001	0.003	0.002	0.002	0.002	0.00167	0.002	0.002	0.003	0.002	0.001	0.002	0.001	0.002	0.002	0.001	0.002	0.185
Cyanobacteria	0.004	0.004	0.002	0.005	0.006	0.005	0.005	0.005	0.004	0.005	0.005	0.004	0.003	0.004	0.006	0.008	0.003	0.003	0.003	0.004	0.004	0.005	0.005	0.005	0.446
Deferribacteres	0	0	0	0	0	0	0	0	0	6E-05	0	0	0	0	0	0	7E-05	0	0	0	0	0	0	0	0.001
Deinococcus-Thermus	0	0	0	0	0	6E-05	0	0	0	3E-05	0	0	0	0	0	0	0	0	0	0	6E-05	0	0	0	0.001
Elusimicrobia	0.005	0.005	0.004	0.006	0.005	0.005	0.003	0.004	0.006	0.006	0.007	0.004	0.00434	0.005	0.006	0.004	0.004	0.006	0.003	0.004	0.003	0.007	0.006	0.003	0.476
Fibrobacteres	0.002	0.002	0.002	0.001	0.003	0.003	0.001	0.003	0.003	0.003	0.002	0.002	0.00189	0.002	0.002	0.001	9E-04	0.003	8E-04	0.001	0.003	0.003	0.002	0.002	0.211

Tab. S4: continued

Treatment	1		3		2		4		6		7		5		8		9		11		10		12		Mean value (%)
Fusobacteria	0	0	5E-05	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.000
Kazan-3B-28	0	0	5E-05	0	1E-04	0	4E-04	0	0	0	0	0	3E-05	0	0	4E-05	0	0	0	0	0	0	0	0	0.002
MVP-21	6E-05	0	0	0	0	4E-05	0	1E-04	0	0	0	0	0	0	1E-04	0	5E-05	0	0	0	0	0	0	4E-05	0.002
NPL-UPA2	0	0	0	0	0	0	0	0	0	0	0	1E-04	0	0	0	0	0	0	0	0	0	0	0	0	0.001
Nitrospirae	0.005	0.005	0.005	0.006	0.004	0.003	0.007	0.003	0.005	0.005	0.005	0.008	0.00396	0.003	0.002	0.005	0.008	0.005	0.004	0.003	0.004	0.002	0.003	0.003	0.454
Planctomycetes	0.01	0.008	0.007	0.004	0.004	0.007	0.007	0.002	0.004	0.005	0.011	0.013	0.00469	0.004	0.01	0.006	0.006	0.005	0.01	0.006	0.002	0.006	0.004	0.003	0.622
SM2F11	8E-04	0.002	0.001	0.001	0.002	7E-04	0.001	0.002	0.003	0.003	0.003	0.002	0.00116	0.001	0.002	0.002	0.002	0.002	0.001	0.002	8E-04	0.001	0.001	9E-04	0.159
Spirochaetes	2E-04	6E-04	3E-04	3E-04	0.001	5E-04	1E-04	4E-04	9E-04	6E-04	7E-04	6E-04	0.00026	2E-04	1E-04	4E-04	2E-04	5E-04	1E-04	2E-04	5E-04	3E-04	8E-04	7E-04	0.044
TA06	0	0	0	0	0	1E-04	1E-04	7E-05	0	0	0	0	7E-05	1E-04	0	5E-05	5E-05	0	5E-05	0	1E-04	0	5E-05	1E-04	0.004
TM6	9E-04	9E-04	7E-04	4E-04	6E-04	6E-04	7E-04	8E-04	0.001	8E-04	9E-04	9E-04	0.00032	6E-04	0.001	9E-04	5E-04	9E-04	3E-04	5E-04	8E-04	3E-04	0.001	1E-03	0.075
Tenericutes	0	7E-05	0	0	0	0	0	0	0	1E-04	0	0	0	0	0	0	0	0	0	0	0	1E-04	0	0	0.001
Thermotogae	0	0	5E-05	0	1E-04	0	2E-04	3E-04	1E-04	1E-04	1E-04	0	0	0	3E-05	4E-05	1E-04	0	9E-05	0	0	1E-04	2E-04	4E-05	0.007
Verrucomicrobia	0.006	0.007	0.002	0.002	0.005	0.005	0.002	0.001	0.005	0.004	0.008	0.006	0.00422	0.005	0.007	0.004	0.003	0.004	0.004	0.002	0.003	0.004	0.004	0.002	0.420
WCHB1-60	0.003	0.003	0.001	0.002	0.002	0.002	0.002	0.002	0.001	0.001	0.002	9E-04	0.0017	0.002	0.005	0.002	0.003	0.002	0.002	0.002	0.003	0.002	0.002	0.002	0.206

Tab. S5: Relative abundances and taxonomic affiliations of the 25 most abundant OTUs with respect to the different treatments (Supplemental Tab. S1) and the above-ground herbivory (c=control, h=herbivory).

OTU ID	taxonomic affiliation	Rel. abundance (%)
15254	Bacteria;Proteobacteria;Alphaproteobacteria;Rhizobiales;Bradyrhizobiaceae;Bradyrhizobium;Bradyrhizobium sp.	4.80
3020	Bacteria;Acidobacteria;Acidobacteria;Candidatus Solibacter;uncultured Acidobacteria bacterium	1.95
15334	Bacteria;Proteobacteria;Alphaproteobacteria;Rhizobiales;Xanthobacteraceae;uncultured;bacterium Ellin6561	1.90
430	Bacteria;Acidobacteria;Acidobacteria;Acidobacteriales;Acidobacteriaceae;Candidatus Koribacter;uncultured bacterium	1.75
31887	Bacteria;Firmicutes;Bacilli;4-15;uncultured Bacillaceae bacterium	1.34
10498	Bacteria;Proteobacteria;Alphaproteobacteria;Rhizobiales;alphaI cluster;uncultured bacterium	1.13
33544	Bacteria;Acidobacteria;Acidobacteria;DA052;uncultured bacterium	1.07
30880	Bacteria;Acidobacteria;Acidobacteria;Order Incertae Sedis;Family Incertae Sedis;Bryobacter;uncultured Acidobacteria	1.05
26950	Bacteria;Acidobacteria;Acidobacteria;Acidobacteriales;Acidobacteriaceae;uncultured;uncultured bacterium	1.03
15204	Bacteria;Proteobacteria;Alphaproteobacteria;Rhizobiales;Family Incertae Sedis;Rhizomicrobium;uncultured bacterium	0.97
35896	Bacteria;Proteobacteria;Betaproteobacteria;Burkholderiales;Comamonadaceae;uncultured;uncultured bacterium	0.91
1434	Bacteria;Bacteroidetes;Sphingobacteriia;Sphingobacteriales;Chitinophagaceae;uncultured;uncultured bacterium	0.91
43557	Bacteria;Acidobacteria;Acidobacteria;Acidobacteriales;Acidobacteriaceae;uncultured;uncultured bacterium	0.88
10655	Bacteria;Bacteroidetes;Cytophagia;Cytophagales;Cytophagaceae;Flexibacter;uncultured bacterium	0.84
42418	Bacteria;Actinobacteria;Actinobacteria;Frankiales;Acidothermaceae;Acidothermus;uncultured bacterium	0.79
10041	Bacteria;Proteobacteria;Alphaproteobacteria;Rhizobiales;Beijerinckiaceae;uncultured;uncultured proteobacterium	0.79
23893	Bacteria;Proteobacteria;Alphaproteobacteria;Rhodospirillales;Acetobacteraceae;uncultured;uncultured bacterium	0.74
24136	Bacteria;Proteobacteria;Alphaproteobacteria;Rhizobiales;Xanthobacteraceae;uncultured;uncultured bacterium	0.72
17761	Bacteria;Acidobacteria;Acidobacteria;Acidobacteriales;Acidobacteriaceae;uncultured;uncultured bacterium	0.68
10097	Bacteria;Acidobacteria;Acidobacteria;Candidatus Solibacter;uncultured Acidobacteria bacterium	0.67
1655	Bacteria;Proteobacteria;Alphaproteobacteria;Rhodospirillales;DA111;uncultured bacterium	0.63
30883	Bacteria;Proteobacteria;Betaproteobacteria;Nitrosomonadales;Nitrosomonadaceae;uncultured;uncultured beta	0.61
41226	Bacteria;Proteobacteria;Alphaproteobacteria;Rhodospirillales;DA111;uncultured bacterium	0.61
22924	Bacteria;Proteobacteria;Alphaproteobacteria;Rhodospirillales;JG37-AG-20;uncultured Rhodospirillaceae bacterium	0.60
38931	Bacteria;Acidobacteria;Acidobacteria;Candidatus Solibacter;uncultured Acidobacteria bacterium	0.58

