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ORIGINAL ARTICLE

Resistance and resilience of the forest soil microbiome to logging-associated compaction

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Soil compaction is a major disturbance associated with logging, but we lack a fundamental understanding of how this affects the soil microbiome. We assessed the structural resistance and resilience of the microbiome using a high-throughput pyrosequencing approach in differently compacted soils at two forest sites and correlated these findings with changes in soil physical properties and functions. Alterations in soil porosity after compaction strongly limited the air and water conductivity. Compaction significantly reduced abundance, increased diversity, and persistently altered the structure of the microbiota. Fungi were less resistant and resilient than bacteria; clayey soils were less resistant and resilient than sandy soils. The strongest effects were observed in soils with unfavorable moisture conditions, where air and water conductivities dropped well below 10% of their initial value. Maximum impact was observed around 6–12 months after compaction, and microbial communities showed resilience in lightly but not in severely compacted soils 4 years post disturbance. Bacteria capable of anaerobic respiration, including sulfate, sulfur, and metal reducers of the Proteobacteria and Firmicutes, were significantly associated with compacted soils. Compaction detrimentally affected ectomycorrhizal species, whereas saprobic and parasitic fungi proportionally increased in compacted soils. Structural shifts in the microbiota were accompanied by significant changes in soil processes, resulting in reduced carbon dioxide, and increased methane and nitrous oxide emissions from compacted soils. This study demonstrates that physical soil disturbance during logging induces profound and long-lasting changes in the soil microbiome and associated soil functions, raising awareness regarding sustainable management of economically driven logging operations.
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Introduction

Soil is an essential component of forest ecosystems, mediating fundamental nutrient and energy flow patterns that ensure forest productivity, sustain biodiversity and regulate climate stability (Bonan, 2008; Reay *et al.,* 2008; Dominati *et al.,* 2010; Normile, 2010). Soils are dynamic biological matrices featuring a complex microbiome that has an integral role in virtually all ecosystem processes (Barrios, 2007). At the system level, microbial metabolism regulates ecosystem functioning and modulates resistance and resilience to perturbations (Allison and Martiny, 2008). It is likely that measuring the microbial community structure and associated functions can improve the ability (1) to monitor alterations of the soil system after disturbances, (2) to evaluate its capacity to recover and perhaps (3) to detect adverse effects in ecosystem functioning before they are irreversible.

Soil compaction has been recognized as a major disturbance associated with forest management (Van-Camp *et al.,* 2004). Economically efficient harvesting requires the use of heavy machines, causing severe compaction of the soil particularly during wet conditions and along skid trails and landings (Grigal, 2000; Marshall, 2000). Alterations in soil porosity affect pore connectivity, water infiltration, air permeability, temperature, rooting space, nutrient flow and biological activity

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(Greacen and Sands, 1980; Kozlowski, 1999; Richard *et al.,* 2001; Mooney and Nipattasuk, 2003), often resulting in increased surface runoff, soil erosion, nutrient leaching and greenhouse gas emission (Worrell and Hampson, 1997; Powers *et al.,* 2005). As a consequence, the soil system can suffer substantial, persistent and sometimes irreversible damage, which ultimately reduces forest productivity and ecosystem functionality. Given that the affected area can range between 10 and 40% of the total logged stand, the impact on the ecosystem can be substantial (Grigal, 2000; Luckow and Guldin, 2007; Frey *et al.,* 2009).

The negative impact of soil compaction caused by logging on physicochemical properties has been demonstrated for years (for example, McNabb *et al.,* 2001; Horn *et al.,* 2007; Ampoorter *et al.,* 2010). In contrast, only few studies have observed significant effects on microbial properties (Dick *et al.,* 1988; Jordan *et al.,* 2003; Schnurr-Pütz *et al.,* 2006), and most such investigations reported inconsistent, equivocal or non-significant effects (Jordan *et al.,* 1999; Chow *et al.,* 2002; Li *et al.,* 2004; Shestak and Busse, 2005; Busse *et al.,* 2006; Mariani *et al.,* 2006; Tan *et al.,* 2008; Jennings *et al.,* 2012). These observations led to the general notion that microbial communities exhibit high degrees of resistance and resilience to compaction and might not adequately reflect the ecological consequences. These previous studies commonly measured bulk parameters such as microbial biomass or were limited by the unavailability of techniques with high taxonomic resolution to resolve the complex structure of the microbiota. With the recent advent of molecular tools, there is increasing evidence that effects of soil compaction on microbial structure and function are probably substantial and long lasting (Frey *et al.,* 2009, 2011; Hartmann *et al.,* 2012).

Applying high-throughput pyrosequencing (Margulies *et al.,* 2005) of bacterial and fungal ribosomal markers, Hartmann *et al.* (2012) recently described the microbial community structure in differently compacted forest soils at far greater depth than previously possible. This large-scale survey demonstrated that logging-induced soil compaction persistently alters the microbiota. However, four factors limited the conclusive evaluation of microbial resistance and resilience in these compacted soils. First, the experimental design did not allow for completely separating effects caused by soil compaction from those caused by biomass removal. Second, the study did not assess soil functions that are directly dependent on physical soil properties such as air permeability and water conductivity in order to relate changes in community structure to its edaphic background. Third, the study did not monitor the microbial response over time in order to evaluate initial resistance and long-term resilience of the system. Finally, the study did not gather process information that could serve as proxy for changes in ecosystem functioning and to evaluate functional redundancy of the structural changes.

Here, we present a study that fills the gaps indicated above and advances our understanding of the resistance and resilience of the forest soil ecosystem to compaction. Recently, Frey *et al.* (2011) reported on alterations in methanogenic community structure and methane fluxes in two controlled field experiments, in which skid trails differing in compaction intensity were generated by logging vehicles. Driven by these findings, we launched a comprehensive assessment of physicochemical and microbial characteristics in these soils to examine resistance and resilience of microbial community structure and associated soil functions to compaction.

Materials and methods

Compaction experiment and soil sampling

The field experiment was conducted in Spring 2007 and 2008 at two forest sites in Switzerland, Ermatingen and Heiteren, respectively. The two independent experiments represented two different scenarios in that the sites differed in their susceptibility to compaction (that is, soil texture) as well as in the degree of compaction induced (that is, ground contact pressure). A detailed description of the study sites and the traffic experiments has been published previously (Frey *et al.,* 2011). The texture at both sites was loamy, but the soil at Ermatingen (17% clay, 47% silt and 36% sand, pH 4.6) was characterized by around 50% more clay and a higher pH when compared with the sandy soil at Heiteren (8% clay, 43% silt and 49% sand, pH 4.0). In order to generate different degrees of compaction, soil moisture contents along projected traffic lanes (independent triplicates within 20 m distance of each other) were adjusted to 0.17 (plastic limit, C1) and 0.35 (liquid limit, C2) gram H_2O per gram of soil and equilibrated for 2 days before compaction. Compaction was induced using a fully loaded forwarder with four passes at Ermatingen (weight of 26 tons, ground contact pressure of 240–320 kPa) and an unloaded skidder with four passes at Heiteren (14 tons, 210–280 kPa). Unaffected areas in the vicinity of the compacted soils (that is, one meter from the center of the traffic lane) served as no impact controls (C0). Thus, the study comprised three independent wheel tracks (triplicates) per forest site with no (C0), light (C1) and severe (C2) soil compaction per lane. The experimental layout at Ermatingen is provided as Supplementary Figure 1.

A detailed soil sampling protocol has been published previously (Frey *et al.,* 2011). Triplicate cores from the topsoil were collected in each replicated traffic lane at a depth of 3–7 cm using steel cylinders with a volume of around 100 cm^3. The tire profiles generate a mixed and sometimes puddled stratum between the tread elements, where

new structure can build up quickly after natural drying-rewetting cycles. Hence, this stratum has limited potential to depict soil compaction and it is more suitable to sample the stratum below this depth. Furthermore, by avoiding the top 3 cm, we also excluded the litter material that was constantly falling into the tire imprint along the skid trails. Samples for soil physical measurements were collected after 1–4 days, and within-lane replicates were analyzed individually. Samples for microbial analyses were collected at the same locations as for the physical measurements after 30, 180, 365, and 1460 days, whereas within-lane replicates were pooled for further analysis.

Measurements of physical soil parameters and greenhouse gas fluxes

Bulk density was determined gravimetrically after oven drying at 105 °C and defined as the mass of dry soil divided by the sample volume. Total pore volume was determined as mass difference between saturated and oven-dried samples. Pore size distribution was determined using the standard pressure-plate procedure for soil moisture retention curve (Hartge and Horn, 1992). Proportions of pore size classes were calculated on the basis of the measured water desorption characteristics (Tebrügge and Düring, 1999). Saturated hydraulic conductivity (kf) was measured using an ICW soil water permeameter model 09.02 (Eijkelkamp Agrisearch Equipment, Giesbeek, the Netherlands) after soil samples have been water saturated for 72 h. Air permeability (ki) was measured after each draining step (30–300 kPa) using an air permeameter model 08.07 (Eijkelkamp) (Gysi *et al.,* 1999).

Net soil-atmosphere fluxes of methane (CH_4), carbon dioxide (CO_2) and nitrous oxide (N_2O) were measured in the triplicate skid trails in monthly intervals between September and December of 2008 (medium-term response) and 2012 (long-term response). Without *a priori* knowledge of the compaction effects, the purpose of measuring the gas fluxes was to assess broad functional end points related to carbon and nitrogen turnover in these soils, which ultimately reflect the degree of disturbance as well as the functional resilience in this system. Fluxes were measured as described recently (Hartmann *et al.,* 2011; Hartmann and Niklaus, 2012; Poll *et al.,* 2013). Static chambers were installed in close proximity to the soil sampling spots a few weeks before the measurements. Headspace gas samples of 30 ml (total headspace volume 7.9 l) were collected at intervals of 5, 20 and 35 min after chamber closure and analyzed using an Agilent 7890 gas chromatograph (Agilent Technologies, Palo Alto, CA, USA). Concentrations were calibrated against standard gas mixtures simultaneously analyzed with every batch of samples, and gas fluxes were calculated by linear regression of gas concentration against time, accounting for air temperature and pressure at the time of sampling. For each period of measurement (medium- and long-term), average gas fluxes were calculated across the 4-month period, representing an unbiased estimate of the fluxes integrated over time.

