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A 30-years vineyard trial: Plant communities, soil microbial communities and litter decomposition respond more to soil treatment than to N fertilization

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ABSTRACT

Soil management strategies in viticulture should not only aim to optimize yield and quality of grapevines, but also sustain soil biodiversity and soil functioning. Here, we report on the combined effects of soil treatment and nitrogen fertilization on parameters related to soil fertility and litter decomposition, as well as plant, fungal, and bacterial communities in a long-term vineyard experiment, where these management practices have been applied since 1987. Plots in this vineyard (Hesse, Germany) were treated with different yearly amounts of nitrogen fertilizer (0, 30, 60, 90, 120, and 150 kg N ha^{-1}) with two types of inter-row soil treatment (tillage vs. permanent cover). We analyzed soil properties of the topsoil, decomposition parameters using the Tea Bag Index approach, the inter-row plant community, and microbial communities (bacteria and fungi) by next generation sequencing techniques.

Long-term tillage decreased soil carbon and nitrogen levels as well as some plant available nutrients, whereas soil pH and plant available phosphorus increased. Elevated amounts of nitrogen fertilization slightly increased soil carbon and nitrogen levels. Relative mass loss of tea bag content due to decomposition was decreased, while stabilization of labile litter components was increased by tillage, compared to permanent cover treatments. Plant community patterns were changed by soil treatment with tillage inter-rows exhibiting annual, ruderal species and being more diverse compared to permanent cover inter-rows. Relative abundance of several bacterial phyla and fungal orders responded strongly to soil treatment. Changes in soil pH and levels of phosphorus could partly explain the underlying mechanisms involved in shifts of bacterial and fungal communities, respectively. In contrast, long-term nitrogen fertilization only slightly shifted plant and microbial community composition. This study shows that over the long-term, soil treatment strongly affects soil functioning and biodiversity, exceeding the effect of even high nitrogen fertilization levels.

1. Introduction

Viticulture is a valuable cropping system in Europe, economically and traditionally. In contrast to annual crops, vineyards provide non-cropped areas within the field allowing non-crop vegetation to develop. Further, vineyard soils are less frequently exposed to mechanical disturbance. For vine-growers, the soil is of particular interest and soil management strategies, including inter-row soil treatment and nitrogen (N) fertilization, are of major concern in order to optimize yield and quality of the grapes and maintain ecosystem functioning (Garcia et al., 2018; Pérez-Álvarez et al., 2015).

The effects of different soil management strategies in vineyards have been extensively addressed in previous research, and were recently reviewed by Garcia et al. (2018). In general, permanent vegetation cover enhances soil organic matter, improves soil structure and aggregate stability, and alleviates the risk of erosion in vineyard inter-rows (Goulet et al., 2004; Ruiz-Colmenero et al., 2013). Yet, inter-row vegetation can lead to a reduction of vegetative growth and fruit yield of grapevines due to competition for nutrients and water during dry periods (Tesic et al., 2007). Moreover, insufficient nutrient supply can have negative effects on subsequent fermentation processes (Guerra and Steenwerth, 2012). However, these findings apply to vineyards in a Mediterranean climate, where most of the published research has been conducted so far. In temperate climate regions, where precipitation during the growing season usually provides sufficient water, moderate competition between grapevines and inter-row vegetation is even

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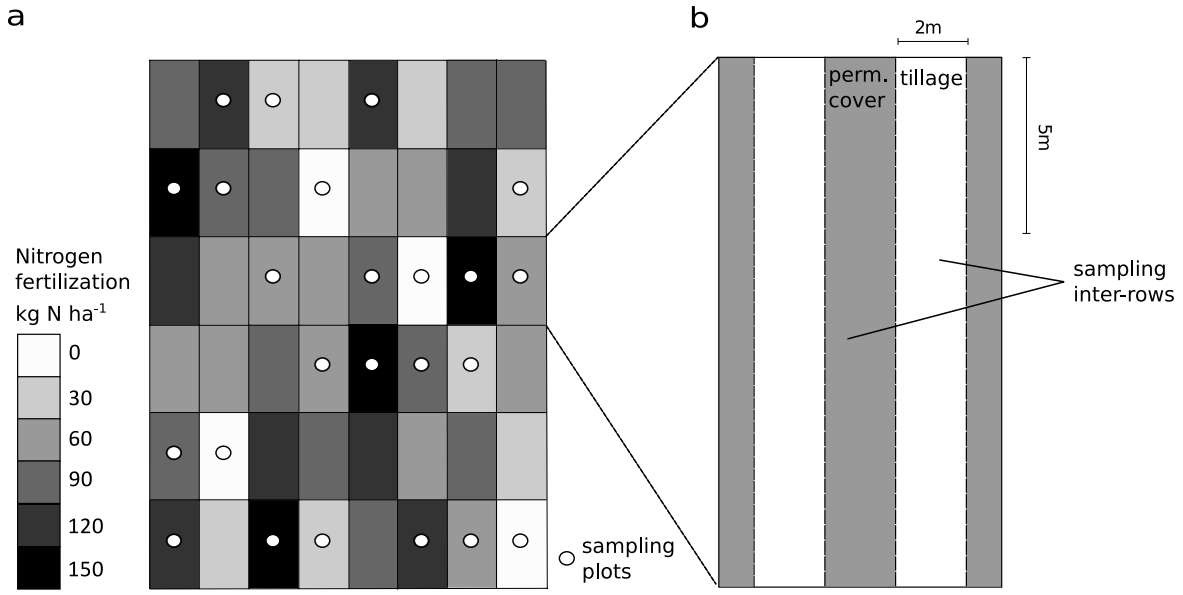

Fig. 1. Plot design of the experimental vineyard, where different yearly amounts of nitrogen fertilizer (shaded in grey according to total N fertilization level per year, subfigure a) and two types of inter-row soil treatments (permanent cover shaded in grey and tillage in white, subfigure b) have been conducted since 1987. Plots sampled for this study are indicated by a white dot. A plot consisted of four grapevine-rows (dashed line) and inter-rows with two different soil treatment types. Two adjacent inter-rows exhibiting both treatment types were sampled.

desirable (Trigo-Córdoba et al., 2015).

Fertilization with mineral N is very common in conventionally managed vineyards to enhance vegetative growth, plant vigor, and nitrogenous compounds in grape berries, in order to optimize the fermentation process and wine quality (Bell and Henschke, 2005). Nitrogen demand of grapevines is rather low for meeting yield and quality requirements, and optimal supply depends on the time of application of N fertilizer (Keller et al., 1999; Linsenmeier et al., 2008).

For defining soil management strategies suitable for regional viticulture, vine-growers should not only consider grape yield and quality, but also take into account possible impacts on ecosystem functions. Litter decomposition is interlinked with a number of soil functions, including nutrient mobilization, carbon sequestration, and soil stability. The rate of litter decomposition depends on climatic conditions, the type of litter, and the composition of the microbial community mediating the decomposition processes (McGuire and Treseder, 2010; Prescott, 2010; Zhang et al., 2008). Vineyards have received little attention regarding litter decomposition experiments (Buchholz et al., 2017). However in annual crops, it has been shown that soil treatment as well as N fertilization influence decomposition rates (Lupwayi et al., 2004; Norris et al., 2013).

The impact of soil management on plant diversity and community composition in vineyards has been addressed in several studies with a strong focus on Mediterranean vine-growing regions(Baumgartner et al., 2008; Kazakou et al., 2016; Steenwerth et al., 2016). Studies on the effect of N deposition on plant diversity have been done across different land-use types, revealing that increased N levels are a major threat to global plant diversity (Field et al., 2014; Payne et al., 2017). Although the majority of N is introduced by fertilization of cropping systems (Good and Beatty, 2011), the direct effects of fertilization on plant communities within the cropped area have gained little attention (Nascimbene et al., 2013).