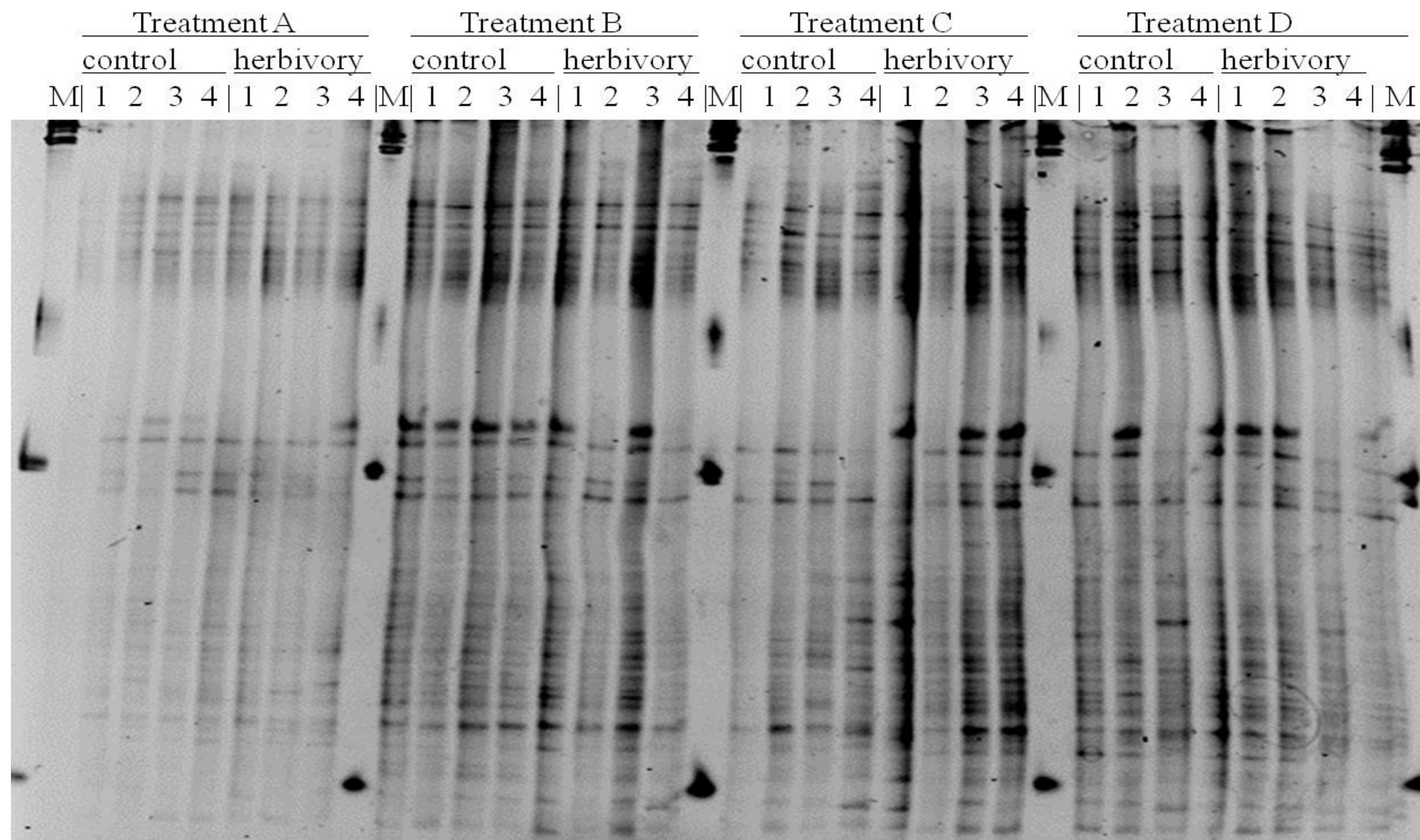

Fig.S1. 16S-DGGE profile of dicot-reduced plots showing the influence of different fertilization and mowing regimes as well as above-ground herbivory on bacterial communities in the rhizosphere. Soil samples were taken in summer 2011. Independent replicates are indicated with numbers from 1 to 4. Treatment A: 1 x mowing/ year, no NPK; treatment B: 3 x mowing/ year, no NPK; treatment C: 1 x mowing/ year, NPK; treatment D: 3 x mowing/ year, NPK. M: GeneRuler *1 kb* DNA *Ladder* (Fermentas, St. Leon-Rot, Germany).

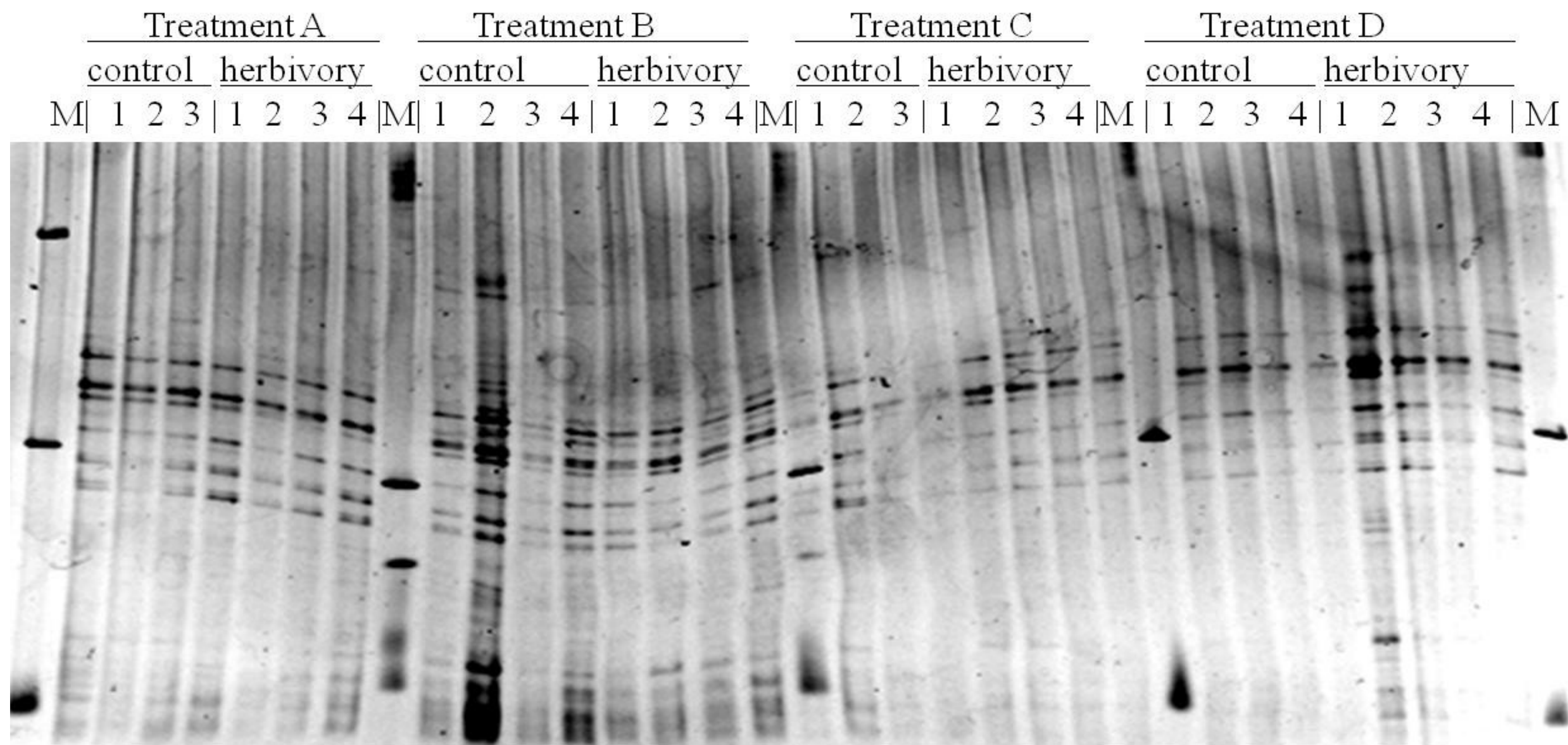

Fig.S2. 16S-DGGE profile of dicot-reduced plots showing the influence of different fertilization and mowing regimes as well as above-ground herbivory on bacterial communities in the rhizosphere. For further details see Fig. S1.