Spatial and temporal treatment effects on physical soil properties and gas fluxes were examined using a repeated measures factorial analysis of variance (ANOVA) followed by Fisher's protected least significant difference (LSD) *post-hoc* test, which reasonably controls the familywise error rate as long as not more than three treatment groups are compared (Meier, 2006). Significance levels of overall pairwise tests were adjusted for multiple comparisons using the Holm method (Holm, 1979). Non-normal data were square-root or log transformed.

Pyrosequencing and quantitative PCR of bacterial and fungal ribosomal markers

Total nucleic acids were extracted in duplicates from 0.5 g sieved soil (2 mm) using a bead-beating procedure (Frey *et al.,* 2008). DNA concentrations were determined using PicoGreen (Molecular Probes, Eugene, OR, USA). PCR amplification of partial bacterial small-subunit ribosomal RNA genes (region V1–V3 of 16S) and fungal ribosomal internal transcribed spacers (region ITS2) was performed using 50 ng of soil DNA as described previously (Hartmann *et al.,* 2012). Each sample was amplified in triplicates and pooled before purification with Agencourt AMPure XP beads (Beckman Colter, Berea, CA, USA) and quantification with the Qubit 2.0 fluorometric system (Life Technologies, Paisley, UK). Amplicons were unidirectionally sequenced using 454 pyrosequencing at the Functional Genomics Center Zurich (Switzerland) using the GS-FLX Titanium technology (Roche 454 Life Sciences, Branford, CT, USA).

Relative abundances of bacterial and fungal communities were determined by quantitative PCR on an ABI7500 Fast Real-Time PCR system (Applied Biosystems, Foster City, CA, USA) with the same primers and cycling conditions as used for the pyrosequencing approach. PCR was performed using 2.5 ng DNA in a total volume of 25 µl containing 0.5 µM of each primer, 0.2 mg ml^{-1} bovine serum albumin and 12.5 µl of QuantiTect SYBR Green PCR master mix (Qiagen, Valencia, CA, USA). Three standard curves per target region (correlations $\geqslant$0.997) were obtained using 10-fold serial dilutions (10^{-1} to 10^{-9} copies) of plasmids generated from cloned targets. Data were converted to represent average copy number of targets per gram of soil dry weight. Spatiotemporal treatment effects were examined using repeated measures factorial ANOVA of log-transformed copy numbers followed by Fisher's least significant difference and Holm adjustments.

Pyrotag processing

Flowgrams were trimmed to low quality signals (Quince *et al.,* 2011) and demultiplexed using MOTHUR (Schloss *et al.,* 2009) allowing one mismatch to the sample-specific barcode and two mismatches to the target-specific primer (Schloss *et al.,* 2011). Flowgrams were denoised using PYRONOISE (Quince *et al.,* 2009) in MOTHUR to eliminate sequencing errors. The bacterial $16S_{V1\text{-}V2}$ (that is, region spanning V1 and V2) and the fungal ITS2 region were verified and extracted using V-XTRACTOR (Hartmann *et al.,* 2010) and its ITS counterpart (Nilsson *et al.,* 2010) in order to remove spurious reads and compare phylogenetically consistent regions (Schloss, 2012). Sequences were further denoised using SEQNOISE (Quince *et al.,* 2011) in MOTHUR to eliminate PCR single-base errors. Potentially chimeric sequences were removed using the *de novo* detection mode in UCHIME (Edgar *et al.,* 2011).

Curated sequences were clustered into operational taxonomic units (OTUs) using the unsupervised Bayesian clustering algorithm CROP (Hao *et al.,* 2011) and an identity threshold of 97%. All reads in a given OTU were assigned to curated taxonomic databases using the naïve Bayesian classifier (Wang *et al.,* 2007) in MOTHUR and a minimum bootstrap support of 60%. Bacterial and fungal reads were queried against GREENGENES (DeSantis *et al.,* 2006; McDonald *et al.,* 2011) and UNITE (Abarenkov *et al.,* 2010), respectively. The consensus taxonomy of each OTU was determined using MOTHUR as the taxonomic path represented by at least 80% of the sequences. On the basis of the consensus taxonomies, abundance data for OTUs at specific taxonomic ranks (species, genus, family, order, class and phylum) were merged and used to generate taxonomic rank-specific matrices that were the basis for the network and the taxa-treatment association analyses.

Analysis of alpha and beta diversity

Estimates of alpha diversity were calculated in MOTHUR. These estimates included the observed OTU richness, the Good's coverage (Good, 1953), the parametric 'best fit' richness estimation CatchAll (Bunge *et al.,* 2012) and the Shannon diversity index (Magurran, 2004, Haegeman *et al.,* 2013). As alpha diversity measures are sensitive to differences in sampling effort, estimates were calculated based on data sets that were randomly subsampled to the same number of sequences. Spatiotemporal treatment effects on alpha diversity estimates were examined using a repeated measures factorial ANOVA followed by Fisher's least significant difference and Holm adjustments.

Multivariate analysis of beta diversity was performed according to the recommendations by Anderson and Willis, 2003 who proposed four components in the analysis of multivariate ecological data: (1) a robust unconstrained ordination to determine structural similarities among communities; (2) a compatible constrained analysis with reference to a specific hypothesis; (3) a rigorous statistical test of the hypothesis; and (4) characterization of the taxa responsible for the multivariate patterns. In accordance with this strategy, we used the following techniques for the corresponding purposes: (1) principal coordinate analysis (PCO; Gower, 1966); (2) canonical analysis of principal coordinates (CAP; Anderson and Willis, 2003); (3) analysis of similarities (ANOSIM; Clarke, 1993) and permutational multivariate analysis of variance (PERMANOVA; Anderson, 2001); and (4) taxon-treatment association analysis (De Cáceres and Legendre, 2009).

Inter-sample Bray-Curtis similarities (Bray and Curtis, 1957) were calculated based on standardized and square root transformed OTU abundances (Hartmann *et al.,* 2012). ANOSIM, PERMANOVA, and CAP were run with 10^5 permutations as routines in PRIMER6+ (Clarke and Gorley, 2006). Both PERMANOVA and ANOSIM were applied in parallel as they are complementary, the first offering analyses of complex designs including interactions and the second providing a universal measure of group separation while being fully non-parametric and thus robust in its application to ecological data (Lek *et al.,* 2011). Permutational analysis of multivariate dispersion (PERMDISP; Anderson, 2006) was used to control the influence of multivariate heterogeneity among *a priori* groups. The discriminative power of permutation-based analyses for pairwise comparisons of triplicates is limited by only 10 possible permutations (Clarke, 1993). In order to test compaction effects within each site and sampling date, the within-group to among-group Bray-Curtis dissimilarities were compared using the Wilcoxon rank sum test (Wilcoxon, 1945) including Holm adjustments for multiple comparisons.

The degree of preference of each taxon for the target group relative to the other groups (that is, the point biserial correlation coefficient R) was determined using taxon-treatment association analyses with all possible group combinations (De Cáceres and Legendre, 2009; De Cáceres *et al.,* 2010). Singletons and doubletons, that is, taxonomic units which were represented by only one or two sequences across the whole data set were not included in the analysis. The analyis was peformed in GINKGO (Bouxin, 2005) with 10^6 permutations. *P*-value adjustments for multiple comparisons were performed using the false discovery rate (FDR; Storey, 2002). *Q*-values were determined using the software QVALITY (Käll *et al.,* 2009), and associations were considered significant with an FDR of 5% ($q<0.05$). On the basis of the taxonomic rank-specific matrices, abundance-based taxonomic association networks were generated using the organic layout algorithm in CYTOSCAPE 2.8 (Smoot *et al.,* 2011) and manual adjustments. Degree of resistance

(overall association strength) and resilience (decrease of association strength between the first and the fourth year post disturbance) was mapped onto these taxonomic association networks.

Results

Soil physical properties and soil functions

Machine passes significantly increased bulk density and reduced porosity, saturated hydraulic conductivity and air permeability in the first week post disturbance (Table 1, Figure 1). Bulk density increased by an average of 16 and 25% after light (C1) and severe (C2) compaction, respectively. Total pore volume simultaneously decreased by 10% and 21%, respectively. Compaction particularly affected large-sized soil pores, reducing macropore volume by 49% (C1) and 73% (C2), respectively. Structural alterations significantly changed soil functional properties, leading to substantial reduction in hydraulic conductivity (-51% and -94%) and air permeability (-94% and -99%), almost completely restricting water infiltration and gas exchange in C2. The response of these properties to compaction was mostly uniform across forest sites, except for the soil texture-driven differences in hydraulic conductivity and macropore volume of the uncompacted reference soils, causing significant *site* × *treatment* interactions (Table 1).

Changes in physical properties altered the greenhouse gas fluxes (Table 2, Figure 1). Compaction significantly reduced net CH_4 consumption, decreased CO_2 emission and increased N_2O emission. Methane consumption decreased by an average of 34% (C1) and 99% (C2), respectively, even resulting in a net CH_4 emission in C2 at Ermatingen. CO_2 emission increased by 16% in C1, but this increase was statistically not significant, whereas CO_2 emission significantly decreased by 24% in C2. N_2O emission significantly increased with increasing compaction by an average of 94% (C1) and 181% (C2). No significant *time* × *treatment* interaction was observed, indicating that differences in greenhouse gas fluxes among treatments remained largely consistent up to around 5 years post disturbance. Treatment differences were also largely consistent across forest sites, showing a significant *site* × *treatment* interaction only for CH_4. The compaction effect on CH_4 was significant at both sites, but the flux alteration was more pronounced at Heiteren than at Ermatingen.

Taxonomic composition

After quality filtering, a total of 473 429 bacterial $16S_{V1\text{-}V2}$ and 423 720 fungal ITS2 sequences remained for community analysis and are provided as Supplementary Data 1. These data correspond to an average of 6575 ± 2947 bacterial $16S_{V1\text{-}V2}$ and 5885 ± 2218 fungal ITS2 pyrotags per sample, with an average read length of 256 ± 2 bp and 276 ± 16 bp, respectively. Sequence clustering yielded 6933 (782 ± 267 per sample) bacterial and 2598 (205 ± 96) fungal OTUs, respectively, representing an average Good's coverage of $95 \pm 3\%$ and $99 \pm 1\%$.