Bacteria and fungi are the main agents mediating soil functions related to nutrient cycling. Recent advances of next generation sequencing methods enable us to study soil microbial communities and their response to soil treatment in agroecosystems at different taxonomic levels (e.g., Dong et al., 2017; Lupwayi et al., 2017; Sharma-Poudyal et al., 2017; Wang et al., 2016a, 2016b). Currently, there are only a few studies focusing on bacterial communities in vineyard soils covering only the Mediterranean climate (Burns et al., 2016, 2015). Numerous studies have focused on the response of bacterial or fungal communities to mineral or organic fertilization (Fierer et al., 2012; Francioli et al., 2016; Hu et al., 2017; Liu et al., 2015a,b; Zhalnina et al., 2014). These studies, however, lack the integration of soil treatment, allowing a comparative analysis of both fertilization and soil treatment effects combined.

Our study aimed at unravelling the long-term changes of soil properties and functions, as well as plant and microbial communities responding to soil treatment and N fertilization. We conducted our study in a long-term vineyard experiment, where different management regimes of both soil treatment and N fertilization have been constantly applied for 30 years. The nested plot design of this vineyard allowed us to study the response of two soil treatment strategies (tillage vs. permanent cover) combined with six levels of N fertilization (0, 30, 60, 90, 120, 150 kg N ha^{-1}). Soil properties reflecting fertility were assessed in the 8-cm topsoil layer; litter decomposition was measured using buried teabags for a period of three months; plant community data were recorded using a species coverage method; soil microbial communities were analyzed using next generation sequencing techniques. Within our experimental framework, we tested the hypotheses that (a) soil organic carbon, soil total nitrogen, and plant available nutrients would be reduced by tillage, but possibly enhanced by high N fertilization rates; (b) litter decomposition would be increased in permanent cover inter-rows and at high N level plots; and (c) community patterns would reflect different soil treatments strongly and would be moderately shifted by increasing N levels.

2. Methods

2.1. Experimental site

The study was conducted in a vineyard in the wine growing region of Rheingau, Germany (50°00′48″ N, 7°59′50″ E). The soil contained 11.6% gravel, soil texture was classified as loamy sand. The vineyard faced south east with a slope of 10°. Detailed information about the study site, cultivation and agricultural practice can be found in Linsenmeier et al. (2008).

The vineyard was split into 48 plots that have been fertilized

annually with different quantities of mineral N as granular ammonium nitrate (27.5% N), covering a gradient from 0 to 150 kg N ha^{-1} (0, 30, 60, 90, 120, 150 kg N ha^{-1}, Fig. 1a). The fertilization regime has been conducted since 1985. For this study, 24 plots were randomly chosen in order to represent each fertilization level by four replicates. Within these sampling plots, N fertilizer applications were split up between grapevine budbreak and fruit set, except for plots receiving 30 kg N ha^{-1}, where fertilization was done only at budbreak. Further annual fertilization of 30 kg P ha^{-1}, 120 kg K ha^{-1}, and 8 kg Mg ha^{-1} did not change between plots. Details concerning the N fertilization regime are described in Linsenmeier et al. (2008).

All fertilization plots have equal dimensions of 15 m length and 8 m width, spanning four grape-vine rows (Fig. 1b). Space between grapevine rows (inter-rows) were managed in an alternating cover system: Every second inter-row was covered with permanent grass vegetation (permanent cover), while the weed vegetation of the other inter-row was removed by mechanical disturbance (tillage). The soil treatments were established in 1987. The permanent cover was initially sown by using a commercial seed mixture “Sedamix Mulchmischung III” containing *Lolium perenne* (10%), *Festuca rubra rubra* (30%), *Festuca rubra communata* (20%), and two varieties of *Poa pratensis* (20% of each type), and has been managed by mulching several times per season. Tillage of inter-rows was performed according to the growth of spontaneous vegetation, but at least twice a year (April and July). For this study, the central inter-row managed by tillage, and the Eastern adjacent inter-row, representing permanent cover, were chosen for sampling. Therefore, our design consisted of two soil treatment entities nested within six levels of N fertilization, each with four replicates per level resulting in 48 sampling units (i.e. two treatments * six N levels * four replicates).

Under-vine vegetation was removed by application of herbicides. Pest control and other vineyard operations were conducted following standard practice in German integrated viticulture.

2.2. Soil properties

Soil samples were taken in August 2015. Soil cores of a depth of 8 cm were taken from each of both sampling inter-rows in all sampling plots, for a total of eight, then mixed and pooled to obtain one compound sample of approx. 500 ml. The field-moist soil was sieved using a mesh size of 2 mm to remove stones and plant particles. An aliquot of 6 g per soil sample was stored at −20 °C until DNA extraction. The remaining soil was used to analyze soil physicochemical parameters reflecting soil fertility: The proportions of fine soil organic carbon (OC) and total nitrogen (TN) were determined following the Dumas combustion method using a “Vario MAX CNS” analyzer (Elementar Analysensysteme GmbH, Germany). To determine the content of OC for calcareous soil samples (pH > 6.9), the calcium-carbonate fraction was determined using the Scheibler method and subtracted, as inorganic C, from the carbon content values obtained by the combustion method. Plant available phosphorus (P) and potassium (K) were measured by applying the CAL (Calcium Acetate Lactate) extraction and measured by photometric methods. Plant available magnesium (Mg) was extracted using 0.01 M $CaCl_2$-solution and measured by atomic absorption spectrometry. Soil pH was measured by suspension of soil samples in 0.01 M $CaCl_2$-solution (1:1.5). All methods are described in Schaller (2000).

2.3. Litter decomposition

To measure litter decomposition the Tea Bag Index (TBI) approach was applied (Keuskamp et al., 2013). This method uses two types of tea (green tea and rooibos tea) with different fractions of hydrolysable material. Green tea, with a high fraction of hydrolysable compounds, is assumed to be decomposed more rapidly than rooibos tea. Assuming a two-phased decomposition model, labile material in the rooibos tea will still be in the process of decomposing at 90 days, whereas decomposition of the green tea will have reached a plateau. Using these underlying assumptions, the TBI method allows the calculation of initial decomposition rate (k) and stabilization (S) of the labile fraction during the second phase of decomposition (Sarneel and Veen, 2017).

Teabags of two types (Lipton green tea, EAN: 87 22700 05552 5 and Lipton rooibos tea, EAN: 87 22700 18843 8) were dried, weighed, and exposed pairwise in each sampling inter-row of all sampling plots in 5 replicates (1 m apart in the central area of each inter-row, depth: 8 cm). After a field exposure period of 90 days (2nd July 2015 to 30th September 2015), tea bags were recovered, dried, and their surfaces carefully cleaned. The weight of the remaining content of each tea bag was determined to calculate decomposition rate k, as well as stabilization factor S, using the mass loss data of both tea types and the equations from Keuskamp et al (2013). Due to the loss of all replicates of the rooibos tea type in the permanent cover treatment in one 150 kg N ha^{-1} plot, we generated the data by drawing the values from a normal distribution with the mean and standard deviation of all measured values obtained from the permanent cover treatment of the three remaining plots with the same fertilization level.

2.4. Plant community

Vascular plants were sampled twice in 2016: (1) in March, before any soil management interventions and (2) in September, close to harvest and approximately eight weeks after the last tillage event. The coverage of plant species were recorded using a modified approach of Nascimbene et al. (2013): In each sampling inter-row of all sampling plots, two 0.5 x 2 m frames were placed randomly in the middle of the inter-row. Within each frame, all vascular plant species was recorded, and the percentage of coverage for each species within the frame was estimated, as well as percentage of total vegetation coverage. Only individuals rooting within the frame were considered. Data about plant species life endurance (annual, biennial, perennial) were obtained from field guides (Jäger, 2011) and the BIOFLOR online database (Klotz et al., 2002).