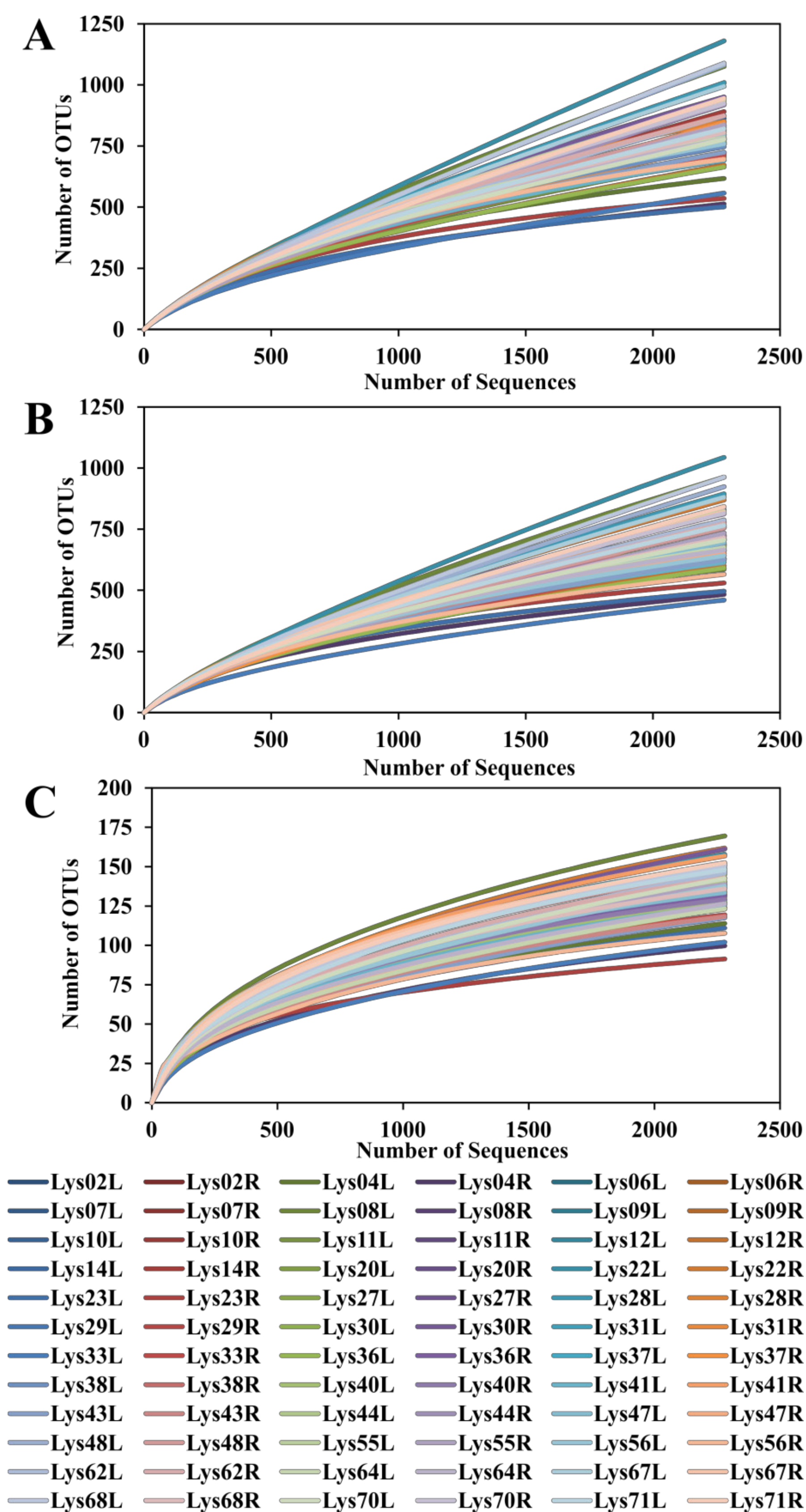

Fig. S3. Rarefaction Curves at 1% (A), 3% (B), and 20% (C) genetic distance for all 72 samples analyzed by pyrosequencing. Curves were calculated in QIIME (Caporaso *et al.*, 2010).

Chapter V

General discussion

Soil is considered to be the most complex environment with respect to microbial species richness and community size (Torsvik et al., 2002; Daniel, 2005; Tringe et al., 2005). Despite their importance for biogeochemical cycling of nitrogen, phosphorous, and carbon, the ecology and functions of bacterial communities in soil ecosystems are not fully understood. The development of culture-independent molecular approaches has greatly advanced the understanding of soil bacterial community structure and diversity. However, most previous studies used DNA-based techniques, which are not able to distinguish between active and inactive community members.

In this thesis, the influence of fertilizer application and season on total and active bacterial communities in a grassland soil (Chapter II) was investigated. In addition, active and total bacterial communities in soil samples from two genetically distinct aspen demes (Chapter III) were analyzed. In total, 216 soil samples were collected over two years (2010 and 2011) in April, July, and September. Environmental DNA and RNA were co-isolated and analyzed by pyrotag-based sequencing. The impacts of fertilizer application, aspen demes, sampling time, and soil properties on bacterial community composition, diversity, and abundance were analyzed by statistical analyses. Furthermore, functional analyses of the active and total bacterial community were performed using Tax4Fun.

In addition to soil as habitat for bacteria, plant-associated bacteria in the rhizosphere of permanent grasslands were investigated with culture-independent approaches (Chapter IV). The impact of fertilizer application, mowing frequency, sward composition, and herbivory exposure on bacterial structure in the rhizosphere was analyzed.

5.1. Bacterial community composition in soil

The majority of studies presented in this thesis focused on investigating bacterial communities and their functions in soils. In the studies from Chapter II and III, more than 4,75 million partial 16S rRNA gene and gene transcript sequences were analyzed. The dominant phyla in the bacterial community of a grassland soil were *Proteobacteria* (DNA 31.1%, RNA 45.7%), *Firmicutes* (DNA 27.4%, RNA 35.7%), *Chloroflexi* (DNA 17%, RNA 9.1%), *Acidobacteria* (DNA 13.3%, RNA 3.4%), and *Actinobacteria* (DNA 6%, RNA 3.4%) (Figure 1A and B). The five phyla accounted for up to 96% of all analyzed

sequences. Similar results were obtained in soil communities of two aspen demes. Here, the dominant phyla were *Proteobacteria* (DNA 32.4%, RNA 44.2%), *Firmicutes* (DNA 27.2%, RNA 36.5%), *Chloroflexi* (DNA 16%, RNA 9.1%), *Acidobacteria* (DNA 13.3%, RNA 3.5%), and *Actinobacteria* (DNA 6.1%, RNA 3.8%) (Figure 1A and B).

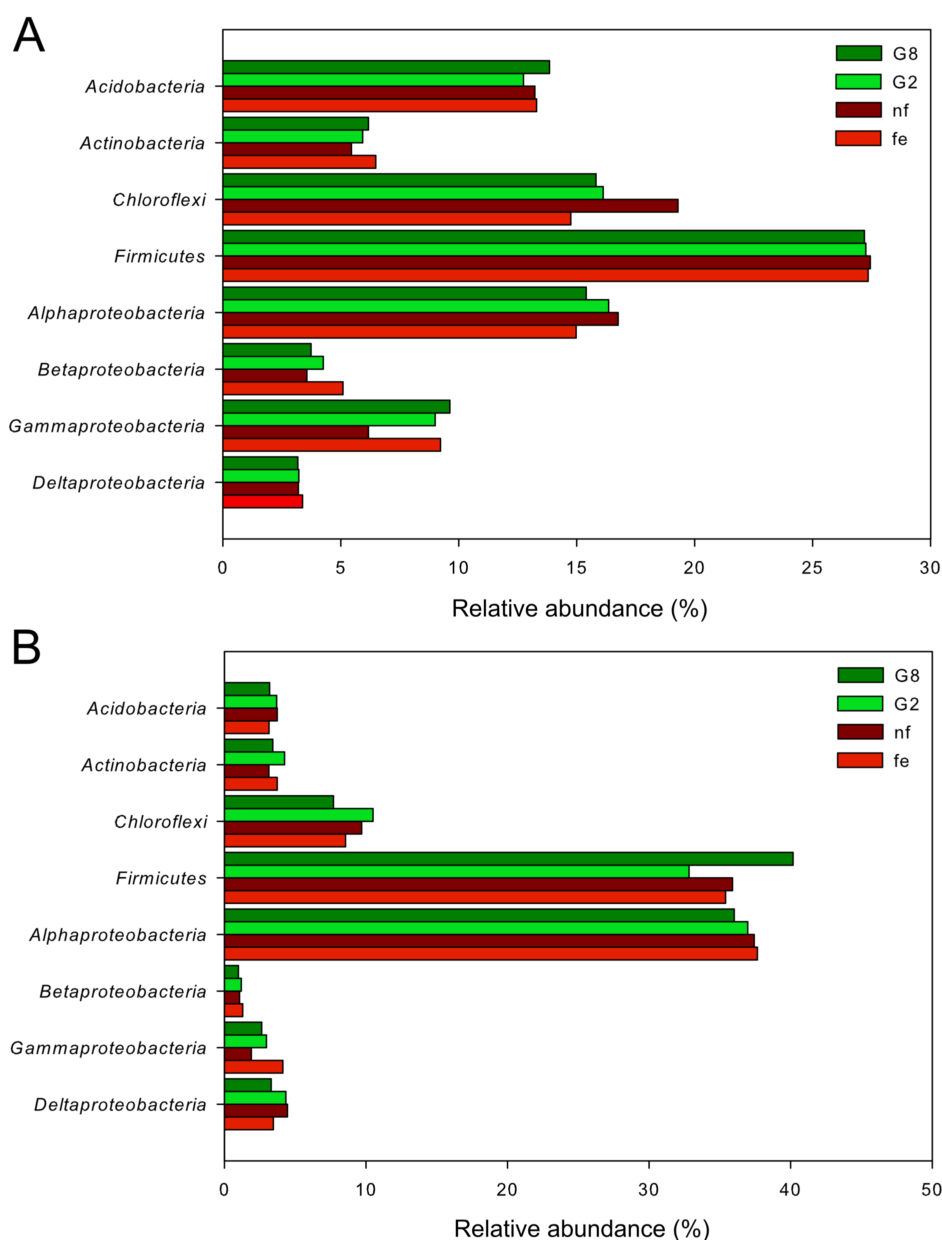

Figure 1. Most abundant phyla and proteobacterial classes of the total (A) and the active (B) bacterial community identified from soil samples of aspen deme Geismar 2 (G2) and aspen deme Geismar 8 (G8) as well as from fertilized (fe) and non-fertilized (nf) grassland soil samples.