A complete list of the detected bacterial and fungal taxa, from phylum to OTU level, including abundance information, is provided as Supplementary Data 2. In brief, a total of 5690 (81%) bacterial OTUs accounting for 97% of the bacterial pyrotags were identified at the phylum level. Proteobacteria (1929 OTUs, 43.2%), Acidobacteria (30.6%, 325 OTUs), and to a lesser extent Bacteroidetes (4.6%, 551 OTUs), Actinobacteria (4.6%, 400 OTUs), Gemmatimonadetes (2.8%, 114

Table 1 Spatial compaction effects on physical soil properties measured in the first week after the disturbance

Main test[a]	*Bulk density* ($g\,cm^{-3}$)	*Total pore volume* (%)[b]	*Macropore volume* (%)[b]	*Hydraulic conductivity* (*m per day*)[b]	*Air permeability* $ki_{60}(\mu m^2)$	*Air permeability* $ki_{150}(\mu m^2)$
Site ($F_{1,12}$)	206.4***	19.9***	44.1***	38.7***	0.6[ns]	0.9[ns]
Compaction ($F_{2,12}$)	124.2***	80.0***	171.5***	82.2***	572.5***	562.4***
Site × compaction ($F_{2,12}$)	0.1[ns]	2.4[ns]	21.0***	22.2***	3.8[ns]	0.3[ns]
Pairwise test[c]						
C0	1.17 ± 0.03^A	53.2 ± 0.8^A	12.8 ± 0.9^A	1.92 ± 0.37^A	184.7 ± 10.6^A	252.6 ± 13.9^A
C1	1.36 ± 0.03^B	47.7 ± 0.8^B	6.1 ± 0.3^B	0.59 ± 0.06^B	7.8 ± 1.1^B	20.3 ± 1.6^B
C2	1.46 ± 0.03^C	42.2 ± 0.5^C	3.4 ± 0.4^C	0.08 ± 0.02^C	0.1 ± 0.1^C	4.1 ± 0.9^C

Abbreviations: ANOVA, analysis of variance; C0, no compaction; C1, light compaction; C2, severe compaction, Hyd, hydraulic; LSD, least significant difference.
[a]Effects of main factors and their interaction assessed by repeated measures factorial ANOVA (degrees of freedom for each factor and the corresponding error term are given in brackets). Factors represent site (Ermatingen, Heiteren) and compaction (C0, C1 and C2). Hydraulic conductivity and air permeability data were square-root transformed in order to obtain more normal distribution of residues. Values in table represent the F-ratio and the level of significance (ns, not significant; $*P<0.05$; $**P<0.01$; $***P<0.001$).
[b]Measurements of soil porosity and hydraulic conductivity in these plots were reported previously (Frey *et al.,* 2011).
[c]Pairwise comparisons between compaction treatments using Fisher's protected LSD *post-hoc* test and Holm-based *P*-value adjustments. Values in table represent means ± s.e. ($n=18$) of the properties. Different superscript capital letters indicate significant differences at $P<0.05$.

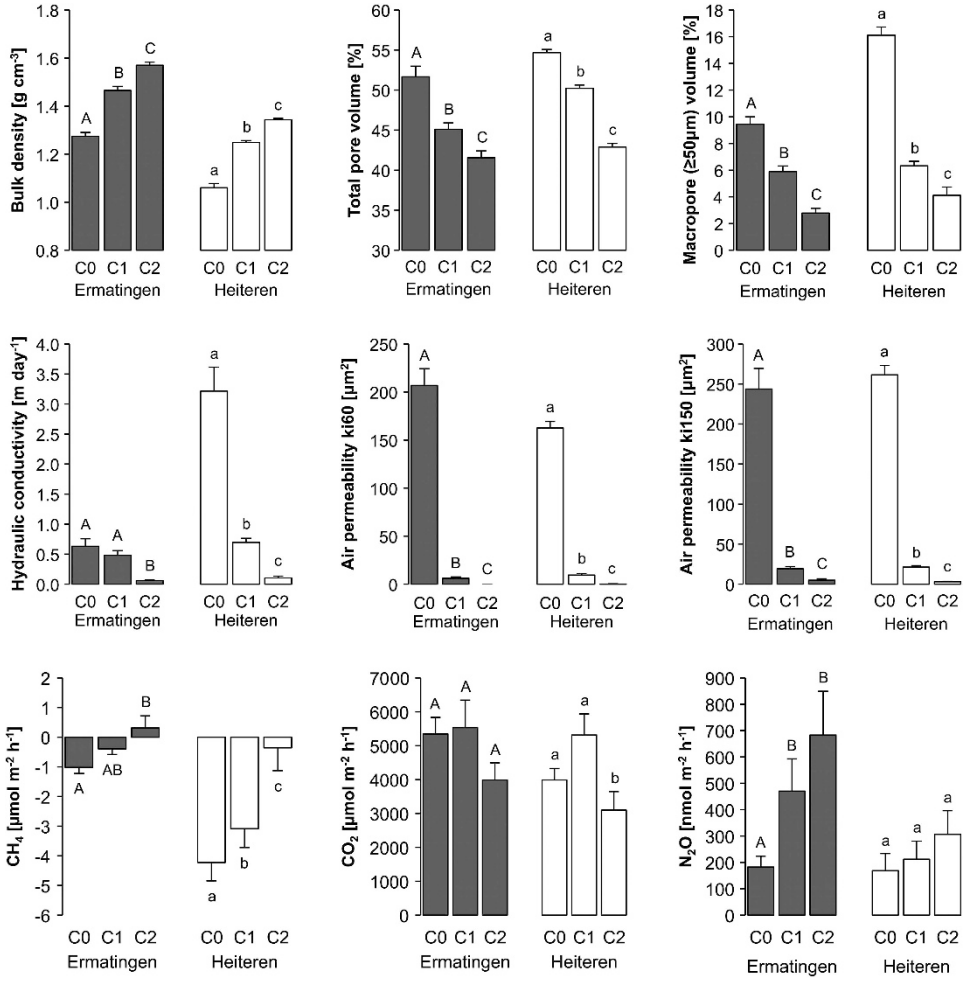

Figure 1 Physical soil properties and trace gas fluxes in the differently compacted soils at the two forest sites Ermatingen (gray shaded bars) and Heiteren (white bars). The two top panels show the physical soil properties measured in triplicate skid trails during the first few days after compaction (means ± s.e. $n = 9$). The bottom panel shows the average CH_4, CO_2 and N_2O fluxes in the triplicate plots as the integrated flux over two 4-month periods in 2008 and 2012 (means ± s.e. $n = 6$). Different letters indicate significant differences within the same forest site at $P < 0.05$ (upper case letters for Ermatingen and lower case letters for Heiteren). The omnibus ANOVA test results are given in Tables 1 and 2. Levels of soil compaction: no compaction (C0); light compaction (C1); and severe compaction (C2).

Table 2 Spatial and temporal compaction effects on greenhouse gas fluxes integrated over 4-month periods 1–2 years (medium-term) and 5–6 years (long-term) after the disturbance

Main test[a]	*CH_4 (μmol m^{-2} h^{-1})*	*CO_2 (μmol m^{-2} h^{-1})*	*N_2O (nmol m^{-2} h^{-1})*
Site ($F_{1,12}$)	61.2***	4.5*	15.7**
Compaction ($F_{2,12}$)	29.8***	7.9**	12.4**
Site × compaction ($F_{2,12}$)	7.6**	1.0^{ns}	2.8^{ns}
Time ($F_{1,12}$)	60.7***	78.5***	1.0^{ns}
Time × site ($F_{1,12}$)	41.7***	0.2^{ns}	8.7*
Time × compaction ($F_{2,12}$)	1.2^{ns}	3.7^{ns}	2.9^{ns}
Time × site × compaction ($F_{2,12}$)	0.3^{ns}	1.3^{ns}	1.3^{ns}
Pairwise test[b]			
C0	-2.62 ± 0.57^{A}	4667 ± 349^{A}	176 ± 37^{A}
C1	-1.74 ± 0.52^{B}	5426 ± 487^{A}	341 ± 77^{B}
C2	-0.02 ± 0.43^{C}	3542 ± 381^{B}	495 ± 106^{C}

Abbreviations: ANOVA, analysis of variance; C0, no compaction; C1, light compaction; C2, severe compaction; LSD, least significant difference.
[a]Effects of main factors and their interaction assessed by repeated measures factorial ANOVA (degrees of freedom for each factor and the corresponding error term are given in brackets). Factors represent site (Ermatingen, Heiteren), compaction (C0, C1 and C2), and time (medium-term, long-term). CO_2 (log) and N_2O (square-root) data were transformed in order to obtain more normal distribution of residues. Values in table represent the F-ratio and the level of significance (ns, not significant; $*P<0.05$; $**P<0.01$; $***P<0.001$).
[b]Pairwise comparisons between compaction treatments using Fisher's protected LSD *post-hoc* test and Holm-based *P*-value adjustments. Values in table represent means ± s.e. ($n = 12$) of the fluxes. Different superscript capital letters indicate significant differences at $P<0.05$.

OTUs), Chloroflexi (2.2%, 516 OTUs), Planctomycetes (1.3%, 565 OTUs), candidate phylum AD3 (1.2%, 8 OTUs) and Firmicutes (1.1%, 288 OTUs) were the abundant bacterial phyla occurring with at least one percent relative abundance. Among the Proteobacteria, class Alpha accounted for 23.4% (527 OTUs), Beta for 5.5% (161 OTUs), Gamma for 7.0% (517 OTUs) and Delta for 5.8% (603 OTUs) of the total abundance. The success for taxonomic assignment decreased at lower taxonomic levels, revealing 5153 OTUs (85%), 3907 OTUs (73%), 2342 OTUs (60%), 904 OTUs (27%) and 63 OTUs (0.3%) that were identified at the class, order, family, genus and species level, respectively. The 10 most abundant bacterial genera were *Candidatus Solibacter* (14.0%), *Rhodoplanes* (4.4%), *Candidatus Koribacter* (1.2%), *Nitrospira* (0.8%), *Flavobacterium* (0.8%), *Geobacter* (0.8%), *Cytophaga* (0.5%), *Phenylobacterium* (0.4%), *Caulobacter* (0.4%) and *Burkholderia* (0.4%).