2.5. Bacterial and fungal community analysis

2.5.1. DNA extraction and sequencing

The soil samples for DNA extraction were taken from the same samples used for the analysis of soil properties. For extraction, the PowerSoil DNA Isolation Kit (MO BIO Laboratories, CA, USA) was used following the manufacturer's protocol with slight modifications: the soil particles were homogenized by a Precellys 24 Homogenizer (VWR International GmbH, Darmstadt, Germany) applying a program of 3 intervals of 30 s at an intensity of 6800; after adding solution C1, samples were incubated in a water bath at 65 °C for 10 min. DNA extracted from the soil was checked for quality by PCR and subsequent gel electrophoresis and quantified using a NanoDrop spectrophotometer.

DNA samples were shipped to Génome Quebec Innovation Centre (Montreal, Canada) for PCR reaction and sequencing. The libraries were created using two separate sets of markers. The V4 region of the bacterial 16S rRNA gene was amplified using the primer pair S-D-Bact-0341F (5′-CCTACGGGNGGCWGCAG-3′) and S-D-Bact-0785R (5′-GACTACHVGGGTATCTAATCC-3′) (Klindworth et al., 2013). The fungal ITS 2 region was amplified using the primer pair ITS4 (5′-TCCTCCGCTTATTGATATGC-3′) and fITS7 (5′-GTGARTCATCGAATCTTTG-3′) (Ihrmark et al., 2012; Schoch et al., 2012). Sequencing of 250bp paired-end amplicons was conducted on a MiSeq Illumina machine.

2.5.2. Data processing

Raw Illumina fastq reads were quality controlled with FastQC (Andrews, 2010), generally showing good quality. Since both reads of those paired-end pairs are overlapping partially at their 3′-ends, they were joined to form contiguous sequences (contigs) including error

correction within overlapping sequences (best scoring base kept). No trimming was applied at this step in order to keep as much of the sequence as possible. In addition, for bacteria, the reads were randomly subsampled to 50% of the total number of reads due to hardware limitations.

For processing the bacterial sequences the software package Mothur version 1.37.1 (Scloss et al., 2009) was used for sequence analysis following the standard operating procedure outlined on http://www.mothur.org/wiki/MiSeq_SOP. Briefly, the two overlapping paired-end reads were combined using *make.contig*. Then, each unique sequence was aligned with *align.seqs* to the SILVA reference alignment release 123 (Quast et al., 2013). A distance matrix was calculated, allowing for four mismatches. Chimeric sequences were identified using *chimera.uchime* and removed (Lupwayi et al., 2017). Sequences matching "Chloroplast-Mitochondria-unknown-Archaea-Eukaryota" were also removed. Next, sequences were clustered using the furthest neighbor clustering algorithm to build operational taxonomic units (OTUs). The resulting file was parsed to separate the data for each sample. OTUs were assigned to a taxonomic group with *classify.seqs* using the RDP reference file (Cole et al., 2014) and a cut-off of 80% of the bootstrap value (Zhou et al., 2015).

The software package PIPITS (version 1.3.x) was used for sequence analysis of fungi (Gweon et al., 2015). PIPITS generates a biom file for OTU with the UNITE fungal ITS reference set, based on the RDP classifier (Kõljalg et al., 2014).

The output from both pipelines was converted to biom files using MEGAN (Huson et al., 2016). The R environment, version 3.4.1 (R Core Team, 2017), was used to extract community matrices from the biom files using the phyloseq-package at different scales of taxonomic resolution: OTU, genus, order, and phylum. To avoid inclusion of erroneous reads that would otherwise lead to inflated estimates of diversity, OTUs represented by less than 0.001% of the total sum of reads were removed (Bokulich et al., 2013; Burns et al., 2015).

2.6. Data analysis

2.6.1. Univariate analysis

All statistical analyses were carried out using the R environment version 3.4.1, (R Core Team, 2017). We applied linear mixed effect models (LMM) using the R-package lme4 (Bates et al., 2015) to test the effects of soil treatment, N fertilization, and the interaction of both on the following response variables: soil physicochemical parameters (OC, TN, C:N ratio, pH, P, K, and Mg), decomposition parameters (relative mass loss of both tea types, k, and S), total vegetation cover and total number of species for two sampling periods (March, September), and number of bacterial and fungal OTUs, which were derived from the filtered - but not rarefied or transformed - community data set of bacteria and fungi. factor plot was included in all models as random intercept. Normality and homogeneity of variance were evaluated by histograms of residuals and by plotting residuals against each explaining variable, respectively. If the assumptions of normality and homogeneity of variance were violated, we transformed the response variable to improve the model as follows: OC, TN, CN, and K were log10-transformed, total vegetation cover for both periods was angular-transformed (arcsine(square-root(x))), number of plant species was square root transformed. Wald-X^2-tests were applied on LMM to test significance of independent variables using the function *Anova* of the *car*-package (Fox and Weisberg, 2011). Marginal and conditional R^2 values were calculated from LMM following the approach of Nakagawa and Schielzeth (2013) implemented in the package MuMIn (Barton et al., 2018).

2.6.2. Multivariate analysis

Multivariate community analyses were performed using the vegan package (Oksanen et al., 2017). Rarefaction curves of the bacterial and fungal data set at the level of OTUs, as well as on different taxonomic levels were examined to evaluate if the diversity of taxa was covered with the number of sequences sampled. For bacterial OTUs the plateau of the rarefaction curves is not reached (see supplementary data, Fig. A1a), but at the level of genera and above, the coverage was sufficient (Fig. A1b-d). The same applied for the fungal data set (Fig. A2). For subsequent analyses, we chose the taxonomic level phyla for bacteria, following Fierer et al. (2007), and the level of orders for fungi. Dissimilarity matrices generated from community data at different taxonomic resolutions were compared by Mantel tests, to test whether matrices are correlated. Further, ordination analyses were performed based on different taxonomic levels and the OTU data to check if major outcomes are similar across different taxonomic levels.

The bacterial and fungal data tables were subsampled so that the number of reads of each observation was equal to the observation representing the minimum number of reads (rarefaction). Taxa occurring only in a single inter-row were removed; this was the case for the combined plant data and the fungal order data. For the plant community, the plant species cover tables from both sampling periods were combined by calculating the mean of each species. Since ordination analyses on the data set separated for the sampling period and on the combined data set showed the same patterns, only the combined data set encompassing 51 species was used for the analyses.

To visualize general patterns of plant, bacterial, and fungal communities and detect the influence of soil treatment and N fertilization on community composition, we applied non-metric multidimensional scaling (NMDS). NMDS input was evaluated by using the Bray-Curtis dissimilarity matrices (Faith et al., 1987) of untransformed and transformed community tables and varying dimensions. We tested square-root-transformation, Wisconsin double transformation (taxa are first standardized by maxima and then observations by observation totals) and the combination of both. The evaluation was based on stress-values and visual examination of the resulting ordination plot. The NMDS results presented in this study were based on three dimensions and 500 random starts using Bray-Curtis dissimilarities of the square-root-transformed community table for plants and fungi, and the Wisconsin-transformed community table for bacteria, respectively. Ordination plots were examined considering all three dimensions. Non-parametric multivariate analyses of variance (PERMANOVA, Anderson, 2001) were performed on dissimilarity matrices of transformed community data analogous to NMDS, using 5000 permutations. The correlation of NMDS axes with N fertilization gradient was further tested using Spearman rank correlation.

In order to test if patterns of the microbial community composition are correlated with the spatial distances of plots within the experimental field, we applied Mantel tests for correlation between Bray-Curtis dissimilarity of bacterial and fungal communities with the Euclidean distance matrix of spatial distances of the inter-rows sampled. We found no correlation between these distance matrices (for bacteria, Mantel $r = 0.005$, $p = 0.41$; for fungi, $r = -0.009$, $p = 0.59$). Therefore, we concluded that patterns of microbial community are unlikely to be biased by spatial effects.