These findings were generally in accordance with previous studies of bacterial communities in forest and grassland soils (e.g. Will et al., 2010; Nacke et al., 2011; Baldrian et al., 2012; Pan et al., 2014). Janssen (2006) identified the dominant bacterial

phyla from 31 libraries of 16S rRNA and 16S rRNA genes in different soils, e.g. grassland and forest soils. In this study, *Proteobacteria*, *Acidobacteria*, *Actinobacteria*, *Verrucomicrobia*, *Bacteroidetes*, *Chloroflexi*, *Planctomycetes*, and *Firmicutes* were the dominant phyla in the libraries and accounted for 92% of all analyzed bacterial sequences. Nacke et al. (2011) found that *Proteobacteria* were the most abundant bacterial phylum in forest (45%) and grassland soils (35%). Will et al. (2010) observed similar high abundances of *Proteobacteria* in grassland soils (42%). This is in line with the results of Uroz et al. (2010) who analyzed the bacterial diversity in the rhizosphere and in the bulk soil of an oak forest. They found the highest abundance of *Proteobacteria* with up to 38% in the bulk soil and 41% in the rhizosphere soil. Thus, *Proteobacteria* is one of most dominant and ubiquitous taxonomic groups in soils. Members of this phylum play a key role as plant growth-promoting bacteria (Mendes et al., 2011, Brown et al., 2012), e.g. *Burkholderiales* (Estrada-De los Santos et al., 2001, Suarez-Moreno, 2012).

The second most abundant phylum was *Firmicutes* (DNA 27.3% and RNA 36.1%). This result is in contrast to other studies (e.g. Janssen, 2006; Will et al., 2010; Nacke et al., 2011; Rampelotto et al., 2013). *Firmicutes* form a large group of Gram-positive bacteria and are divided into three main classes, *Bacilli*, *Erysipelotrichi*, and *Clostridia* (Ludwig et al., 2009). In this study, sequences affiliated to the *Firmicutes* were mostly assigned to the genus *Bacillus*. Members of the *Bacillus* are aerobic bacteria with the ability to form UV-resistant endospores that also endure drought and oxidizing agents (Popham et al., 1995). Members of the genus *Bacillus* are common in soil, well accommodated to this habitat, and known as beneficial for plant growth and health (Berg, 2009). The high number of *Firmicutes* in this study may result from the former land-use history of the study site, which was for hay-making or for grazing.

In this thesis, the phylum *Chloroflexi* represents 16.5% of all analyzed sequences in the total and 9.1% in the active bacterial community. Janssen (2006) found that the abundance of this phylum varied between 0 % and 16% in the entire soil bacterial community. We found several subphyla of *Chloroflexi* with *Ktedonobacteria* as most abundant class (5.39% of all analyzed 16S rRNA gene and gene transcript sequences). Davis et al. (2005 and 2011) isolated some members of the *Chloroflexi* subphyla such as *Ktedonobacteria* and *Thermomicrobia* from paddock soil by inoculation experiments. They characterized these groups as slow-growing and mini-colony-forming bacteria. Yamada et al. (2005) investigated the community of *Chloroflexi* subphylum I in mesophilic and thermophilic sludge granules. They isolated and analyzed 3 strains belonging to this subphylum and

suggested that *Chloroflexi* might contribute to the degradation of carbohydrates and other cellular components such as amino acids. Thus, this observation may give us a hint about the role of *Chloroflexi* in soil.

Another abundant phylum in the total community was *Acidobacteria*. Members of this phylum form a highly abundant and diverse group (Quaiser et al., 2003) and their abundance is often linked to soil pH (Lauber et al., 2009) and an oligotrophic lifestyle (Fierer et al., 2007; Naether et al., 2012). Oligotrophic bacteria show a high substrate affinity and low growth rate but are well adapted to poor soil conditions. As consequence, they have an advantage compared to bacteria with a copiotrophic lifestyle. Copiotrophic bacteria such as *Betaproteobacteria* exhibit high growth rates at high-nutrient conditions (Naether et al., 2012). In this thesis, *Acidobacteria* represents 13.3% and 3.5% of all analyzed sequences at DNA and RNA level, respectively. Correlation studies with soil parameters such as pH, C/N, and water content had shown that the abundance of this phylum is significantly correlated with pH. In a study by Jones et al. (2009), the relative abundance of *Acidobacteria* within 87 different soil samples varied from 2.4 to 78.5%. The abundance of this phylum correlated strongly with pH, with higher abundances at low pH values. Within the *Acidobacteria*, the distinct subclasses correlated differently with pH. While the acidobacterial subclasses 1, 2, 3, 12, 13, and 15 decreased, the acidobacterial subclasses 4, 6, 7, 10, 11, 16, 17, 18, 22, and 25 increased with rising pH. The abundance of the active members of subgroup 1 correlated significantly negatively with pH, while that of subgroup 7 is significantly positively correlated with pH.

The phylum *Actinobacteria* (DNA 6% and RNA 3.6%) forms a large group of mainly Gram-positive bacteria. *Actinobacteria* are divided into six classes and are characterized as an extremely diverse group with high GC-content (Stackebrandt and Schumann, 2006; Lu and Zhang, 2012). In a study by Lauber et al. (2009), the relative abundances of *Actinobacteria* varied between 5 and 24% with an average of 13%. They found approximately 7% abundance of *Actinobacteria* within a pH range of 4 to 6 and this is in line with the result from our study of the total soil bacterial community.

5.2. Active and total bacterial communities differs with respect to their diversity and abundance

To study the metabolic active soil bacterial community, RNA was isolated from 216 soil samples, transcribed into cDNA and alpha- and beta diversity analyses were performed (Chapter II and III).

The total bacterial community is dominated by *Proteobacteria* (32%), *Firmicutes* (27%), *Chloroflexi* (17%), *Acidobacteria* (13%), and *Actinobacteria* (6%) (Figure 1A), whereas the active bacterial community is predominated by the two phyla *Proteobacteria* (45%) and *Firmicutes* (36%) (Figure 1B). This is in accordance with a study of Baldrian et al. (2012). They observed that the active bacterial community in forest soils was less evenly distributed and less diverse compared to the corresponding total bacterial community. Generally, both phyla *Proteobacteria* and *Firmicutes* have more 16S rRNA gene copy numbers and a higher degree of overestimation by amplifying the variable regions between the V1-V3 16S rRNA gene region (Sun et al., 2013, Větrovský and Baldrian, 2013). This could also result in high abundances of these phyla in the active as well as in the total bacterial community. Additionally, estimated numbers of OTUs differ significantly between DNA and RNA level at 20% and 3% genetic distance, indicating a higher diversity in the total bacterial community compared to the active bacterial community (Figure 2). This result could be underlined by several studies (Baldrian et al., 2012; Schneider et al., 2015; Stibal et al., 2015). Stibal et al. (2015) found a significant higher diversity in the total bacterial community compared to the active bacterial community from margin sites of an ice sheet in Greenland. Additionally, they identified significant differences between the active and total community composition as mentioned in this thesis. Thus, DNA-based approaches, which include also dead cells, extracellular DNA, and dormant microorganisms (Lennon and Jones, 2011) may lead to a higher diversity of the total bacterial community compared to the active bacterial community.

However, approximately 91% of all analyzed 16S rRNA gene and gene transcript sequences were shared between the active and the total bacterial community. This suggests that the core community consisted of also metabolic active members. This is in line with a comprehensive stable isotope study of DNA and RNA with $H_2{}^{18}O$ (Rettedal and Brözel, 2015). Here, total and active members of the same type of nucleic acid exhibited similar

community structures. The authors suggested that the most abundant OTUs in the total nucleic acid extracts contained active members.