A total of 2239 (86%) fungal OTUs accounting for 97% of the fungal pyrotags were identified at the phylum level. Ascomycota (55%, 843 OTUs) and Basidiomycota (29%, 1239 OTUs) were the predominant phyla, with the former Zygomycota, Chytridiomycota and allies accounting for 13% (157 OTUs). Below the phylum level, 1686 OTUs (92%), 1365 OTUs (88%), 1150 OTUs (86%), 1007 OTUs (77%) and 382 OTUs (45%) were identified at the class, order, family, genus and species level, respectively. Thus, the classification success at lower taxonomic levels was much better for the fungal data set than for the bacterial data set. The 10 most abundant fungal genera were *Russula* (17.3%), *Mortierella* (12.3%), *Inocybe* (4.3%), *Clavulina* (3.9%), *Cryptococcus* (3.0%), *Laccaria* (2.8%), *Hydnotrya* (2.1%), *Neobulgaria* (2.1%), *Hygrophorus* (1.8%) and *Trichosporon* (1.8%).

Community-level abundance and alpha diversity

Soil compaction significantly reduced bacterial and fungal abundance (Table 3). Light compaction had no impact, whereas severe compaction reduced the number of bacterial and fungal targets at both sites. No significant *time* × *treatment* interaction was observed, indicating that differences in relative abundance among treatments remained largely consistent between 1 and 4 years post disturbance. The decline in fungal abundance was significantly greater at Ermatingen than at Heiteren, whereas the decline in bacterial abundance was similar at both sites (Figure 2).

Compaction generally increased alpha diversity, but effects were often site and time-dependent (Table 4). Compaction increased alpha diversity at least in C2, but the bacterial response at Ermatingen was not consistent with the overall observation. In the following, we discuss the response of Shannon diversity as representative measure (Figure 2). Fungal diversity increased with compaction. Diversity in the sandy soils at Heiteren only increased in the severely compacted soils, whereas diversity in the clayey soils at Heiteren increased at both compaction levels. At both sites, fungal diversity changed little after 30 days, then increased in compacted soils after 180–365 days, and was increasingly resilient after 4 years (Supplementary Figure 2). The bacterial response at Heiteren was similar to the fungal response, showing an increase in diversity in C2 but not in C1, as well as in the medium-term but not in the short or long-term. Bacterial diversity at Ermatingen showed a unique response. In C1, diversity was reduced after 30 days and 4 years, but did not differ from the control soils after 180 and 365 days. In C2, diversity was reduced in the medium-term but not in the short and long-term.

Given the sensitivity of alpha diversity to sampling effort, the above results are based on rarefied data sets. However, differences in alpha diversities based on the full data sets were identical (data not shown), which was not surprising given the high Good's coverage of 95% and 99% for the bacterial and fungal data sets and the observed robustness of the Shannon diversity to sampling effort (Supplementary Figure 3). Thus, patterns of alpha diversity can directly be compared with the following beta diversity measures, which are based on the complete data sets.

Beta diversity

Soil compaction significantly and persistently altered the bacterial and fungal community

Table 3 Spatial and temporal compaction effects on relative bacterial and fungal abundance

Main test[a]	*Relative bacterial abundance [16 S copies g^{-1} soil]*	*Relative fungal abundance [ITS copies g^{-1} soil]*
Site ($F_{1,12}$)	70.7***	56.5***
Compaction ($F_{2,12}$)	22.4***	111.7***
Site × Compaction ($F_{2,12}$)	2.8^{ns}	53.0***
Time ($F_{1,12}$)	6.9*	74.4***
Time × Site ($F_{1,12}$)	22.1***	100.7***
Time × Compaction ($F_{2,12}$)	3.7^{ns}	3.3^{ns}
Time × Site × Compaction ($F_{2,12}$)	4.9*	25.5*
Pairwise test[b]		
C0	5.96 ± 1.20 [× 10^{10}]^{A}	1.41 ± 0.22 [× 10^{8}]^{A}
C1	7.00 ± 2.10 [× 10^{10}]^{A}	2.62 ± 0.81 [× 10^{8}]^{A}
C2	2.36 ± 0.54 [× 10^{10}]^{B}	0.44 ± 0.14 [× 10^{8}]^{B}

Abbreviations: C0, no compaction; C1, light compaction; C2, severe compaction.
[a]Effects of main factors and their interactions assessed by repeated measures factorial ANOVA (degrees of freedom for each factor and the corresponding error term are given in brackets). Main factors represent site (Ermatingen, Heiteren), time (30, 180, 365, 1460 days), and compaction (C0, C1, C2). Values in table represent the F-ratio and the level of significance (ns, not significant; *$P<0.05$; **$P<0.01$; ***$P<0.001$).
[b]Pairwise comparisons between compaction treatments using Fisher's protected LSD *post-hoc* test and Holm-based *P*-value adjustments. Values in table represent means ± s.e. ($n=12$). Different superscript capital letters indicate significant differences at $P<0.05$.

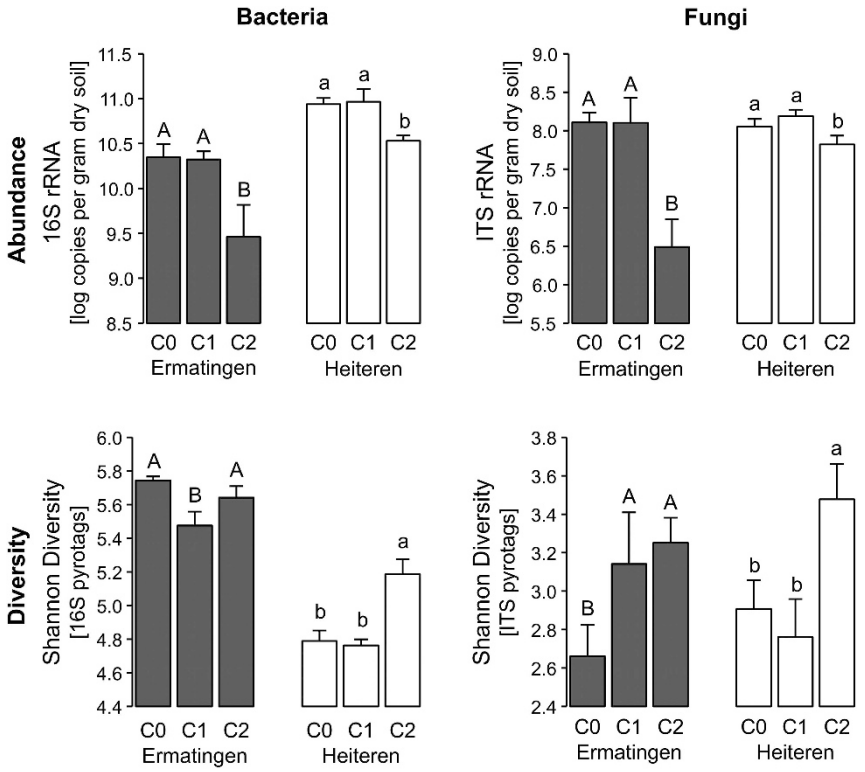

Figure 2 Bacterial and fungal abundance (target copy number; means ± s.e. $n=6$) and alpha diversity (Shannon index; means ± s.e., $n=12$) in the differently compacted soils at the two forest sites Ermatingen (gray shaded bars) and Heiteren (white bars) averaged over time. Different letters indicate significant differences within the same forest site at $P<0.05$ (upper case letters for Ermatingen and lower case letters for Heiteren). The omnibus ANOVA test results are given in Tables 3 and 4. Shannon indices were calculated based on rarefied OTU abundance matrices that were obtained by randomly subsampling each sample to the smallest data size (that is, 3465 bacterial and 2208 fungal sequences per sample). Levels of soil compaction: no compaction (C0); light compaction (C1); and severe compaction (C2).

Table 4 Spatial and temporal compaction effects on bacterial and fungal alpha diversity[a]

Main test[b]	*Bacteria*			*Fungi*		
	S_{obs}	*CatchAll*	H	S_{obs}	*CatchAll*	H
Site ($F_{1,12}$)	689.7***	204.7***	652.5***	8.0*	2.2^{ns}	0.1^{ns}
Compaction ($F_{2,12}$)	45.4***	23.1***	37.5***	24.3***	9.4**	12.4**
Site × compaction ($F_{2,12}$)	26.1***	3.7^{ns}	26.9***	10.9**	3.3^{ns}	4.4*
Time ($F_{3,36}$)	15.0***	1.9^{ns}	11.0***	26.2***	14.0***	28.3***
Time × site ($F_{3,36}$)	21.2***	3.6*	10.5***	4.4**	2.0^{ns}	0.4^{ns}
Time × compaction ($F_{6,36}$)	5.4***	1.4^{ns}	3.2*	7.1***	3.6**	4.7**
Time × site × compaction ($F_{6,36}$)	21.5***	3.3*	14.0***	5.2***	2.3^{ns}	2.1^{ns}
Pairwise test[c]						
C0	589 ± 39^{B}	1535 ± 188^{A}	5.27 ± 0.10^{B}	112 ± 8^{A}	264 ± 26^{A}	2.78 ± 0.11^{A}
C1	544 ± 31^{A}	1337 ± 126^{A}	5.12 ± 0.09^{A}	145 ± 15^{B}	286 ± 32^{A}	2.95 ± 0.17^{A}
C2	661 ± 31^{C}	2001 ± 166^{B}	5.41 ± 0.07^{C}	170 ± 12^{C}	422 ± 52^{B}	3.36 ± 0.11^{B}

Abbreviations: S_{obs}, observed richness; CatchAll, parametric 'best fit' richness estimate; H, Shannon diversity index; C0, no compaction; C1, light compaction; C2, severe compaction.
[a]Estimates are based on rarefied data sets (that is subsampled to the same number of sequences per sample, that is, 3465 bacterial and 2208 fungal sequences).
[b]Effects of main factors and their interactions assessed by repeated measures factorial ANOVA (degrees of freedom for each factor and the corresponding error term are given in brackets). Main factors represent site (Ermatingen, Heiteren), time (30, 180, 365, 1460 days) and compaction (C0, C1 and C2). Values in table represent the F-ratio and the level of significance (ns, not significant; $*P<0.05$; $**P<0.01$; $***P<0.001$).
[c]Pairwise comparisons between compaction treatments using Fisher's protected LSD *post-hoc* test and Holm-based *P*-value adjustments. Values in table represent means ± s.e. ($n=24$). Different superscript capital letters indicate significant differences at $P<0.05$.