Stepwise variable selection combined with redundancy analysis (RDA) was applied on plant, bacterial, and fungal community data with soil treatment, N fertilization, and soil parameters included as constraining variables, in order to (1) find parsimonious models explaining the variability of communities, (2) assess the importance of constraining variables for community patterns, and (3) identify taxa responding to constraining variables. The data tables of combined plant species cover, bacterial phyla, and fungal orders were Hellinger-transformed, as proposed by Legendre and Gallagher (2001). Stepwise variable selection based on the AIC criterion was performed using the ordistep function implemented in vegan, combining forward and backward selection (options: Pin = 0.05, Pout = 0.1, permutations = 1000). P-values of marginal effects of retained variables were calculated using permutation tests (function anova.cca) using 5000 permutations.

Table 1
Mean values of soil properties at different soil treatment regimens (permanent cover vs. tillage) and yearly N fertilization levels in the experimental vineyard.

Parameter[b]	Unit	Treatment[a]	N fertilization (kg N ha^{-1})					
			0	30	60	90	120	150
OC	% dry soil	pc	2.12	2.75	2.49	2.67	2.95	2.77
		ti	1.06	1.36	1.26	1.36	1.56	1.33
TN	% dry soil	pc	0.18	0.21	0.21	0.24	0.26	0.25
		ti	0.08	0.10	0.10	0.12	0.13	0.12
C:N		pc	11.9	13.2	11.9	11.0	11.6	11.0
		ti	13.0	13.9	12.2	11.9	12.0	11.5
pH		pc	6.9	7.1	6.9	6.8	6.9	6.8
		ti	7.3	7.4	7.3	7.2	7.2	7.2
P	mg 100g^{-1} soil	pc	29.5	41.3	28.0	25.9	46.5	26.6
		ti	44.8	47.7	38.3	41.0	51.5	43.5
K	mg 100g^{-1} soil	pc	46	52	44	46	61	47
		ti	28	31	29	32	42	33
Mg	mg 100g^{-1} soil	pc	18.0	17.6	19.6	19.8	18.9	19.2
		ti	10.3	9.0	10.1	10.7	10.6	11.2

[a] pc: permanent cover; ti: tillage.
[b] OC: organic carbon; TN: total nitrogen; P: available phosphorous; K: available potassium; Mg: available magnesium.

3. Results

3.1. Effects of soil treatment and fertilization on soil properties

Means of soil properties in the experimental vineyard are given in Table 1. All of them responded significantly to soil treatment (Table 2). The permanent cover treatment showed higher content of OC and TN as well as higher concentrations of plant available K and Mg compared to the tillage treatment, which in return exhibited higher soil pH-values, a slightly higher C:N ratio, and slightly higher concentrations of plant available P.

Nitrogen fertilization significantly increased OC and TN. The C:N ratio was decreased weakly by increased N fertilization. There was a slight increase of K with N fertilization, which was, however, only significant in soils under tillage. Soil pH seemed to decrease with increasing N fertilization, although this effect was not significant. Phosphorus, Mg and K did not respond to N fertilization.

Table 2
ANOVA table of soil properties in the experimental vineyard responding to soil treatment (permanent cover vs. tillage) and yearly N fertilization. X^2 and p-values obtained from Wald-X^2-test, marginal R^2 (mR^2) and conditional R^2 (cR^2) calculated from LMM following Nakagawa and Schielzeth (2013).

Parameter[a]	Management factor	X^2	P-value		mR^2	cR^2
OC	soil treatment	257.81	< 0.001	***[b]	0.68	0.89
	N fertilization	4.40	0.036	*		
	interaction	0.33	0.563			
TN	soil treatment	219.85	< 0.001	***	0.79	0.85
	N fertilization	18.36	< 0.001	***		
	interaction	0.09	0.759			
C:N	soil treatment	4.83	0.028	*	0.22	0.54
	N fertilization	6.03	0.014	*		
	interaction	0.20	0.658			
pH	soil treatment	95.09	< 0.001	***	0.41	0.82
	N fertilization	1.78	0.182			
	interaction	0.03	0.868			
P	soil treatment	40.54	< 0.001	***	0.13	0.85
	N fertilization	0.004	0.951			
	interaction	0.06	0.812			
K	soil treatment	63.66	< 0.001	***	0.35	0.76
	N fertilization	0.66	0.418			
	interaction	2.90	0.088			
Mg	soil treatment	229.87	< 0.001	***	0.70	0.86
	N fertilization	0.90	0.336			
	interaction	0.01	0.934			

[a] OC: organic carbon; TN: total nitrogen; P: available phosphorous; K: available potassium; Mg: available magnesium.
[b] Significance levels: ***, P < 0.001, **, P < 0.01; *, P < 0.05.

3.2. Decomposition

The decomposition reflected by the relative mass loss of buried tea bags was significantly higher in inter-rows with permanent cover compared to tillage inter-rows (Table 3 and 4). While the decomposition rate k did not respond to soil treatment, the stabilization factor S was higher in inter-rows where tillage was applied. None of the decomposition variables responded to N fertilization.

3.3. Effects of soil treatment and N fertilization on plant, bacterial, and fungal communities

3.3.1. Total vegetation cover and plant, bacterial, and fungal richness

Total vegetation cover did not differ between soil treatments in either time points (March and September, Fig. 2, Table 5 and 6). However, it responded to N fertilization depending on soil treatment type: Vegetation cover increased with the amount of N in inter-rows with tillage in both months, whereas it remained constant in permanent cover inter-rows in March, and even declined in September.

A total number of 34 and 31 plant species were found in March and September 2016, respectively with a combined data set encompassing 51 species (supplementary Table A1). At both time points plant species richness was significantly higher in tillage inter-rows than in inter-rows with permanent cover (Table 5 and 6), and a slight but significant decrease of species richness was observed with increasing N fertilization.

The filtered bacterial data set encompassed 924 250 sequences with sample sizes ranging from 13 209 – 25 024. For fungi, the data set encompassed a total of 1 667 111 sequences with sample sizes ranging from 6210 – 69 094. The minimum sample size was used as the threshold for subsampling. Sequences were assigned to 7265 OTUs for bacteria and 953 OTUs for fungi. The mean number of bacterial OTUs, assumed as a proxy for bacterial richness, responded significantly to soil treatment and was slightly higher in the tillage inter-rows than in the permanent cover inter-rows (Table 5 and 6). In contrast, mean OTU richness of fungi was significantly higher in permanent cover treatments than in tillage treatments. There was no effect of N fertilization on bacterial or fungal OTU richness.

The bacterial OTUs could be assigned to 17 taxonomically accepted or candidate phyla and two candidate divisions (supplementary Table A2), while 11% of bacterial OTUs could not be classified. The fungal OTUs could be assigned to 37 taxonomically accepted orders and 13 unique taxa that were not unambiguously assignable to order level (supplementary Table A3).

3.3.2. Community patterns

The NMDS ordination plots for the Bray-Curtis dissimilarities of plant, bacterial, and fungal communities are given in Fig. 3. Across all communities, samples were clustered into two clearly separated groups that reflected soil treatment along the first dimension of the NMDS ordination plot. Non-metric multivariate ANOVA confirmed that soil treatment had a strong effect on the composition of plant and soil microbial communities (Table 7). Furthermore, N fertilization level was reflected best by the second dimension for plants and bacteria, and by the third dimension for fungi. Overlaps between groups of adjacent N fertilization levels suggest that community composition changed rather slightly along the N fertilization gradient. Examining Spearman rank correlations of N fertilization gradient and NMDS scores confirmed the visual observation that the distribution of plant and bacterial communities along the second dimension of the corresponding ordination plot correlated with the N fertilization gradient (Table 7). For the fungal

Table 3
Mean values of decomposition parameters at different soil treatment regimens (permanent cover vs. tillage) and yearly N fertilization levels in the experimental vineyard.