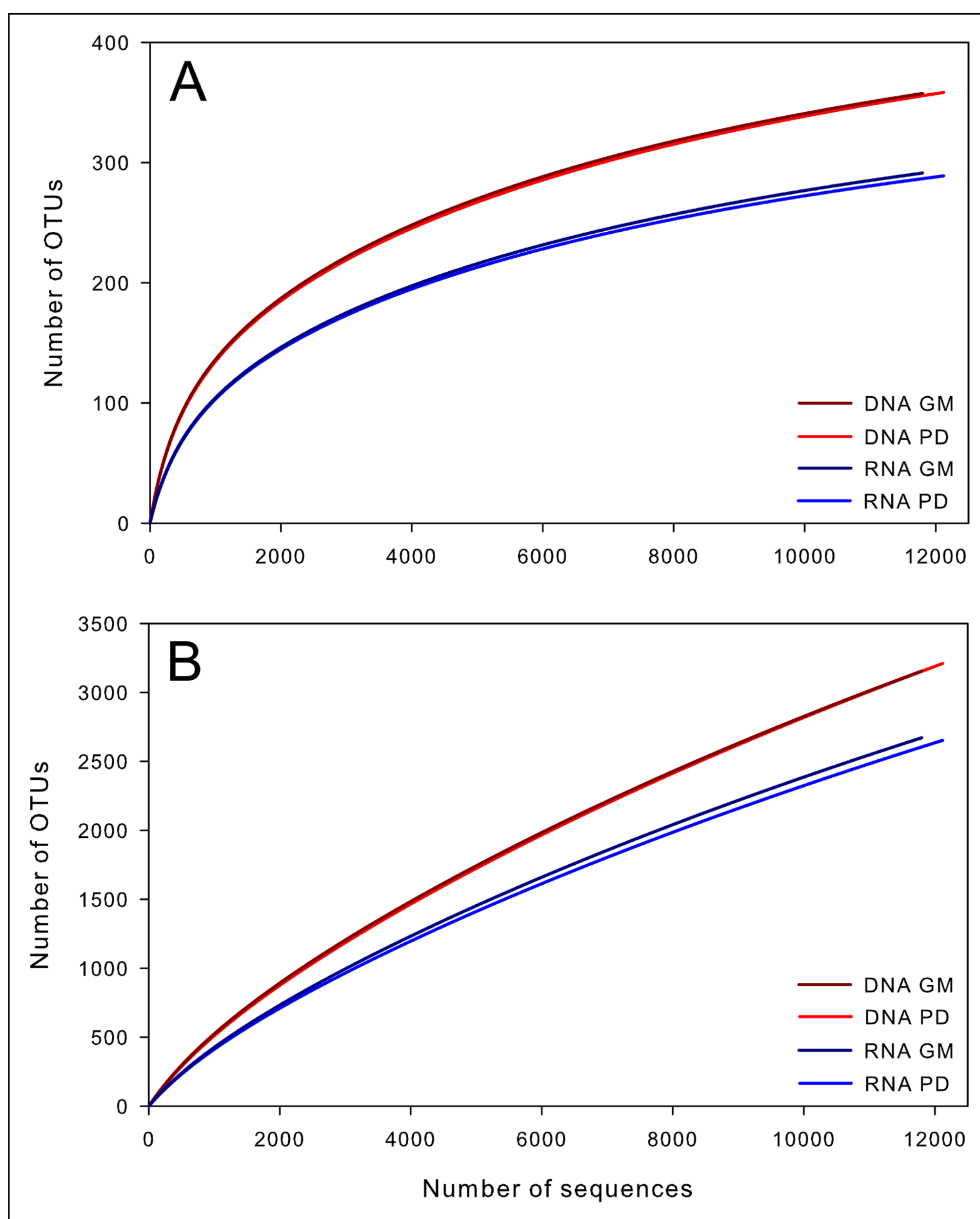

Figure 2: Rarefaction curves at 20% (A) and 3% (B) genetic distance derived from the total (DNA) and active (RNA) bacterial community. Depicted were rarefaction curves from the GrassMan (GM) and PopDiv (PD) experimental site.

Direct RNA extraction from soil and analysis allow the exploration of the metabolic activity of bacteria as the abundance of rRNA per cell nearly correlates with bacterial growth activity (Molin and Givskov, 1999). Although this technique has some drawbacks such as varying ribosome content per cell and remaining RNA reserves in dormant cells (Sukenik et al., 2012; Blazewicz et al., 2013), RNA-based approaches represent a useful

tool for analyzing the metabolic active bacterial community in soil. As we observed differences between total and active bacterial community composition, a combined analysis is the best way to investigate main drivers of bacterial communities in soils and other ecosystems.

5.3. Total and active bacterial communities and functions in a grassland soil are influenced by fertilizer application and environmental conditions

The results of Chapter II demonstrate that fertilizer application altered the total as well the active bacterial community in different ways. While the diversity of the total bacterial community is higher in the fertilized soils, the diversity in the active bacterial community as response to fertilization was reduced. The DNA-based results were in accordance with previous studies (Nacke et al., 2011; Shange et al., 2012; Poulsen et al., 2013). For example, Nacke et al. (2011) found similar OTU values at 3% genetic distance in fertilized and non-fertilized grassland soil bacterial communities. Fertilizer application influenced not only the diversity, but also the composition of the bacterial community (Shange et al., 2012; Udikovic-Kolic et al., 2014). In previous studies, it could be shown that *Gammaproteobacteria* increased with rising N inputs (Ramirez et al., 2010; Broszat et al., 2014) or with long-term fertilization (Campbell et al., 2010). However, very little is known so far about responses of the active bacterial community to fertilizer application as most previous studies used DNA as template (Campbell et al., 2010; Broszat et al., 2014). Especially, the active members of the class *Gammaproteobacteria* were significantly more abundant in the fertilizer-treated plots than in all other analyzed treatments. For example, we observed a higher activity of fewer groups such as *Xanthomonadales* which can use N compounds as energy sources. This is in line with previous studies (Patra et al., 2006; Ramirez et al., 2010; Fierer et al., 2012; Rampelotto et al., 2013).

Spearman rank correlation analysis revealed that several taxonomic groups correlated significantly with environmental parameters. This is in accordance with previous studies (Fierer et al., 2007; Jones et al., 2009; Lauber et al., 2009; Rousk et al., 2010; Tripathi et al., 2012; Zhou et al., 2014). Lauber et al. (2009) found strong positive correlations for *Acidobacteria* (r = 0.72), *Alphaproteobacteria* (r = 0.70), and *Actinobacteria* (r = 0.63) with pH and Rousk et al. (2010) observed that the relative abundance of *Acidobacteriales*

decreased with increasing soil pH, while the relative abundance of *Acidobacteria* subgroups 5, 6 and 7 increased with soil pH.

We found that also the active members of the orders *Acidobacteriales* and *Myxococcales* significantly correlated with pH and C/N, respectively (Jones et al., 2009; Zhou et al., 2014). This is in line with a study of myxobacterial communities in different soils by Zhou et al. (2014). The authors observed a strong correlation between pH and the relative abundance of *Myxobacteria*. This group plays a key role in the carbon turnover in soils (Lueders et al., 2006). Jones et al. (2009) found that *Acidobacteria* strongly correlated with pH and that subgroups of this phylum correlated positively or negatively with pH.

Additionally, functional predictions were performed using Tax4Fun (Aßhauer et al., 2015). Tax4Fun is an open-source R package that links the functional potential of microbial communities based on 16S rRNA genes and gene transcript sequences (Aßhauer et al., 2015). Higher abundances of genes encoding for subunits for nitrate reductases (*nar*IJ) and nitrite reductase (*nir*B) were observed at active bacterial community level in fertilizer-treated plots. Furthermore, genes facilitating the first step of the nitrification reaction (*amo*ABC) were more abundant in the fertilized soils. Especially, we could show that the active bacterial community response is more sensitive to soil parameter and fertilizer application than that of the total bacterial community.

This thesis gave first insights in the active bacterial community composition in different managed soils, indicating that it is of great importance to analyze both, the active and total bacterial structure to understand the mutual influence of management regimes and bacterial dynamics in soils.

5.4. Diversity of the active bacterial community are altered by two aspen demes

In Chapter III, the effect of two aspen demes (Geismar2 and Geismar8) on the diversity of active and total soil bacterial community was investigated. Most previous studies showed an effect of tree species diversity and tree identity on total bacterial community structure and diversity in forest soils (Nacke et al., 2011; Oh et al., 2012; Sun et al., 2014). In this study, only the diversity of the active bacterial community was influenced and the total bacterial community in soils of the two aspen demes did not differ. For example, comparison of mean Shannon indices from aspen deme Geismar2 and deme Geismar8 revealed a significantly higher diversity in the active soil bacterial community of Geismar2

compared to Geismar8 at 3% and 20% genetic distance (P = 0.018 and P = 0.005, respectively), whereas no differences were recorded in the total bacterial community. To underline this result, Pfeiffer et al. (2013), investigated the influence of beech and ash on the active bacterial community composition and diversity and found that beech and ash as tree species impacts the soil bacterial diversity. Urbanova et al. (2015) observed that the effect of tree species on the total microbial-community composition was alone not significant, but was partly mediated by soil pH. Thus, it is of great importance, to analyze both, the active and total bacterial community composition and their diversity to understand soil microbial interaction with tree identity as well as tree diversity.

5.5. Influence of sampling time on total and active bacterial communities in soils

We showed that sampling time impacts the bacterial diversity and structure in bulk soil (see Chapter II and III), including the diversity of the active bacterial community in grassland soil (Chapter II). Estimated numbers of operational taxonomic units (OTUs) in summer samples in 2010 differed significantly from those of the summer samples in 2011 at 20% and 3% genetic distance due to higher temperature and drier soil conditions during summer 2010 compared to summer 2011. A possible explanation is that the bacterial community is altered as response to seasonal changes of temperature, water availability, and plant growth activity (Jonasson et al., 1999; Cruz-Martinez et al., 2009; Liu et al., 2009; Angel et al., 2010; Castro et al., 2010; DeAngelis et al., 2015). A rainfall manipulating experiment showed little differences in soil bacterial community composition in grasslands after 5 years of manipulation (Cruz-Martinez et al., 2009). Changes in microbial abundance and composition were detected in response to extreme weather conditions, but sampling repeatly across seasons and years showed that these changes were only short-lived. Furthermore, Angel et al. (2010) investigated the diversity of soil bacteria along a steep precipitation gradient ranging from the Negev Desert in the south of Israel (<100 mm annual rain) to the Mediterranean forests in the north (>900 mm annual rain). The difference in community compositions was not statistically significant within sites, but it differed profoundly by ecosystem type. They explained these differences by the precipitation gradient combined with the vegetation cover.