Table 5 Spatial and temporal compaction effects on bacterial and fungal beta diversity

Main test[a]	*Bacteria*			*Fungi*		
	PERMANOVA		*ANOSIM*	*PERMANOVA*		*ANOSIM*
	F(p)	R^2	R	*F(p)*	R^2	R
Site	58.9***	32.8	0.975***	35.3***	34.1	0.988***
Time	2.9***	8.5	0.523***	4.6***	15.6	0.557***
Compaction	5.6***	11.3	0.708***	9.3***	20.6	0.783***
Site x time	2.3***	9.8		3.3***	17.6	
Site x compaction	4.2***	13.3		6.4***	23.4	
Time x compaction	2.1***	10.9		3.2***	21.3	
Site x time x compaction[b]	2.1***	16.0		3.1***	29.1	
Pairwise test[b]	*t(p)*	*Øsim*	R	*t(p)*	*Øsim*	R
C0 vs C1	1.6***	46.9	0.481***	2.5***	21.7	0.588***
C0 vs C2	2.8***	43.0	0.884***	3.9***	19.1	0.926***
C1 vs C2	2.5***	44.1	0.801***	2.8***	22.9	0.838***

Abbreviations: PERMANOVA, permutational multivariate analysis of variance; ANOSIM, analysis of similarities; C0, no compaction; C1, light compaction; C2, severe compaction.
[a]Effects of main factors and their interactions assessed by PERMANOVA and ANOSIM. Main factors represent site (Ermatingen, Heiteren), time (30, 180, 365 and 1460 days) and compaction (C0, C1 and C2). Values in table represent the F-ratio (F), the level of significance (ns, not significant; $*P<0.05$; $**P<0.01$; $***P<0.001$) and the estimation of the variance component (R^2) for PERMANOVA as well as the global R and the level of significance for ANOSIM. ANOSIM was performed using a two-way crossed design in order to solely consider similarities between samples within the same level of the second factor. Thus, in the 3-factorial setup, the second factor was the combination of the remaining factors (Lek, 2011 #11199). ANOSIM does not calculate interaction effects.
[b]Pairwise comparisons between compaction treatments using PERMANOVA and ANOSIM. Values in table represent the univariate t-statistic (t), the level of significance (ns, not significant; $*P<0.05$; $**P<0.01$; $***P<0.001$), and the average between-group Bray-Curtis similarity (Øsim) for PERMANOVA as well as the pairwise R and the level of significance for ANOSIM. Significance levels of all pairwise tests were Holm adjusted. None of the pairwise tests were influenced by significant differences in multivariate dispersion as determined by PERMDISP (data not shown).

structures, and the different compaction intensities resulted in significantly distinct communities (Table 5, Figure 3). Compaction treatments explained 11% and 21% of the variance in the bacterial and fungal data sets, respectively. The spatial and temporal components were also important drivers explaining 33–34% and 9–16% of the variance, respectively. Equivalent to alpha diversity, compaction effects on beta diversity were strongly dependent on forest site and time since disturbance (Table 5). Soil microbial community structures at Heiteren appeared to be more resistant and resilient than at Ermatingen. Fungi were less resistant and resilient than bacteria. Compaction effects were most pronounced in the medium-term, whereas communities were moderately affected in the short-term and showed trends of resilience in the long-term. The strong compaction effects were confirmed by CAP ordinations maximizing differences in community structures among the different levels of compaction (Supplementary Figure 4). Canonical discriminant analysis associated with CAP revealed high reclassification rates between 88% and 96%.

At Ermatingen, community structures were substantially altered in both C1 and C2 (Figure 3a). Light compaction altered both communities in the medium-term, with no response in the short-term and resilience after 4 years (Figure 3b). Severe compaction caused significant structural differences of both communities at all time points with some resilience after 4 years. At Heiteren, community structures changed little in C1, but were strongly altered in C2 (Figure 3a). The only effect of light compaction was observed on the fungal community after 180 days (Figure 3b). In contrast, severe compaction significantly altered both communities at all time points (bacteria after 30 days with $P=0.052$) with a trend to resilience over time. Notably, the 2-dimensional representation of the principal coordinate analysis was not able to adequately resolve differences in C2 after 4 years that became obvious when comparing within-group with among-group dissimilarities.

Resistant, sensitive and resilient microbial taxa

Taxonomic treatment association networks revealed the complex structure of the bacterial (Figure 4) and fungal (Figure 5) communities in these soils and demonstrated which taxonomic groups were significantly ($q<0.05$) associated with the compacted or undisturbed soils. A complete discussion of compaction-sensitive taxa is beyond the scope of this study, and we show only salient cases; however, treatment association statistics for all taxa are provided as Supplementary Data 2. At the OTU level, a total of 127 bacterial and 117 fungal OTUs

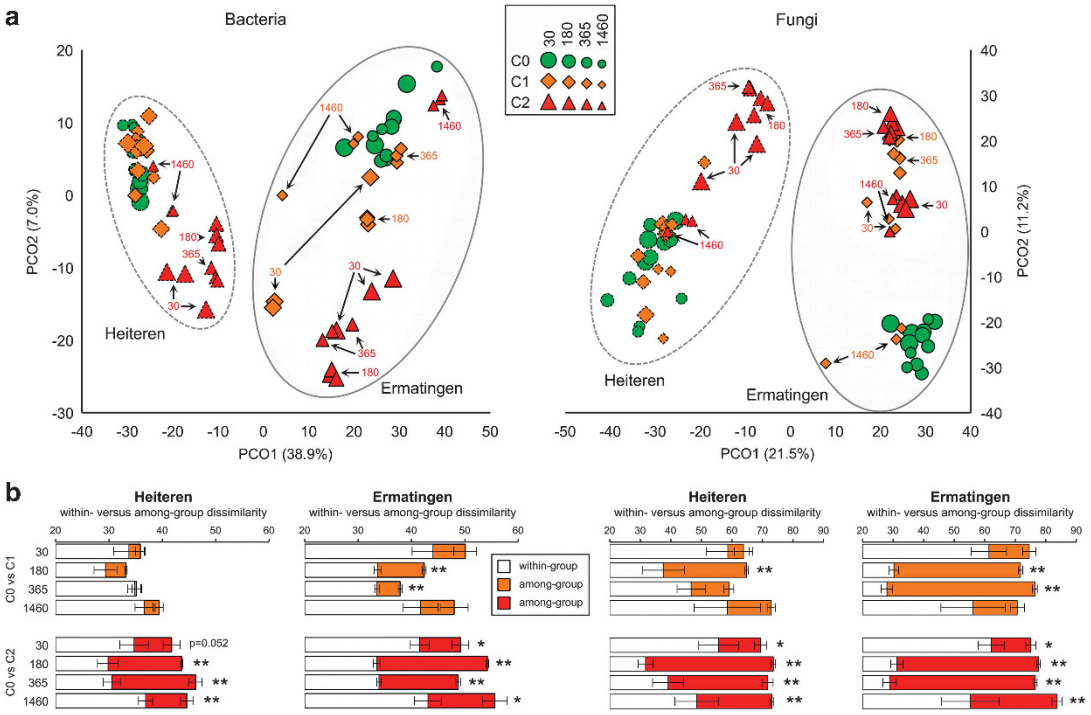

Figure 3 Differences in bacterial and fungal beta diversity in the differently compacted soils across the four different sampling dates at the two forest sites Ermatingen and Heiteren. (**a**) Principal coordinate analysis (PCO) ordinations of Bray-Curtis similarities calculated based on relative OTU abundances. Decreasing symbol size, as shown in the legend, indicates time since compaction and compaction samples showing strong deviation from the controls have been labeled. Symbols with straight lines represent samples from Ermatingen; symbols with hashed lines represent samples from Heiteren. Variance explained by each PCO axis is given in parentheses. Levels of soil compaction: no compaction (C0), green circles; light compaction (C1), orange diamonds; and severe compaction (C2), red triangles. (**b**) Pairwise comparisons of within- to among-group Bray-Curtis dissimilarities between control and compacted soils (C0 vs C1; C0 vs C2). White bars (means ± s.e. $n=3$) represent average within-group dissimilarity, whereas colored bars represent among-group dissimilarities (bars are overlapping, not stacked). Larger colored bars represent stronger differences between control and compacted soils. Asterisks indicate significant differences ($*P<0.05$, $**P<0.01$).

representing 7.3% and 24.6% of the pyrotags, respectively, were significantly affected by soil compaction.

Bacterial taxa that were significantly associated with compacted soils were assigned to taxonomic groups such as Delta- and Betaproteobacteria, Firmicutes, Acidobacteria, Bacteroidetes or Chloroflexi (Figure 4). Members adapted to environments with low oxygen availability—such as: the deltaproteobacterial genera *Geobacter*, *Anaeromyxobacter*, *Desulfuromonas*, *Desulfovibrio*, *Desulfobulbus*, *Pelobacter*, *Syntrophobacter* and *Sulfurospirillum*; the betaproteobacterial genera *Rhodoferax*, *Rhodocyclus* and *Dechloromonas*; or the firmicute genera *Clostridium*, *Desulfosporosinus*, *Sporotalea*, *Desulfitobacterium*, *Thermosinus*, *Bacillus*, *Paenibacillus*, *Acetivibrio*, *Thermincola* and *Ethanoligenens*—were all significantly increased in compacted soils (examples are listed in the order of decreasing abundance). Members that were primarily associated with the undisturbed soils were assigned to groups such as Alpha- and Gammaproteobacteria, Actinobacteria, as well as several candidate divisions. The proteobacterial genera *Candidatus Odysella* and *Steroidobacter* or the verrucomicrobial genus *Opitutus* were prominent genera that were reduced in compacted soils. Other taxa showed special patterns. *Nitrospira,* for example, was slightly increased in C0, strongly increased in C2, but negatively associated with C1, whereas *Candidatus Koribacter* revealed the optimum association with C1.