Parameter	Unit	Treatment[a]	N fertilization (kg N ha^{-1})					
			0	30	60	90	120	150
Mass loss green tea	% dry weight	pc	69.5	66.9	67.1	68.0	67.3	66.9
		ti	65.3	64.1	63.2	63.3	64.8	62.3
Mass loss rooibos tea	% dry weight	pc	24.6	23.4	25.2	25.3	26.3	25.2
		ti	21.6	22.0	24.2	22.8	23.1	23.2
Decomposition rate k		pc	0.00852	0.00850	0.00942	0.00944	0.01014	0.00975
		ti	0.00876	0.00823	0.00968	0.00875	0.00884	0.00926
Stabilization factor S		pc	0.174	0.206	0.203	0.192	0.201	0.205
		ti	0.225	0.239	0.250	0.248	0.231	0.260

[a] pc: permanent cover; ti: tillage.

Table 4
ANOVA table of decomposition parameters responding to soil treatment (permanent cover vs. tillage) and yearly N fertilization. X^2 and p-values obtained from Wald-X^2-test, marginal R^2 (mR^2) and conditional R^2 (cR^2) calculated from LMM following Nakagawa and Schielzeth (2013).

Parameter	Management factor	X^2	P-value		mR^2	cR^2
Mass loss green tea	soil treatment	52.62	< 0.001	***[a]	0.47	0.61
	N fertilization	2.82	0.09			
	interaction	0.02	0.87			
Mass loss rooibos tea	soil treatment	16.31	< 0.001	***	0.25	0.42
	N fertilization	2.32	0.13			
	interaction	0.02	0.89			
Decomposition rate k	soil treatment	1.54	0.21		0.13	0.31
	N fertilization	3.55	0.06			
	interaction	1.53	0.22			
Stabilization factor S	soil treatment	52.62	< 0.001	***	0.47	0.61
	N fertilization	2.81	0.09			
	interaction	0.03	0.87			

[a] Significance levels: ***, $P < 0.001$, **, $P < 0.01$; *, $P < 0.05$.

community, there was a significant correlation with both the second and third axis.

The patterns are similar if NMDS analyses are applied on plant species cover data at each sampling time point separately (supplementary Fig. A3) and, for microbial communities, if NMDS analyses are applied on data at different taxonomic levels (OTU, genus, order, and phylum, supplementary Fig. A4 and A5) – except for fungal phyla, which exhibited a less strong effect of soil treatment. For bacterial communities, Mantel test for correlation between dissimilarity matrices revealed that Bray-Curtis dissimilarities at different taxonomic resolutions (OTU, genus, order, and phylum) are correlated with a Pearson correlation coefficient of $r > 0.8$ for all pairwise comparisons (supplementary Table A4). For fungi, correlations between dissimilarities were below $r = 0.75$ if the matrix at phylum level was compared to the other matrices, indicating that patterns shown by OTU, genus and order level may not be returned if the fungal phylum data are applied.

3.3.3. Responses of taxa to soil treatment, N fertilization and soil parameters

Stepwise forward selection RDA retained soil treatment and N fertilization gradient as significant explanatory variables determining patterns of plant, bacterial, and fungal communities (Fig. 4), and confirms the patterns of the NMDS analysis (Fig. 3). Soil treatment, correlated with the first axes, acted independently from N fertilization, correlated with the second axis. Considering the variation explained by RDA axes, the influence of soil treatment on the community compositions was much higher than the long-term N fertilization (22% vs. 6.7% for plants, 33.4% vs. 4.9% for bacteria, and 14.0% vs. 4.1% for fungi).

The RDA for the plant community showed that the life endurance strategy of plant species was closely linked to soil treatment (Fig. 4a and supplementary Fig. A6). Species associated with permanent cover treatment were mainly perennials, whereas inter-rows facing tillage were dominantly covered with annual species. Shifts of species composition as response to N fertilization were less distinct: Increasing fertilization was associated with the exclusion of some species at high N levels (e.g. *Draba verna*, *Bellis perennis*) and increased coverage of individual species (*Stellaria media*, *Lamium purpureum*). Regarding soil parameters, no variable was retained in the final model explaining the plant community pattern since they were correlated with the selected ones.

For bacteria, several phyla were strongly associated with soil treatment type (Fig. 4b and supplementary Fig. A4), although none of the most abundant phyla were exclusively found in tillage or permanent cover inter-rows. Within the ten most abundant phyla, *Acidobacteria*,

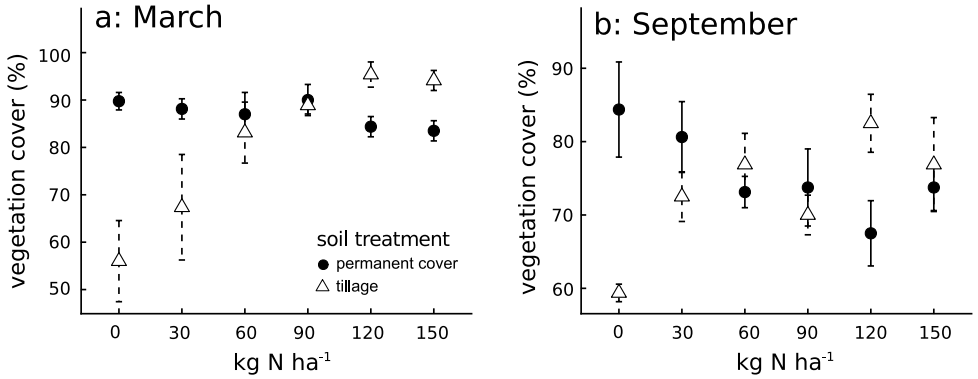

Fig. 2. Response of total vineyard inter-row vegetation cover to soil treatment and yearly N fertilization level in March (a) and September (b) 2016. Symbols indicate mean values with circles indicating permanent cover and triangles indicating tillage. Bars indicate standard error (n = 4).

Table 5
Mean values of vegetation cover and number of plant species at two time points (March and September), and bacterial and fungal OTUs in the experimental vineyard at different soil treatment regimens (permanent cover vs. tillage) and yearly N fertilization levels.

Parameter	unit	Treatment[a]	N fertilization (kg N ha^{-1})					
			0	30	60	90	120	150
vegetation cover (March)	% area	pc	90	88	87	90	84	84
		ti	56	67	83	89	95	94
vegetation cover (September)	% area	pc	84	81	73	74	68	74
		ti	59	73	77	70	83	77
number of plant species (March)		pc	11	10	9	9	9	8
		ti	11	11	12	13	9	10
number of plant species (September)		pc	7	8	7	6	7	5
		ti	10	9	9	9	8	10
number of bacterial OTUs		pc	1762	1849	1767	1745	1915	1847
		ti	1839	1838	1869	1888	1885	1845
number of fungal OTUs		pc	135	143	123	120	138	143
		ti	109	123	112	117	126	110

[a] pc: permanent cover; ti: tillage.

Table 6
ANOVA table of vegetation cover and number of plant species (at two time points: March and September), and bacterial and fungal OTUs in the experimental vineyard responding to soil treatment (permanent cover vs. tillage) and yearly N fertilization. X^2 and p-values obtained from Wald-X^2-test, marginal R^2 (mR^2) and conditional R^2 (cR^2) calculated from LMM following Nakagawa and Schielzeth (2013).

Parameter	Management factor	X^2	P-value		mR^2	cR^2
vegetation cover (March)	soil treatment	1.934	0.16		0.51	0.53
	N fertilization	15.37	< 0.001	***[a]		
	interaction	31.99	< 0.001	***		
vegetation cover (September)	soil treatment	1.13	0.29		0.25	0.25
	N fertilization	0.02	0.90			
	interaction	14.61	< 0.001	***		
number of plant species (March)	soil treatment	19.46	< 0.001	***	0.35	0.48
	N fertilization	7.45	0.006	**		
	interaction	0.50	0.48			
number of plant species (September)	soil treatment	27.7	< 0.001	***	0.40	0.45
	N fertilization	4.00	0.045	*		
	interaction	1.60	0.21			
number of bacterial OTUs	soil treatment	5.49	0.02	*	0.06	0.54
	N fertilization	0.29	0.59			
	interaction	0.002	0.96			
number of fungal OTUs	soil treatment	8.33	0.004	**	0.012	0.32
	N fertilization	0.05	0.824			
	interaction	0.0003	0.99			

[a] Significance levels: ***, P < 0.001, **, P < 0.01; *, P < 0.05.