Sampling time had a minor effect on the total bacterial community composition in the aspen demes. This is in line with a study by Kuffner et al. (2012). They observed in a

warming experiment, established in a 130-years-old mountain forest, that seasonal community dynamics were slight compared to the dynamics of soil respiration. Despite a pronounced respiration response to soil warming, they did not detect warming effects on community structure or composition. De Angelis et al (2015) studied changes of soil bacterial community as response to soil warming in a long-term forest ecological research site in which soil was warmed 5°C above ambient temperatures for 5, 8, and 20 years. They detected only a significant change in bacterial community structure after 20 years of warming.

In this thesis, the effect of sampling time was earlier detectable at active bacterial community level than at total bacterial community level. This is in line with a study by Maaløe and Kjeldgaard (1966). They showed in shift experiments that ribosome synthesis was immediately affected by changing environmental conditions.

5.6. The total bacterial community in the rhizosphere is influenced by management regimes as well as plant species composition

In Chapter IV it was shown that mowing frequency in combination with fertilizer application significantly altered the bacterial community composition in the rhizosphere, but fertilization alone did not significantly influence bacterial richness (Maron and Jeffries, 2001; Patra et al., 2006; Fierer et al., 2012). Fierer et al. (2012) analyzed the structure and functional characteristics of soil microbial communities from replicated plots in two long-term N fertilization experiments by pyrosequencing 16S rRNA gene sequences and found no significant effects of N fertilization on bacterial diversity, but significant effects on community composition. The effect of mowing influenced the rhizospheric bacterial community, which is in accordance with Patra et al. (2006), who showed that grazing and mowing can affect the size and composition of key microbial functional groups driving N dynamics. On the other hand Denef et al. (2009) did not detect an effect of mowing frequency on rhizospheric soil bacterial community composition. This contrast might be due to different approaches used such as phospholipid fatty acid (PLFA) analysis (Denef et al., 2009) and restriction fragment length polymorphism (RFLP) in combination with denaturing gradient gel electrophoresis (DGGE) analysis (Patra et al., 2006) in these studies which lead to different results. Additionally, the study sites represent different land

use histories and soil types, which impact the bacterial community and richness in rhizospheric soils in one way or another (Singh et al., 2007; Garbeva et al., 2008).
The impact of sward composition was investigated, resulting in a reduction of bacterial richness in plots with either monocotyledonous or dicotyledonous grass plants compared to control plots without grass species reduction. Additionally, the bacterial composition in the rhizosphere was influenced. It is well known that plant diversity influences bacterial community composition in soil (Grayston et al., 1998; Stephan et al., 2000; Zak et al., 2003; Costa et al., 2006; Garbeva et al., 2008; Mitchell et al., 2010). Stephan et al. (2000) showed that the catabolic activity and catabolic diversity of cultivable soil bacteria increased with plant species number and number of plant functional groups in experimental grassland ecosystems. Mitchell et al. (2010) investigated if vegetation composition or soil chemistry best predict the soil microbial community. They found that above-ground vegetation composition may be a better predictor of the soil microbial community than one-off measurements of soil properties.
However, the sward composition had a weaker influence on the bacterial richness compared to the effect of sward composition in combination with mowing frequency and/or fertilizer applications. Thus, the combination of various factors influenced the bacterial community composition and diversity in the rhizosphere differently.

5.7. Concluding remarks

The majority of studies presented in this thesis investigated the diversity and dynamics of bacterial communities in bulk and rhizospheric soil using different culture-independent approaches. Although being intensively studied over the past years, our knowledge on ecology and functions of these communities is still limited. Understanding how bacterial communities in soil and rhizosphere are structured and how they react towards different factors such as fertilizer application is of crucial interest due to their important role for plant growth and ecosystem functioning.
We showed further that fertilizer application affected both total and active bacterial communities in a grassland soil. However, the active bacterial community showed a stronger response to fertilizer application and sampling time. This was confirmed by the analysis of soils from two aspen demes. While the active bacterial community was influenced by aspen demes, the total bacterial community did not differ. The results of this

thesis highlighted the importance of a combined metagenomic and metatranscriptomic approach for in-depth analysis of soil bacterial communities and their functions.

The total bacterial community composition in the rhizosphere was significantly affected by different management regimes. Moreover, the combination of mowing frequency with fertilizer application, and sward composition had significant effects on the community composition. As several bacteria in the rhizosphere promote plant growth and health, the understanding of community dynamics and functions is of fundamental importance.

5.8. References

Angel, R., Soares, M.I., Ungar, E.D., and Gillor, O. (2010). Biogeography of soil archaea and bacteria along a steep precipitation gradient. *ISME J* 4**,** 553-563.

Aßhauer, K.P., Wemheuer, B., Daniel, R., and Meinicke, P. (2015). Tax4Fun: predicting functional profiles from metagenomic 16S rRNA data. *Bioinformatics*. 31, 2882-2884.

Baldrian, P., Kolařík, M., Stursová, M., Kopecký, J., Valášková, V., Větrovský, T., Zifčáková, L., Snajdr, J., Rídl, J., Vlček, C., and Voříšková, J. (2012). Active and total microbial communities in forest soil are largely different and highly stratified during decomposition. *ISME J* 6**,** 248-258.

Berg, G. (2009). Plant–microbe interactions promoting plant growth and health: perspectives for controlled use of microorganisms in agriculture. *Appl. Microbiol. Biotechnol.* 84**,** 11-18.

Blazewicz, S., Barnard, R., Daly, R., and Firestone, M. (2013). Evaluating rRNA as an indicator of microbial activity in environmental communities: limitations and uses. *ISME J.* 7**,** 2061-2068.

Broszat, M., Nacke, H., Blasi, R., Siebe, C., Huebner, J., Daniel, R., and Grohmann, E. (2014). Wastewater Irrigation Increases the Abundance of Potentially Harmful *Gammaproteobacteria* in Soils in Mezquital Valley, Mexico. *Appl. Environ. Microbiol.* 80**,** 5282-5291.

Brown, S., Utturkar, S., Klingeman, D., Johnson, C., Martin, S., Land, M., Lu, T., Schadt, C., Doktycz, M., and Pelletier, D. (2012). Twenty-One Genome Sequences from Pseudomonas Species and 19 Genome Sequences from Diverse Bacteria Isolated from the Rhizosphere and Endosphere of *Populus deltoides*. *J. Bacteriol.* 194**,** 5991-5993.

Campbell, B., Polson, S., Hanson, T., Mack, M., and Schuur, E. (2010). The effect of nutrient deposition on bacterial communities in Arctic tundra soil. *Environ. Microbiol.*12**,** 1842-1854.

Castro, H.F., Classen, A.T., Austin, E.E., Norby, R.J., and Schadt, C.W. (2010). Soil microbial community responses to multiple experimental climate change drivers. *Appl. Environ. Microbiol.* 76**,** 999-1007.

Costa, R., Gotz, M., Mrotzek, N., Lottmann, J., Berg, G., and Smalla, K. (2006). Effects of site and plant species on rhizosphere community structure as revealed by molecular analysis of microbial guilds. *Fems Microbiol. Ecol.* 56**,** 236-249.

Cruz-Martinez, K., Suttle, K., Brodie, E., Power, M., Andersen, G., and Banfield, J. (2009). Despite strong seasonal responses, soil microbial consortia are more resilient to long-term changes in rainfall than overlying grassland. *ISME J.* 3**,** 738-744.

Daniel, R. (2005). The metagenomics of soil. *Nature Rev. Microbiol.* 3**,** 470-478.

Davis, K., Joseph, S., and Janssen, P. (2005). Effects of growth medium, inoculum size, and incubation time on culturability and isolation of soil bacteria. *Appl. Environ.l Microbiol.* 71**,** 826-834.

Davis, K., Sangwan, P., and Janssen, P. (2011). *Acidobacteria*, *Rubrobacteridae* and *Chloroflexi* are abundant among very slow-growing and mini-colony-forming soil bacteria. *Environ. Microbiol.* 13**,** 798-805.

Deangelis, K.M., Pold, G., Topçuoğlu, B.D., Van Diepen, L.T.A., Varney, R.M., Blanchard, J.L., Melillo, J., and Frey, S.D. (2015). Long-term forest soil warming alters microbial communities in temperate forest soils. *Front. Microbiol.* 6. doi: 10.3389/fmicb.2015.00104

Denef, K., Roobroeck, D., Wadu, M., Lootens, P., and Boeckx, P. (2009). Microbial community composition and rhizodeposit-carbon assimilation in differently managed temperate grassland soils. *Soil Biol. Biochem.* 41**,** 144-153.