A majority of the abundant fungal taxa were significantly affected by soil compaction (Figure 5). Generally, Basidiomycota were negatively associated with compacted soils, whereas Ascomycota proportionally increased in compacted soils, suggesting an overall negative impact of compaction on ectomycorrhizal fungi and proportionally positive effects on saprobic fungi and the like. Indeed, among the abundant and most strongly affected taxa, many known or putative mycorrhizal genera such as *Russula*, *Inocybe*, *Clavulina*, *Hygrophorus*, *Elaphomyces*, *Hyphodontia*, *Boletus*, *Cortinarius* and *Tarzetta* were negatively affected by compaction. Conversely, many fungal genera with putative saprobic or parasitic lifestyles such as *Cryptococcus*, *Neobulgaria*, *Trichosporon*, *Lecythophora*, *Pseudeurotium*, *Chalara*, *Scutellinia*, *Penicillium*, *Leptodontidium*, *Hypocrea*, *Asterophora* and *Cheilymenia* proportionally increased in compacted soils.

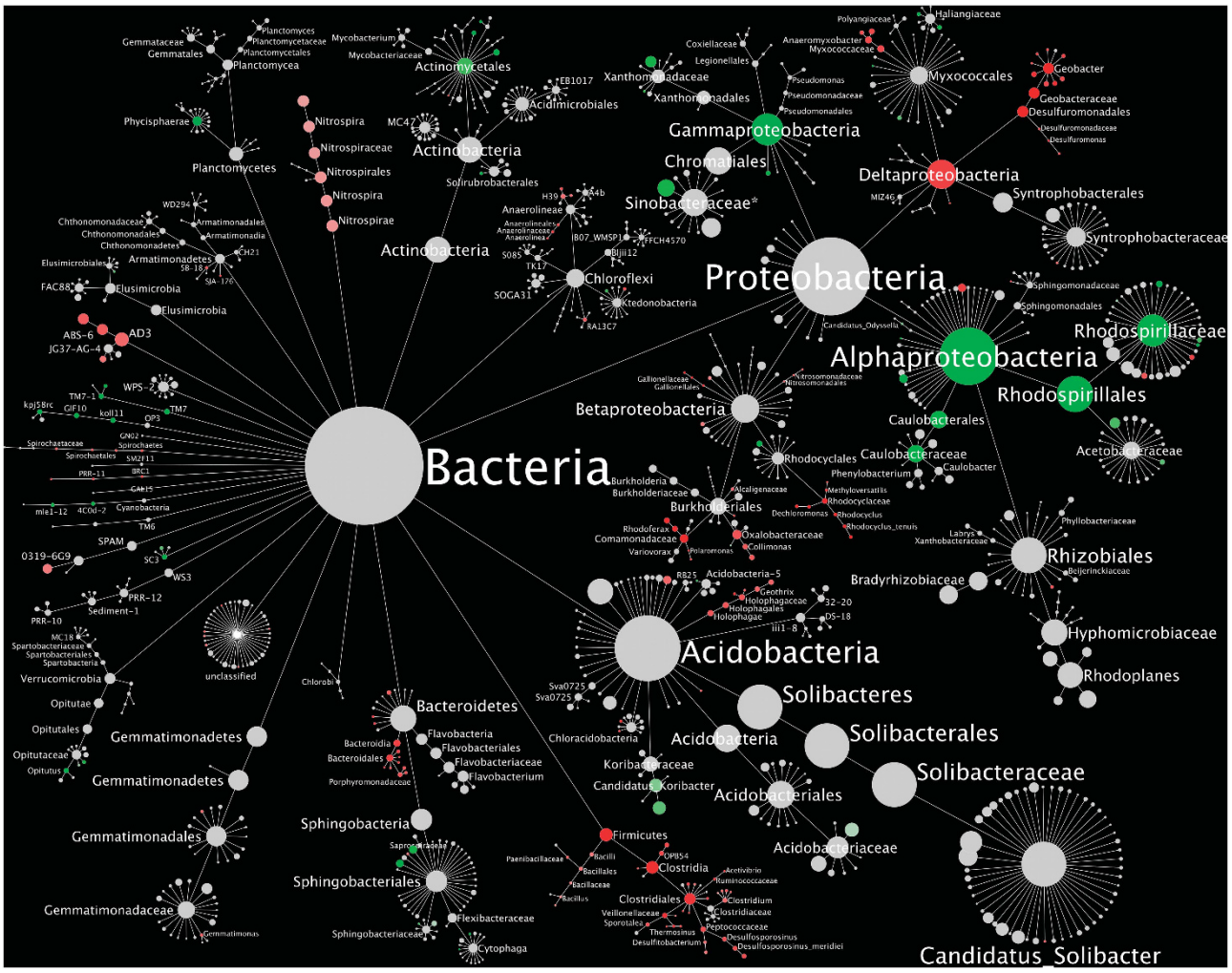

Figure 4 Taxonomic treatment association network (from domain to OTU) of abundant ($\geqslant 0.01\%$) bacterial taxa. The size of the nodes and the labels represents the relative abundance (square-root) of the corresponding taxonomic group in the data set. Colored nodes represent taxa that were significantly ($q<0.05$) influenced by compaction, and the color gradient represents the degree of association with either the undisturbed reference soils C0 (green, that is, increased relative abundance in C0) or with the severely compacted soils C2 (red, that is, increased relative abundance in C2). Given the intermediate role of light compaction, that is, strong compaction effects in Ermatingen but weaker effects in Heiteren, associations to C1 were ignored for this network. Nodes in light gray represent taxa with no significant treatment association. Labels are not shown at the OTU level and for taxa with less than 0.1% abundance (unless they were indicators or phylum-level assignments). An electronic, high-resolution image of the network is available online, allowing exploration of the network in more detail. The complete taxa-treatment association statistics is available in Supplementary Data 2.

We furthermore examined the long-term resilience of compaction-sensitive taxa as the reduction in treatment association strength over time (Supplementary Figures 5 and 6). A substantial proportion of the compaction-sensitive taxa showed no or little resilience after 4 years, but patterns specific for certain taxonomic groups were observed. For example, members of the Deltaproteobacteria and the Firmicutes were among the strongest bacterial indicators, proportionally increasing in compacted soils; however, whereas none of the deltaproteobacterial indicators showed substantial resilience, firmicute indicators largely recovered after 4 years (Figure 6). In conclusion, these taxonomic treatment association networks do not only allow visualizing the complex structure of the soil microbiota, but also help to detect patterns of resistance and resilience that are consistent across the complete taxonomic range.

Discussion

Allison and Martiny (2008) suggested microbial community structure as an indicator of environmental change, because this parameter is sensitive and not immediately resilient to disturbances, and structural shifts are often associated with changes in ecosystem processes. In line with this notion, we demonstrated that changes in physical soil properties after compaction significantly and persistently altered the soil microbiota and associated ecosystem

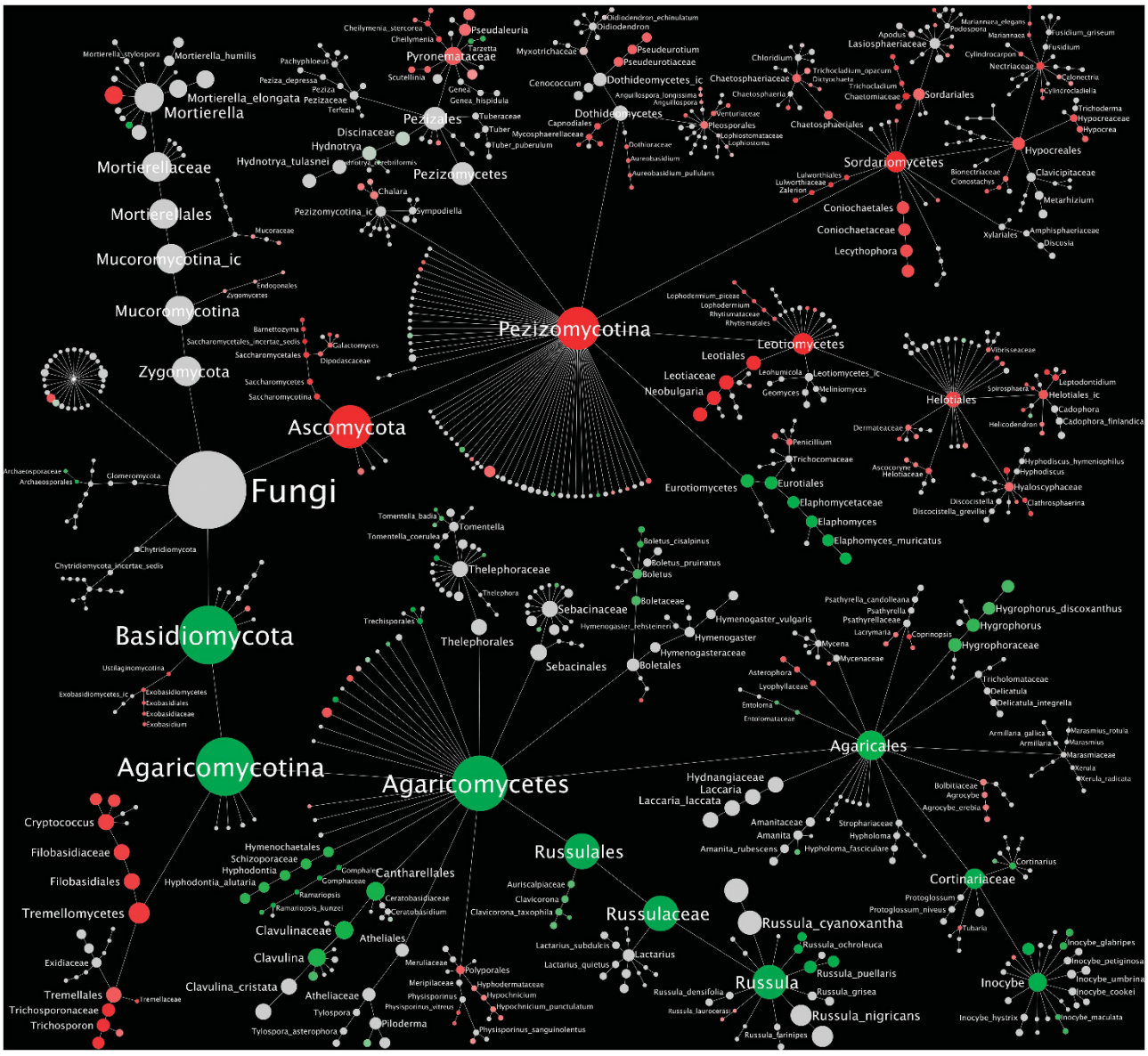

Figure 5 Taxonomic treatment association network (from domain to OTU) of the most abundant ($\geqslant 0.01\%$) fungal taxa. The information displayed is equivalent to the bacterial network in Figure 4. An electronic, high-resolution image of the network is available online, allowing exploration of the network in more detail. The complete taxa-treatment association statistics is available in Supplementary Data 2.