Actinobacteria, and *Verrucomicrobia* were more abundant in permanent cover inter-rows, whereas *Proteobacteria*, *Chloroflexi*, *Bacterioidetes*, *Gemmatimonadetes,* and *Firmicutes* were more abundant in tillage inter-rows. *Planctomycetes* were more abundant at low fertilization levels, whereas *Proteobacteria* and *Firmicutes* where associated with high fertilization levels. However, the marginal effect of N fertilization was not significant (ANOVA F = 1.95, p = 0.067). Soil pH (F = 2.98, p = 0.013), which was also correlated with soil treatment, was associated with the phylum *Latescibacteria* (see Fig. A4) and the large group of unclassified bacteria.

For fungi, within the ten most abundant orders, *Chaetothyriales*, *Pleosporales,* and *Xylariales* were more abundant in permanent cover inter-rows, whereas *Helotiales*, *Tremellales*, and *Hypocreales* were more abundant in inter-rows under tillage (Fig. 4c and supplementary Fig. A5). *Pleosporales* were associated with high N fertilization levels and a high concentration of plant available K. *Mortierellales*, *Chaetothyriales,* and *Agaricales* were more abundant at low N fertilization levels. The soil variables retained in the final set of variables were P, which was correlated with tillage treatment, and K, which is correlated with N fertilization and permanent cover treatment.

4. Discussion

4.1. Soil treatments affected soil properties more than N fertilization

The undisturbed, permanently covered vineyard inter-rows exhibited substantially increased values of soil parameters reflecting soil fertility. Most prominent was soil OC, which was almost doubled when compared to inter-rows with tillage. Total N, primarily bound in soil organic matter (SOM), was also significantly higher in inter-rows with permanent cover compared to tillage inter-rows. Regarding these parameters, our study confirmed general results found in annual cropping systems (Al-Kaisi et al., 2005; West and Post, 2002) as well as Mediterranean vineyards (Peregrina et al., 2012; Steenwerth and Belina, 2008). The above-ground biomass within permanent cover inter-rows, which was mulched frequently, as well as dead roots and root exudates contributed to carbon and nitrogen pools by decomposition and incorporation into SOM (Agnelli et al., 2014). We did not include measurements of biomass of inter-row vegetation in our study, but surveys conducted on our experimental field confirmed that above-ground biomass production of permanent cover is higher compared to the tillage treatment (Weber, 2015). Plant available nutrients also responded significantly to soil treatment: Plant available Mg and K were increased by permanent cover, while plant available P and soil pH were decreased. This partly supports the hypothesis that SOM improves availability of nutrients (Linares et al., 2014).

Compared to soil treatment, the response of the measured soil parameters to N fertilization was rather small: Soil OC, TN, which are the main components of SOM, increased with increasing N fertilization level, while the C:N ratio decreased. It has been shown that fertilization enhances vegetative growth contributing to higher SOM levels (Mazzoncini et al., 2011). This finding was confirmed by our study, since OC and TN levels were increased. However, vegetative growth of inter-row vegetation may not only be limited by N, but also light, water and other nutrients. The comparatively small increase of OC suggests that excess N was not transferred to vegetation cover biomass, but may have left the system by N leakage or greenhouse gas emissions (Snyder et al., 2009).

In our study, a slight non-significant decrease of soil pH was observed. Soil pH is often reported to decrease with increasing mineral fertilization (Zhou et al., 2015), in particular if high amounts of mineral N up to 300 kg N ha^{-1} are applied.

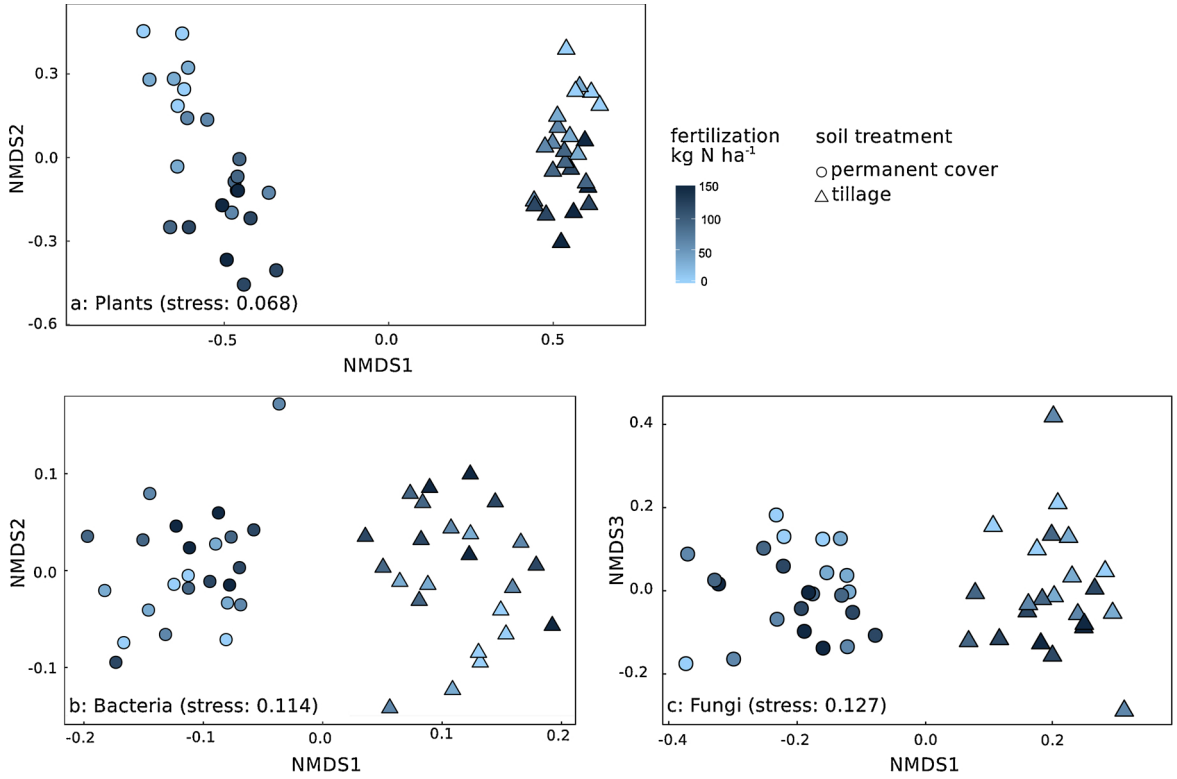

Fig. 3. Nonmetric multidimensional scaling (NMDS) of the combined plant species community (a), bacterial phyla (b) and fungal orders (c). NMDS was based on the Bray-Curtis dissimilarity matrix of the transformed community data sets (square-root transformation for plants and fungi, Wisconsin transformation for bacteria). Point shapes of samples indicate soil treatment (with circles representing permanent cover and triangles, tillage treatment), point color intensity signifies the amount of yearly N fertilization (from 0 kg N ha^{-1}, pale blue, to 150 kg N ha^{-1}, dark blue). Ordination plots show dimensions that optimally reflect soil treatment and N fertilization gradient. Stress values for the three-dimensional solutions are given.