Estrada-De Los Santos, P., Bustillos-Cristales, R., and Caballero-Mellado, J. (2001). Burkholderia, a genus rich in plant-associated nitrogen fixers with wide environmental and geographic distribution. *Appl. Environ. Microbiol.* 67**,** 2790-2798.

Fierer, N., Bradford, M., and Jackson, R. (2007). Toward an ecological classification of soil bacteria. *Ecology.* 88**,** 1354-1364.

Fierer, N., Lauber, C., Ramirez, K., Zaneveld, J., Bradford, M., and Knight, R. (2012). Comparative metagenomic, phylogenetic and physiological analyses of soil microbial communities across nitrogen gradients. *ISME J.* 6**,** 1007-1017.

Garbeva, P., Van Elsas, J., and Van Veen, J. (2008). Rhizosphere microbial community and its response to plant species and soil history. *Plant Soil.* 302**,** 19-32.

Grayston, S., Wang, S., Campbell, C., and Edwards, A. (1998). Selective influence of plant species on microbial diversity in the rhizosphere. *Soil Biol. Biochem.* 30**,** 369-378.

Janssen, P. (2006). Identifying the dominant soil bacterial taxa in libraries of 16S rRNA and 16S rRNA genes. *Appl. Environ. Microbiol.* 72**,** 1719-1728.

Jonasson, S., Michelsen, A., Schmidt, I.K., and Nielsen, E.V. (1999). Responses in microbes and plants to changed temperature, nutrient, and light regimes in the arctic. *Ecology* 80**,** 1828-1843.

Jones, R., Robeson, M., Lauber, C., Hamady, M., Knight, R., and Fierer, N. (2009). A comprehensive survey of soil acidobacterial diversity using pyrosequencing and clone library analyses. *ISME J.* 3**,** 442-453.

Kuffner, M., Hai, B., Rattei, T., Melodelima, C., Schloter, M., Zechmeister-Boltenstern, S., Jandl, R., Schindlbacher, A., and Sessitsch, A. (2012). Effects of season and experimental warming on the bacterial community in a temperate mountain forest soil assessed by 16S rRNA gene pyrosequencing. *Fems Microbiol. Ecol.* 82**,** 551-562.

Lauber, C.L., Hamady, M., Knight, R., and Fierer, N. (2009). Pyrosequencing-based assessment of soil pH as a predictor of soil bacterial community structure at the continental scale. *Appl. Environ. Microbiol.* 75**,** 5111-5120.

Lennon, J., and Jones, S. (2011). Microbial seed banks: the ecological and evolutionary implications of dormancy. *Nature Rev. Microbiol.* 9**,** 119-130.

Liu, W., Zhang, Z.H.E., and Wan, S. (2009). Predominant role of water in regulating soil and microbial respiration and their responses to climate change in a semiarid grassland. *Glob. Chang. Biol.* 15**,** 184-195.

Lu, Z., and Zhang, W. (2012). Comparative Phylogenies of Ribosomal Proteins and the 16S rRNA Gene at Higher Ranks of the Class Actinobacteria. *Curr. Microbiol.* 65**,** 1-6.

Ludwig, W., Schleifer, K.-H., and Whitman, W.B. (2009). "Revised road map to the phylum *Firmicutes*," in Bergey's Manual of Systematic Bacteriology. Michigan State Universitiy: Springer, 1-13.

Lueders, T., Kindler, R., Miltner, A., Friedrich, M., and Kaestner, M. (2006). Identification of bacterial micropredators distinctively active in a soil microbial food web. *Appl. Environ. Microbiol.* 72**,** 5342-5348.

Maaløe, O., and Kjeldgaard, N.O. (1966). *Control of macromolecular synthesis; a study of DNA, RNA, and protein synthesis in bacteria.* New York: W. A. Benjamin.

Maron, J., and Jeffries, R. (2001). Restoring enriched grasslands: Effects of mowing on species richness, productivity, and nitrogen retention. *Ecol. Appl.* 11**,** 1088-1100.

Mendes, R., Kruijt, M., De Bruijn, I., Dekkers, E., Van Der Voort, M., Schneider, J., Piceno, Y., Desantis, T., Andersen, G., Bakker, P., and Raaijmakers, J. (2011). Deciphering the Rhizosphere Microbiome for Disease-Suppressive Bacteria. *Science* 332**,** 1097-1100.

Mitchell, R.J., Hester, A.J., Campbell, C.D., Chapman, S.J., Cameron, C.M., Hewison, R.L., and Potts, J.M. (2010). Is vegetation composition or soil chemistry the best predictor of the soil microbial community? *Plant Soil* 333**,** 417-430.

Molin, S., and Givskov, M. (1999). Application of molecular tools for in situ monitoring of bacterial growth activity. *Environ. Microbiol.* 1**,** 383-391.

Nacke, H., Thurmer, A., Wollherr, A., Will, C., Hodac, L., Herold, N., Schoning, I., Schrumpf, M., and Daniel, R. (2011). Pyrosequencing-Based Assessment of Bacterial Community Structure Along Different Management Types in German Forest and Grassland Soils. *Plos One* 6. doi: 10.1371/journal.pone.0017000

Naether, A., Foesel, B., Naegele, V., Wust, P., Weinert, J., Bonkowski, M., Alt, F., Oelmann, Y., Polle, A., Lohaus, G., Gockel, S., Hemp, A., Kalko, E., Linsenmair, K., Pfeiffer, S., Renner, S., Schoning, I., Weisser, W., Wells, K., Fischer, M.,

Overmann, J., and Friedrich, M. (2012). Environmental Factors Affect Acidobacterial Communities below the Subgroup Level in Grassland and Forest Soils. *Appl. Environ. Microbiol.* 78**,** 7398-7406.

Oh, Y.M., Kim, M., Lee-Cruz, L., Lai-Hoe, A., Go, R., Ainuddin, N., Rahim, R.A., Shukor, N., and Adams, J.M. (2012). Distinctive bacterial communities in the rhizoplane of four tropical tree species. *Microb. Ecol.* 64**,** 1018-1027.

Pan, Y., Cassman, N., Hollander, M., Mendes, L.W., Korevaar, H., Geerts, R.H.E.M., Veen, J.A., and Kuramae, E.E. (2014). Impact of long-term N, P, K, and NPK fertilization on the composition and potential functions of the bacterial community in grassland soil. *Fems Microbiol. Ecol*. 90, 195-205.

Patra, A., Abbadie, L., Clays-Josserand, A., Degrange, V., Grayston, S., Guillaumaud, N., Loiseau, P., Louault, F., Mahmood, S., Nazaret, S., Philippot, L., Poly, F., Prosser, J., and Le Roux, X. (2006). Effects of management regime and plant species on the enzyme activity and genetic structure of N-fixing, denitrifying and nitrifying bacterial communities in grassland soils. *Environ. Microbiol.* 8**,** 1005-1016.

Pfeiffer, B., Fender, A.-C., Lasota, S., Hertel, D., Jungkunst, H.F., and Daniel, R. (2013). Leaf litter is the main driver for changes in bacterial community structures in the rhizosphere of ash and beech. *Appl. Soil Ecol.* 72**,** 150-160.

Popham, D.L., Senguta, S., and Seltow, P. (1995). Heat, hydrogen-peroxide, and UV resistance of *Bacillis subtilis* spores with increased core water-content and with or without major DNA-binding proteins. *Appl. Environ. Microbiol.* 61**,** 3633-3638.

Poulsen, P.H.B., Al-Soud, W.A., Bergmark, L., Magid, J., Hansen, L.H., and Sørensen, S.J. (2013). Effects of fertilization with urban and agricultural organic wastes in a field trial – Prokaryotic diversity investigated by pyrosequencing. *Soil Biol. Biochem.* 57**,** 784-793.

Quaiser, A., Ochsenreiter, T., Lanz, C., Schuster, S., Treusch, A., Eck, J., and Schleper, C. (2003). Acidobacteria form a coherent but highly diverse group within the bacterial domain: evidence from environmental genomics. *Mol. Microbiol.* 50**,** 563-575.

Ramirez, K.S., Lauber, C.L., Knight, R., Bradford, M.A., and Fierer, N. (2010). Consistent effects of nitrogen fertilization on soil bacterial communities in contrasting systems. *Ecology.* 91**,** 3463-3470.

Rampelotto, P.H., De Siqueira Ferreira, A., Barboza, A.D., and Roesch, L.F. (2013). Changes in Diversity, Abundance, and Structure of Soil Bacterial Communities in Brazilian Savanna Under Different Land Use Systems. *Microb. Ecol*. 66, 593-607.

Rettedal, E.A., and Brözel, V.S. (2015). Characterizing the diversity of active bacteria in soil by comprehensive stable isotope probing of DNA and RNA with $H_2^{18}O$. *MicrobiologyOpen* 4**,** 208-219.

Rousk, J., Baath, E., Brookes, P., Lauber, C., Lozupone, C., Caporaso, J., Knight, R., and Fierer, N. (2010). Soil bacterial and fungal communities across a pH gradient in an arable soil. *ISME J.* 4**,** 1340-1351.