functions, such as turnover of carbon and nitrogen. As summarized in Figure 7, the combined assessment of these properties has therefore great potential to define compaction thresholds below which there is no detrimental and irreversible impact on the soil ecosystem. This finding is in agreement with recent studies in this field (Frey *et al.,* 2011; Hartmann *et al.,* 2012), but providing an unprecedentedly comprehensive view on the complex microbial response to compaction. First, the combined assessment of physicochemical and biological characteristics in a uniquely controlled experiment provides an integrative view on how changes in physical soil properties are linked to major shifts in the microbiota and associated soil processes. Second, to the best of our knowledge, this study is the first high-throughput sequencing assessment of compaction effects on microbial diversity, acknowledging that effects observed in the only other high-throughput sequencing study were confounded by effects from forest biomass removal (Hartmann *et al.,* 2012). The power of new sequencing technologies to assess structural shifts in the soil microbiota at deep coverage and high phylogenetic resolution provided novel information regarding the resistance and resilience of the forest soil microbiome to compaction.

Logging operations can increase the frequency and duration of anoxic conditions in forest soils (Goutal *et al.,* 2012). In this study, logging vehicle

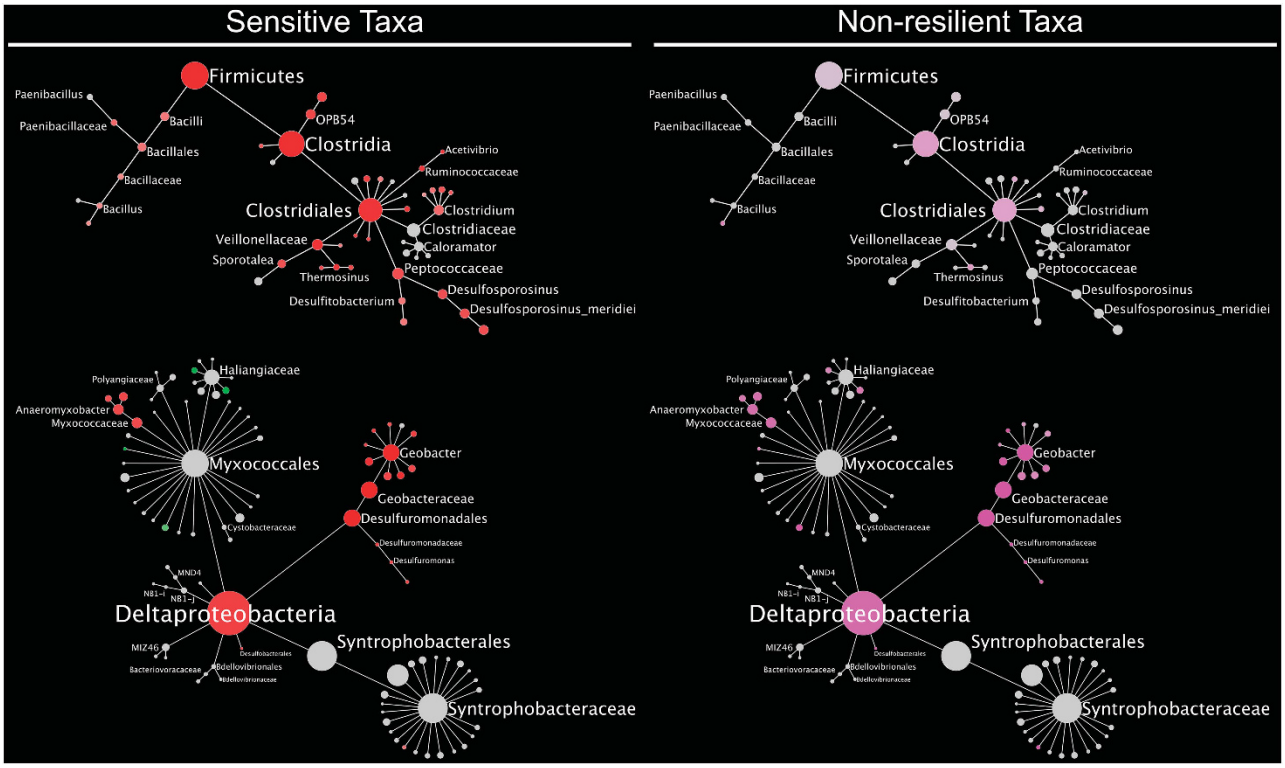

Figure 6 Compaction-sensitive (left panel) and non-resilient (right panel) taxa among the abundant members (⩾0.01%) of the Deltaproteobacteria and Firmicutes (unlabeled nodes represent OTUs). The information displayed in the left panel is equivalent to the information provided in the complete association network (see Figure 4 for details), namely that colored nodes (that is, red and green) represent taxa that were sensitive to compaction (with color intensity equivalent to the overall treatment association strength). Colored nodes (purple) in the right panel represent compaction-sensitive taxa that lack resilience. Nodes with intense purple color represent taxa with low resilience (taxon-treatment association strength remains largely unchanged up to 4 years post disturbance), whereas nodes with fading purple color represent resilient taxa (association strength decreased between the first and the fourth year post disturbance). Thus, nodes in gray represent taxa that were either resistant to begin with or resilient after 4 years. Information about resilience for all abundant bacterial and fungal taxa can be found in Supplementary Figures 5 and 6.

traffic induced profound changes in soil structure, which in turn drastically reduced water and air conductivity in the compacted skid trails (Table 1, Figure 1). It has been reported that an increase in bulk density beyond 15% can become harmful (Lacey and Ryan, 2000), a threshold that was reached after light compaction and substantially exceeded after severe compaction. Measuring bulk density alone is, however, not sufficient to predict the consequences for soil functional properties such as water infiltration and air permeability, as these properties are less controlled by total porosity (and thus bulk density) than by macroporosity (Young and Ritz, 2000). Macropore volume was reduced by up to 73% in the first week post disturbance, almost completely restricting water and air infiltration in the severely compacted soils.

Altered conditions in the compacted soils reduced abundance, increased alpha diversity and shifted the composition of the microbiota (Figures 2 and 3). Whereas the decrease in abundance suggests a potentially detrimental effect on microbial activity, the increase in alpha diversity and shift in beta diversity might indicate a loss of functional organization in these communities. The decrease in microbial abundance is in agreement with only few previous reports (Dick *et al.,* 1988; Frey *et al.,* 2009) and in contradiction with the majority of studies that did not observe an impact of forest soil compaction on microbial biomass (Jordan *et al.,* 1999; Ponder and Tadros 2002; Li *et al.,* 2004; Shestak and Busse, 2005; Tan *et al.,* 2005; Busse *et al.,* 2006; Tan *et al.,* 2008). Contradictory results have also been reported for bacterial and fungal alpha diversity, as we have not observed any compaction effects in a previous study (Hartmann *et al.,* 2012). However, given that traditional methods were limited in adequately measuring alpha diversity, this property has yet rarely been assessed in such systems. In contrast to the integrated parameters like biomass or alpha diversity, effects on community composition have been frequently reported (Ponder and Tadros, 2002; Shestak and Busse, 2005; Busse *et al.,* 2006; Schnurr-Pütz *et al.,* 2006; Frey *et al.,* 2009, 2011; Hartmann *et al.,* 2012). However, most of these studies were based on the first generation of molecular techniques such as phospholipid fatty

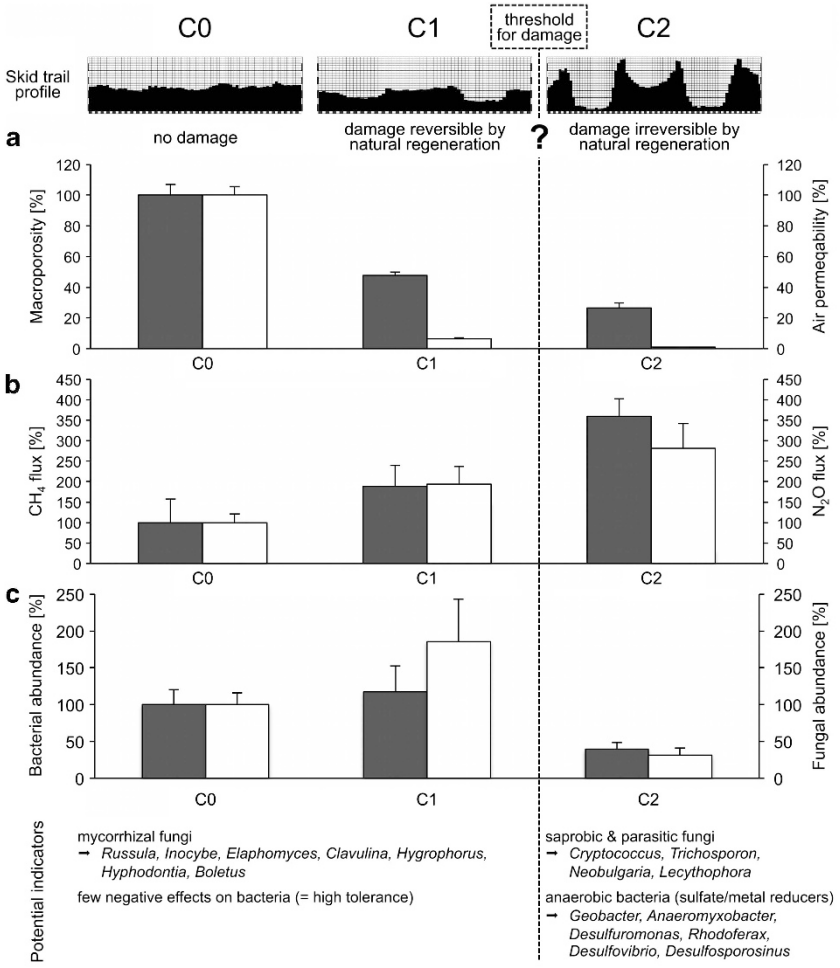

Figure 7 Diagram summarizing the compaction effects on soil physical properties (**a**), soil processes (**b**) and (**c**) microbial characteristics. The combined assessment of these properties has the potential to define a compaction threshold below which there is no detrimental and irreversible impact on the soil ecosystem. Effects are shown as percent change compared with the uncompacted reference soil (means ± s.e.). The upper panel shows representative rut types for the different compaction levels.

acid or terminal restriction fragment length polymorphism analysis and were thus limited in the structural resolution as well as in the taxonomic identification of compaction-sensitive groups. In the present study, using an array of cutting-edge molecular techniques, the results dismantle the notion that the forest soil microbiome is largely resistant or resilient to logging-induced compaction (Shestak and Busse, 2005; Busse *et al.,* 2006). Considering that the observed effects on microbial characteristics were strongly dependent on the degree of disturbance (for example, ground contact pressure, soil type), the contradiction with many previous studies is most likely based on the different impacts examined in the different surveys. The lack of a unifying concept highlights the need for properly controlled experiments to determine compaction thresholds below which there is no negative impact on the soil microbiota.