4.2. Soil treatment and litter decomposition: effect on S, but not on k

Regarding litter decomposition, we found a greater mass loss in permanent cover inter-rows for the labile green tea as well as for the more resistant rooibos tea suggesting a higher biological activity of decomposing microorganisms. This was in contrast to Lupwayi et al. (2004), who found that mass loss of litter bag content was higher under tillage conditions in an annual cropping system. However, in Lupwayi et al.'s (2004) work litter bags in tillage plots were buried, whereas in no-till plots, litter bags were laid on the surface. We decided to the bury tea bags in both soil treatment plots in order to draw conclusions of decomposition processes involving soil microbes within the topsoil.

The application of the 2-phase-model of litter decomposition underlying the Tea Bag Index method revealed that only the stabilization factor S responded to soil treatment, while the decomposition rate k was not affected (Keuskamp et al., 2013). The stabilization of labile organic matter compounds is mediated by soil organisms (Prescott, 2010) and intermediate protection of compounds within aggregates and mineral complexes (Stockmann et al., 2013). Our results suggest a higher stabilization within soils facing tillage, compared to soils under permanent cover. Assuming the hypothesis outlined by Stockmann et al. (2013), we speculate that conditions within tilled soils promote intermediate immobilization of introduced soil organic matter. The underlying mechanisms, however, are not yet fully understood.

In our experiment the decomposition rate k did not respond to soil management. We assume that the different soil conditions between treatments were not sufficient to resolve significant effects on decomposition rates. Comparison of global data from Keuskamp et al. (2013) with decomposition rates obtained in our study revealed that our data fall into the lower part of the entire range of decomposition rates observed, suggesting that conditions for rapid decomposition were rather unfavorable in our study.

4.3. Response of plant, bacterial, and fungal communities to management practice

4.3.1. Effect of soil treatment and fertilization on plant community

Soil treatments in vineyard inter-rows induce different plant

Table 7
Results of non-parametric multivariate analysis of variance (PERMANOVA) of soil treatment and N fertilization on community patterns, and correlation of N-fertilization level with ordination axes of NMDS. Significant results in bold face with threshold level $p < 0.05$.

	PERMANOVA				Spearman rank correlation					
	soil treatment		N fertilization		N fertilization vs dimension 1		N fertilization vs dimension 2		N fertilization vs dimension 3	
	R^2	P(> F)	R^2	P(> F)	ρ^2	p-value	ρ^2	p-value	ρ^2	p-value
Plants	**0.58**	**< 0.001**	**0.09**	**< 0.001**	0.02	0.36	**0.65**	**< 0.001**	0.01	0.48
Bacteria	**0.42**	**< 0.001**	**0.03**	**0.047**	< 0.01	0.86	**0.25**	**< 0.001**	0.05	0.13
Fungi	**0.30**	**< 0.001**	**0.04**	**0.015**	< 0.01	0.90	**0.11**	**0.024**	**0.23**	**< 0.001**

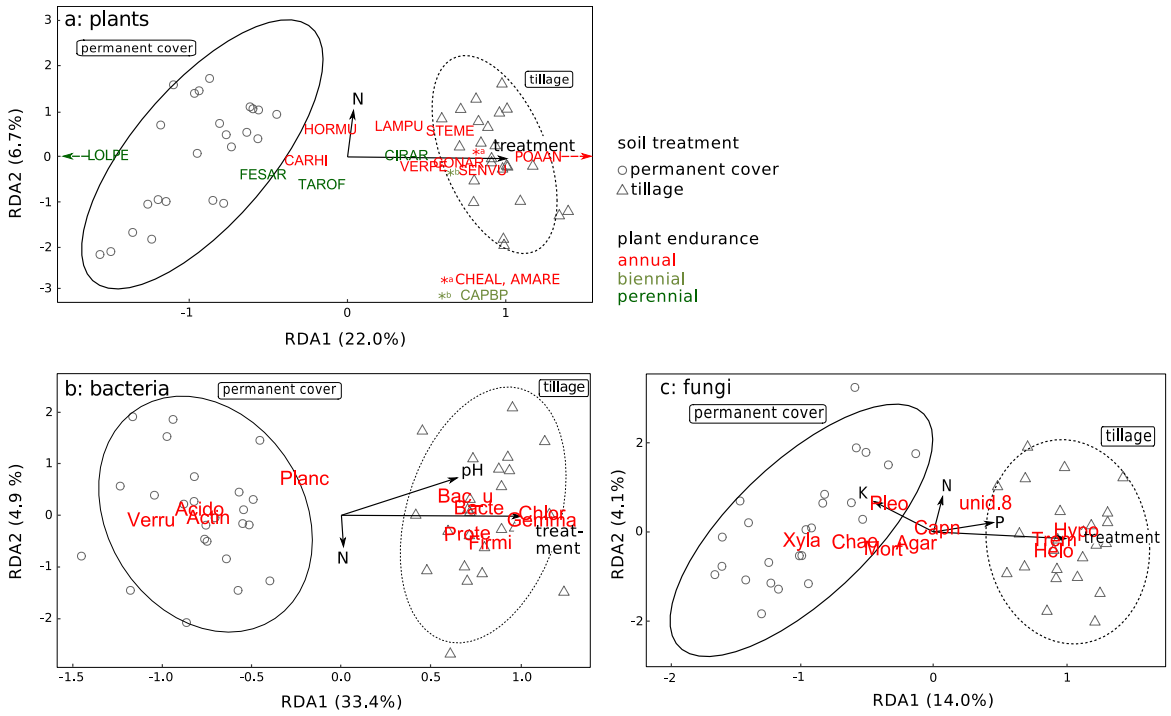

Fig. 4. RDA based on the Hellinger-transformed data set of combined plant species community (a), bacterial phyla (b), and fungal orders (c), combined with management factors and soil parameters as constraining variables. Only variables retained by stepwise selection are shown (black vectors, 'treatment' pointing towards 'tillage'). Ellipsoids indicate position of samples grouped by soil treatment at 95% confidence level (circles indicating permanent cover and triangles indicating tillage). Axis titles indicate percentage of explained variation. For plants, the 15 most abundant species, and for bacteria and fungi the ten most abundant taxa were plotted. The taxa were scaled to unit variance and coded for better representation (plants: AMARE, *Amaranthus retroflexus*; CAPBP, *Capsella bursa-pastoris*; CARHI, *Cardamine hirsuta*; CHEAL, *Chenopodium album*; CIRAR, *Cirsium arvense*; CONAR, *Convolvulus arvensis*; FESAR, *Festuca arundinacea*; HORMU, *Hordeum murinum*; LAMPU, *Lamium purureum*; LOLPE, *Lolium perenne*; POAAN, *Poa annua*; SENVU, *Senecio vulgaris*; STEME, *Stellaria media*; TAROF, *Taraxacum officinale*; VERPE, *Veronica persica*; bacteria: Acido, *Acidobacteria*; Actin, *Actinobacteria*; Bacte, *Bacteroidetes*; Bac_u, Bacteria_unclassified; Chlor, *Chloroflexi*; Firmi, *Firmicutes*; Gemma, *Gemmatimonadetes*; Planc, *Planctomycetes*; Prote, *Proteobacteria*; Verru, *Verrucomicrobia*; fungi: Agar, *Agaricales*; Capn, *Capnodiales*; Chae, *Chaetothyriales*; Helo, *Helotiales*; Hypo, *Hypocreales*; Mort, *Mortierellales*; Pleo, *Pleosporales*; unid.8, unidentified.8; Xyla, *Xylariales*). Color of plant species names represents plant life endurance. Positions of plant species LOLPE and POAAN are not shown, because they exceed axis limits (direction of position indicated by dashed arrows).

communities. These results confirm observations of studies in Californian vineyards (Baumgartner et al., 2008; Steenwerth et al., 2016). Further, soil perturbation of vineyard inter-rows by tillage led to a higher plant species richness compared to permanent cover treatments, which resembled the work of Steenwerth et al. (2016), who found that the highest plant species richness was observed when tillage was applied combined with spontaneous vegetation. Our findings contribute to the 'intermediate-disturbance hypothesis' proposed by J. Philip Grime that points out that an increase of disturbance does not necessarily lead to the reduction of species richness in herbaceous flora (Grime, 1973; Kazakou et al., 2016). In our case, the permanent cover treatment represents a low disturbance with few strong competitors dominating the vegetation (e.g. perennial grasses like *Lolium perenne*). The tillage treatment can be considered as an intermediate disturbance, acknowledging that frequency (twice a year) and intensity (chisel ploughing of the 10-cm-horizon) are rather moderate compared to intensive mono-cropping systems. Tillage excludes advantages of strong competitive species by disrupting the plant cover. It further creates bare soil allowing recolonization by species that are less competitive but tolerant to moderate stress, for example ruderal species and therophytes (*Cardamine hirsuta*, *Stellaria media*, *Poa annua*). In order to further prove the applicability of Grime's hypothesis on vineyard inter-row vegetation, an experimental design that implements additional treatments with varying intensities of disturbance would be needed.