Schneider, D., Reimer, A., Hahlbrock, A., Arp, G., and Daniel, R. (2015). Metagenomic and metatranscriptomic analyses of bacterial communities derived from a calcifying karst water creek biofilm and tufa. *Geomicrobiol. J.* 32**,** 316-331.

Shange, R., Ankumah, R., Ibekwe, A., Zabawa, R., and Dowd, S. (2012). Distinct Soil Bacterial Communities Revealed under a Diversely Managed Agroecosystem. *Plos One.* 7. doi: 10.1371/journal.pone.0040338

Singh, B., Munro, S., Potts, J., and Millard, P. (2007). Influence of grass species and soil type on rhizosphere microbial community structure in grassland soils. *Appl. Soil Ecol.* 36**,** 147-155.

Stackebrandt, E., and Schumann, P. (2006). "Introduction to the taxonomy of *Actinobacteria*," in The Prokaryotes. Springer, 3, 297-321.

Stephan, A., Meyer, A., and Schmid, B. (2000). Plant diversity affects culturable soil bacteria in experimental grassland communities. *J. Ecol.* 88**,** 988-998.

Stibal, M., Schostag, M., Cameron, K., Hansen, L., Chandler, D., Wadham, J., and Jacobsen, C. (2015). Different bulk and active bacterial communities in cryoconite from the margin and interior of the Greenland ice sheet. *Environ. Microbiol. Rep.* 7**,** 293-300.

Suarez-Moreno, Z., Caballero-Mellado, J., Coutinho, B., Mendonca-Previato, L., James, E., and Venturi, V. (2012). Common Features of Environmental and Potentially Beneficial Plant-Associated *Burkholderia*. *Microbial. Ecol.* 63**,** 249-266.

Sukenik, A., Kaplan-Levy, R., Welch, J., and Post, A. (2012). Massive multiplication of genome and ribosomes in dormant cells (akinetes) of *Aphanizomenon ovalisporum* (*Cyanobacteria*). *ISME J.* 6**,** 670-679.

Sun, D., Jiang, X., Wu, Q., and Zhou, N. (2013). Intragenomic Heterogeneity of 16S rRNA Genes Causes Overestimation of Prokaryotic Diversity. *Appl. Environ. Microbiol.* 79**,** 5962-5969.

Sun, H., Terhonen, E., Koskinen, K., Paulin, L., Kasanen, R., and Asiegbu, F.O. (2014). Bacterial diversity and community structure along different peat soils in boreal forest. *Appl. Soil Ecol.* 74**,** 37-45.

Torsvik, V., Øvreås, L., and Thingstad, T.F. (2002). Prokaryotic diversity--magnitude, dynamics, and controlling factors. *Science.* 296**,** 1064-1066.

Tringe, S., Von Mering, C., Kobayashi, A., Salamov, A., Chen, K., Chang, H., Podar, M., Short, J., Mathur, E., Detter, J., Bork, P., Hugenholtz, P., and Rubin, E. (2005). Comparative metagenomics of microbial communities. *Science.* 308**,** 554-557.

Tripathi, B., Kim, M., Singh, D., Lee-Cruz, L., Lai-Hoe, A., Ainuddin, A., Go, R., Rahim, R., Husni, M., Chun, J., and Adams, J. (2012). Tropical Soil Bacterial Communities in Malaysia: pH Dominates in the Equatorial Tropics too. *Microb. Ecol.* 64**,** 474-484.

Udikovic-Kolic, N., Wichmann, F., Broderick, N., and Handelsman, J. (2014). Bloom of resident antibiotic-resistant bacteria in soil following manure fertilization. *Proc. Natl. Acad. Sci.s U.S.A.* 111**,** 15202-15207.

Urbanova, M., Snajdr, J., and Baldrian, P. (2015). Composition of fungal and bacterial communities in forest litter and soil is largely determined by dominant trees. *Soil Biol. Biochem.* 84**,** 53-64.

Uroz, S., Buee, M., Murat, C., Frey-Klett, P., and Martin, F. (2010). Pyrosequencing reveals a contrasted bacterial diversity between oak rhizosphere and surrounding soil. *Environ. Microbiol. Rep.* 2**,** 281-288.

Větrovský, T., and Baldrian, P. (2013). The variability of the 16S rRNA gene in bacterial genomes and its consequences for bacterial community analyses. *PLoS One* 8**.** doi: 10.1371/journal.pone.0057923

Will, C., Thurmer, A., Wollherr, A., Nacke, H., Herold, N., Schrumpf, M., Gutknecht, J., Wubet, T., Buscot, F., and Daniel, R. (2010). Horizon-Specific Bacterial Community Composition of German Grassland Soils, as Revealed by Pyrosequencing-Based Analysis of 16S rRNA Genes. *Appl. Environ. Microbiol.* 76**,** 6751-6759.

Yamada, T., Sekiguchi, Y., Imachi, H., Kamagata, Y., Ohashi, A., and Harada, H. (2005). Diversity, localization, and physiological properties of filamentous microbes belonging to Chloroflexi subphylum I in mesophilic and thermophilic methanogenic sludge granules. *Appl. Environ.l Microbiol.* 71**,** 7493-7503.

Zak, D.R., Holmes, W.E., White, D.C., Peacock, A.D., and Tilman, D. (2003). Plant diversity, soil microbial communities, and ecosystem function: are there any links? *Ecology* 84**,** 2042-2050.

Zhou, X., Li, S., Li, W., Jiang, D., Han, K., Wu, Z., and Li, Y. (2014). Myxobacterial community is a predominant and highly diverse bacterial group in soil niches. *Environ. Microbiol. Rep.* 6**,** 45-56.

Summary

Bacteria are key players in nutrient cycles and energy transduction in soil. Although soil bacterial communities have been studied for several decades, our knowledge on their structure, dynamics ecosystem function is still limited. The aim of this thesis was to contribute to the understanding of these communities.

In the first two studies, the impact of fertilizer treatment, two distinct aspen demes, soil properties (pH, water content, and C/N ratio), and sampling time on the total (DNA level) and the metabolic active (RNA level) bacterial community was analyzed. Thus, soil samples were collected in April, July, and September over two consecutive years. Community compositions were further assessed by pyrotag sequencing of 16S rRNA amplicons generated from environmental DNA and RNA, respectively. Additionally, functional analyses were performed based on the prediction of functional traits from taxonomic community composition.

In the first study, all factors investigated influenced the bacterial community composition and diversity. Fertilizer application leaded to a diversity loss in the active bacterial community at phylum as well as at species level. Relative abundances of active bacterial community members showed a shift to bacterial groups such as *Xanthomonadales*, which are specialized to use nitrogen compounds as energy source. In addition, genes encoding for the uptake of nitrate/nitrite, nitrification, and denitrification steps were significantly more abundant in fertilized plots at active bacterial community level.

In the second study, an influence of two different aspen demes Geismar2 and Geismar8 on soil bacterial community and diversity was observed at the active community level. The comparison of mean Shannon indices revealed a significantly higher diversity in the active soil bacterial community of aspen deme Geismar2 compared to Geismar8 at 3% and 20% genetic distance. Moreover, several of the main abundant phyla and proteobacterial classes were either more abundant in aspen deme Geismar2 or Geismar8, respectively.

The effect of sampling time on bacterial community was more pronounced at active bacterial community level, indicating that the metabolic active community members responded earlier to environmental changes. This result was supported by correlation analyses of relative abundances and soil properties. Additionally, we observed more significant positive and negative correlations of soil properties at many taxonomic levels

(at phylum, proteobacterial class, and order level) in the active bacterial community than in the total bacterial community. As a consequence, seasonal change has to be regarded in further studies as it might alter the effects of different grassland management regimes or aspen demes on soil bacterial communities.

In the third study, the effect of management regimes, mowing frequency, sward composition, and above-ground herbivory on the bacterial community composition in the rhizosphere was investigated. For this purpose, a lysimeter experiment was established in autumn 2010. Following a two-week exposure to herbivory by grasshoppers and snails, soil samples were collected from the lysimeters in summer 2011. DNA was extracted from the collected samples and subjected to 16S rRNA gene analysis. Community structure and bacterial diversity were assessed either by DGGE analysis or pyrosequencing of 16S rRNA gene amplicons. Sward composition and lower mowing frequencies decreased the bacterial richness in the rhizosphere. Despite that differences in bacterial richness between fertilized and non-fertilized plots were not recorded, the bacterial community composition responded to different management regimes. For example, *Acidobacteria* were significantly more abundant in non-fertilized plots, whereas *Actinobacteria* were significantly more abundant in fertilized plots.

In conclusion, bacterial communities in soil and in the rhizosphere are affected by different factors such as fertilizer application. Evaluating the main drivers of bacterial communities may results in a better understanding of the complex interactions between plants and bacterial communities. Furthermore, the results of this study will help to predict the impact of different factors onto bacterial communities in rhizosphere and soil and related effects on soil ecosystems.

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List of publications

Klaus Schützenmeister, Marco Gronwald, Diana Grubert, Sarah Herzog, Christina Lödige & Dirk Gansert, 2015, The species-specific influence of beech and ash saplings on CO_2, CH_4 and N_2O fluxes from soil during frondescence: GEO-ÖKÖ, XXXV, p. 206-233.

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