Although altered physical conditions established immediately after compaction, structural shifts in the microbiota peaked in the medium-term around 6–12 months after the disturbance and showed less response in the first few weeks after compaction. Whereas it can be expected that microbial activity will be immediately affected by water and oxygen limitations, structural shifts of mostly slow-growing soil microorganisms likely manifest at a slower rate, in particular, as the compaction experiment was conducted in spring, where soil temperatures were low. Four years after compaction, the community structure has recovered in lightly but not in heavily compacted soils. The lack of structural resilience correlated with the lack of functional resilience in terms of altered greenhouse gas fluxes, supporting the notion that changes in composition are often associated with changes in ecosystem processes (Allison and Martiny, 2008). One could argue that the observed treatment differences originated from comparing different soil horizons due to the formation of ruts and associated soil displacements. However, differences in carbon contents among samples were minimal, suggesting that equivalent horizons were compared (Frey *et al.,* 2011). Furthermore, we

examined compaction effects within the topsoil, but the treatment will likely have an impact along the complete depth profile, knowing that alterations in bulk density after compaction are even more persistent in subsoil (Page-Dumroese *et al.,* 2006).

Oxygen and water limitation in the compacted tracks introduced specific structural shifts to the microbiota (Figures 4 and 5). In line with previous observations but at much higher resolution (Schnurr-Pütz *et al.,* 2006, Frey *et al.,* 2011), bacteria adapted to low oxygen availability and capable of anaerobic respiration strongly increased in compacted soils. These taxa included many known sulfate, sulfur and metal reducers like *Geobacter*, *Desulfuromonas*, *Anaeromyxobacter*, *Rhodoferax*, *Desulfovibrio*, *Desulfosporosinus*, *Desulfobulbus* and *Geothrix* (Dworkin *et al.,* 2006). Among other compaction-associated taxa, we observed genera capable of anoxygenic photosynthesis (*Rhodocyclus*), anaerobic perchlorate reduction (*Dechloromonas*) or exhibiting other strategies of anaerobic growth with metabolically versatile or largely cryptic lifestyles (for example, *Clostridium*, *Thermosinus*, *Sporotalea*, *Acetivibrio*, *Anaerolinea*). Accordingly, the increased bacterial diversity in compacted soils might have been caused by the high number of anaerobically respiring species in combination with a considerable tolerance of aerobic bacteria and increased protection from protozoan grazing (Wright *et al.,* 1995), unless conditions became critically limited as potentially observed after 6–12 months at Ermatingen (Figure 2). Whereas some groups such as the Firmicutes showed almost complete resilience within 4 years, other groups such as the Deltaproteobacteria did not yet recover (Figure 6). According to Allison and Martiny (2008), Deltaproteobacteria such as *Geobacter* might therefore serve as indicators of compaction in otherwise well-aerated soils, as they were sensitive and not immediately resilient to compaction, reflect the functional status of the system (for example, in terms of oxygen availability and greenhouse gas emission), and are abundant thereby facilitating detection. Despite the significant impact on bacteria, many highly abundant bacterial taxa such members of the orders Solibacterales, Rhizobiales and Gemmatimonadales did not significantly respond to compaction, indicating that certain bacterial groups indeed exhibit considerable tolerance to these disturbances as reported by other studies (Shestak and Busse, 2005).

Fungi appeared to be more sensitive and less resilient to compaction when compared with bacteria (Figures 3 and 5). This difference can in part be explained by the generally higher sensitivity of eukaryotes to low oxygen pressures (Schnurr-Pütz *et al.,* 2006). The fact that mycorrhizal species were almost exclusively reduced in compacted soils also suggests negative effects on plant hosts, mechanical disruption of existing mycorrhizal networks and limited network reformation owing to restricted hyphal penetration. Abundant compaction-sensitive mycorrhizae included genera such as *Russula*, *Inocybe*, *Clavulina* and *Elaphomyces* (Figure 5), whereas *Inocybe* was largely resilient, hypogeous *Elaphomyces* did not recover 4 years post disturbance (Supplementary Figure 6). Non-mycorrhizal taxa proportionally increased in the compacted soils. Abundant saprobic fungi like *Neobulgaria*, *Cryptococcus*, *Trichosporon* and *Lecythophora* likely benefited from freshly exposed organic matter after vegetation dieback and physical breakdown of soil aggregates, although being largely tolerant to lower oxygen concentrations. Some compaction-associated fungi such as the aeroaquatic *Cylindrocarpon* are reportedly adapted to periodically low availability of oxygen (Medeiros *et al.,* 2009). Compaction temporarily increased fungal diversity, suggesting a stimulating effect of fresh organic matter on saprobic fungi in the first year post disturbance (Figure 2). In conclusion, the profound changes in the fungal community suggest significant and persistent alterations with respect to plant–microbe interactions and nutrient cycling, and raise concern regarding forest productivity, juvenile tree regeneration and long-term ecosystem functioning.

Structural shifts in the soil microbiota were accompanied by changes in soil processes, reducing methane consumption, decreasing carbon dioxide emission and increasing nitrous oxide emission (Table 2, Figure 1). These changes are consistent with previous findings (for example, Teepe *et al.,* 2004; Keller *et al.,* 2005; Frey *et al.,* 2011; Goutal *et al.,* 2012). We already reported on the higher abundance of methanogenic archaea linked to increased methanogenesis in the compacted skid trails (Frey *et al.,* 2011). Despite perfect matches of the primers, we recovered only very few methanotroph pyrotags in these soils and cannot conclude on potentially co-occurring negative effects on methane oxidation. However, it can be hypothesized that the anaerobic conditions largely limited the predominantly aerobic methanotrophs and contributed to methane emission by reducing methane oxidation.

The response of the CO_2 flux was highly variable and appeared to be bivalent. Moderate compaction tended to increase CO_2 emission, whereas severe compaction reduced the CO_2 flux. CO_2 production is driven by soil organic matter decomposition and root respiration, and it has been reported that soil CO_2 production is greater under aerobic than anaerobic conditions (Ball *et al.,* 1999). After moderate compaction, elevated CO_2 emission could be linked to enhanced microbial mineralization of freshly exposed organic matter (Novara *et al.,* 2012). Once water infiltration and air permeability have reached critical limits, CO_2 emissions decrease due to reduced microbial activity, root respiration and gas diffusivity (Conlin and van den Driessche, 2000; Shestak and Busse, 2005; Goutal *et al.,* 2012). Furthermore, soil organic matter in severely

compacted soils might become physically protected from decomposition (Fleming *et al.,* 2006).

Nitrous oxide emission steadily increased with increasing compaction, but the large flux fluctuations indicate the interplay of aerobic and anaerobic processes that generate N_2O. Generally, nitrous oxide is produced anaerobically during denitrification and aerobically during nitrification at suboptimal oxygen concentrations (Bremner, 1997), although the two processes have also been observed under contrasting oxygen conditions (Hayatsu *et al.,* 2008). The relative ecological importance of these processes under varying oxygen availability is not completely understood, but it has been suggested that denitrification becomes the key process for N_2O production in compacted soils (Ruser *et al.,* 2006). Considering the wide range of microbial species across all domains of life that are involved in denitrification including their varying oxygen requirements (Hayatsu *et al.,* 2008), it is difficult to directly link the structural shifts of the microbiota to changes in nitrous oxide emission, but we can assume that nitrous oxide production is stimulated in compacted soils by favoring species involved in the denitrification process (Skiba and Smith, 2000).

Conclusion

Soil compaction is a major problem inherently linked to economically efficient logging operations. Once a soil has been compacted, a return to the initial state can be very slow, and recovery from severe compaction might take centuries rather than decades (Webb, 2002; von Wilpert and Schäffer, 2006). As the degree of disturbance depends on factors like harvesting equipment, operation condition and site characteristics, careful operational design can substantially mitigate the environmental impact. We observed that site characteristics such as soil type were important determinants of the degree of impact, with clayey soils exhibiting less resistance and resilience than sandy soils. However, high moisture contents as simulated in the severely compacted skid trails led to a strong and persistent impact on the soil microbiota and functions at both forest sites. Ultimately, site conditions and characteristics (for example, soil moisture, texture) should drive the decisions about the time of logging (for example, rainfall, storms) and type of equipment used (for example, machine load, type of tires). We demonstrated that the combined investigation of soil physical, microbial and functional characteristics represents a powerful tool to measure resistance and resilience of the soil system to compaction (Figure 7). The deep sequencing approach identified microbial indicators that can assist in monitoring such disturbances in forest ecosystems and determining compaction thresholds below which there is no detrimental impact on ecosystem functioning in the long term.

Conflict of Interest

The authors declare no conflict of interest.

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Disclaimer

We confirm that the material discussed in the manuscript is original research, has not been previously published, and has not been submitted for publication elsewhere.

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