Compared to the impact of soil treatment on plant community composition, the influence of the long-term N fertilization gradient was rather small. Although identified as a main threat to plant species richness in many ecosystems around the globe (Payne et al., 2017), the number of species decreased only slightly as a result of increasing N fertilization in our study (by one to two species between control and highest N level, depending on sampling period). We also did not observe a pronounced community shift with the N fertilization gradient, but the exclusion of rather few species was observed at high fertilization levels, particularly in tillage inter-rows. However, the plant vegetation cover of tillage inter-rows responded strongly to N fertilization. Pioneer species recolonizing the bare soil of inter-rows after tillage benefit from N fertilization, transferring it into vegetative growth and increasing ground coverage. Within permanent cover inter-rows, the already dense vegetation cover did not enable further expansion, regardless of the N nutrition status of the soil.

4.3.2. Microbial community patterns respond to soil treatment, N fertilization, and soil parameters

The microbial richness, represented by the number of bacterial and fungal OTUs, differed between soil treatment types but not between N fertilization levels. Interestingly, the response of OTU richness to soil treatment depended on the group of microorganisms: the number of bacterial OTUs increased when tillage was applied, whereas for fungi, the OTU number was higher in soils under permanent cover.

Soil microbial communities, both bacteria and fungi, responded strongly to soil treatment but rather weakly to long-term N fertilization. Surprisingly, the general patterns of bacterial and fungal communities resembled the general pattern of the plant community response, suggesting that disturbance does not only affect above-ground plant communities but also organisms living in the soil. For bacterial phyla, our findings partly resembled the results described by Burns et al. (2016) –

to our knowledge so far the only study that investigated soil treatment effects on bacteria in vineyard soils using next generation sequencing. Likewise, strong impacts of soil treatment practices on the soil bacteria community were observed by several studies conducted in annual cropping systems (Dong et al., 2017; Lupwayi et al., 2017; Wang et al., 2016b). The authors concluded that changes of soil treatment practice cause changes of soil properties, which in turn alter resource availability for bacterial groups, thus leading to community shifts.

Various studies identified soil pH as the integrating soil variable determining the pattern of bacterial communities at a coarse taxonomic level (Fierer and Jackson, 2006; Lauber et al., 2008; Zhalnina et al., 2014). It has been shown that pH determines microbial composition by regulating the nutrient availability but also by acting as a stressor for prokaryotic cells (Zhalnina et al., 2014). The response of bacterial phyla to soil treatment in our study could partly be explained by the change of soil pH that was increased in soil under tillage. *Acidobacteria*, for example are associated with low pH values and permanent cover treatment. Studies across land-use types confirm a negative correlation of the abundance of *Acidobacteria* with soil pH (Lauber et al., 2008). *Proteobacteria*, *Gemmatimonadetes*, *Chloroflexi*, and *Verrucomicrobia* are more abundant in tillage soil, which likewise exhibited slightly higher soil pH than soil with permanent cover. Previous studies confirm the positive correlation of these groups and soil pH (Zhalnina et al., 2014; Zhou et al., 2015). An investigation of certain optimal pH ranges, where specific bacterial groups can thrive, should be a focus of future studies in microbial ecology.

Proteobacteria and *Firmicutes* were positively correlated with increasing N fertilization. It has been shown that *Proteobacteria* profit from fertilization (Zhou et al., 2015). *Planctomycetes* were strongly associated with low N fertilization levels. This group is known to be more abundant in non-cultivated soils (Eo and Park, 2016). Zhou et al. (2015) identified a number of taxa that responded to mineral fertilization. However, in their experiment on a black soil wheat field, they applied a fertilization gradient with a maximum of 300 kg N ha^{-1}, which was two-fold the amount of N fertilizer as applied in our study.

Regarding soil fungi, recent work in annually cropped systems demonstrated that tillage practices have profound effects on fungal communities (Sharma-Poudyal et al., 2017; Wang et al., 2016a). Our results from a perennial vineyard support the conclusions drawn from these studies that fungal communities benefit from minimal mechanical disturbance and high OC contents, since we found higher numbers of fungal OTUs in inter-rows with permanent cover. We can further confirm that SOM and nutrients like plant available P are additional contributing factors shaping the fungal community (Lauber et al., 2008; Liu et al., 2015a,b). For fungi, it has been shown that nutrient pools affected by N fertilization are putative drivers of change in fungal communities (Francioli et al., 2016; Hu et al., 2017). Our study confirmed that fungal orders are associated with N fertilization, as well as P and K contents in the soil.

5. Conclusions

The present study showed that almost 30 years of constant vineyard soil treatment practice had strong impacts on soil porperties, functions and communities, whereas long-term N fertilization, even at high N application rates, influenced rather few soil parameters and slightly shifted plant and soil microbial community patterns. Although intensive mineral fertilization has been identified to have a strong negative effect on biodiversity and soil properties, we observed only a rather small effect on plant diversity and soil SOM. This might be attributed to the fact that only comparatively moderate levels of mineral N are applied in viticulture, compared to annual cropping systems like corn, rice or wheat with fertilization rates of up to over 300 kg N ha^{-1}.

This study confirmed that the detrimental effects of tillage on soil C and N levels, known from Mediterranean vineyards, are also apparent under temperate climate conditions. Soil organic matter is a crucial soil component to ensure stability and erosion prevention. Since vineyards in Central Europe are increasingly exposed to long droughts during the summer with occasional heavy rainfalls, it is necessary to adopt inter-row cover strategies to prevent vineyard soils from erosion and degradation. We also observed that litter decomposition was higher in soils under permanent cover, indicating a higher microbial activity and an improved mobilization of nutrients compared to soils facing tillage. Improvement of soil fertility by establishing a permanent cover is a desirable aim of sustainable viticulture. However, whether favorable soil characteristics of permanently covered and undisturbed soils are restricted to the upper soil layer providing a potential benefit to the grapevine requires further studies.

Regarding plant diversity, moderate tillage not only increased species richness but also promoted a ruderal plant community with fast life cycles and herbaceous plants that provide a food source for pollinating insects. Since sustainable agriculture aims at enhancing biodiversity and ecosystem functions, the current practice of inter-row-wise alternating soil treatments, applied in many German vineyards, combines both advantages of tillage and permanent cover. If tillage is not desirable due to negative effects on soil properties, it is advisable to consider seed mixtures that not only contain few strong grass species but also herbs and annual plants to enhance plant diversity in vineyard inter-rows.

To our knowledge, this study is the first that combined the assessment of the plant community with the communities of soil bacteria and fungi in an agricultural field experiment. Surprisingly, all three communities responded similarly to soil treatment and N fertilization. We concluded that for plants, these patterns are dominantly driven by mechanical disturbance directly, whereas microbial communities might not only react to the disturbance but also to soil parameters, like pH and SOM levels that are changed by long-term soil treatment. Future research should focus on the responses of specific bacterial and fungal taxa to soil management strategies and their role in soil processes related to fertility.

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Declaration of interest

none

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.agee.2018.11.005.

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