A peer-reviewed version of this preprint was published in PeerJ on 17 October 2017.

Anderson C, Beare M, Buckley HL, Lear G. 2017. Bacterial and fungal communities respond differently to varying tillage depth in agricultural soils. PeerJ 5:e3930 https://doi.org/10.7717/peerj.3930

Bacterial and fungal communities respond differently to varying tillage depth in agricultural soils

Craig Anderson Corresp., [1] , **Mike Beare** [1] , **Hannah L Buckley** [2] , **Gavin Lear** [3]

[1] Plant and Food Research, Lincoln, New Zealand

[2] School of Science, Auckland University of Technology, Auckland, New Zealand

[3] School of Biological Sciences, University of Auckland, Auckland, New Zealand

Corresponding Author: Craig Anderson
Email address: Craig.Anderson@plantandfood.co.nz

In arable cropping systems, reduced or conservation tillage practices are linked with improved soil quality, C retention and higher microbial biomass, but most long-term studies rarely focus on depths greater than 15 cm nor allow comparison of microbial community responses to agricultural practices. We investigated microbial community structure in a long-term field trial (12-years, Lincoln, New Zealand) established in a silt-loam soil over four depth ranges down to 30 cm. Our objectives were to investigate the degree of homogenisation of soil biological and chemical properties with depth, and to determine the main drivers of microbial community response to tillage. We hypothesised that soil microbiological responses would depend on tillage depth, observed by a homogenisation of microbial community composition within the tilled zone. Tillage treatments were mouldboard plough and disc harrow, impacting soil to ~20 and ~10 cm depth, respectively. These treatments were compared to a no-tillage treatment and two control treatments, both permanent pasture and permanent fallow. Bacterial and fungal communities collected from the site were not impacted by the spatial location of sampling across the study area but were affected by physicochemical changes associated with tillage induced soil homogenisation and plant presence. Tillage treatment effects on both species richness and composition were more evident for bacterial communities than fungal communities, and were greater at depths <15 cm. Homogenisation of soil and changing land management appears to redistribute both microbiota and nutrients deeper in the soil profile while consequences for soil biogeochemical functioning remain poorly understood.

Title: Bacterial and fungal communities respond differently to varying tillage intensities in agricultural soils

Craig Anderson[a*], Mike Beare[a], Hannah Buckley[b], Gavin Lear[c]

[a] Plant and Food Research, Gerald Street, Lincoln 7600, New Zealand

[b] School of Science, Auckland University of Technology, Auckland 1142, New Zealand

[c] School of Biological Sciences, The University of Auckland, Auckland 1010, New Zealand

*Corresponding Author: (T) +64 3 325 9397
(F) +64 3 325 2074
(email) craig.anderson@plantandfood.co.nz

Abstract

In arable cropping systems, reduced or conservation tillage practices are linked with improved soil quality, C retention and higher microbial biomass, but most long-term studies rarely focus on depths greater than 15 cm nor allow comparison of microbial community responses to agricultural practices. We investigated microbial community structure in a long-term field trial (12-years, Lincoln, New Zealand) established in a silt-loam soil over four depth ranges down to 30 cm. Our objectives were to investigate the degree of homogenisation of soil biological and chemical properties with depth, and to determine the main drivers of microbial community response to tillage. We hypothesised that soil microbiological responses would depend on tillage depth, observed by a homogenisation of microbial community composition within the tilled zone. Tillage treatments were mouldboard plough and disc harrow, impacting soil to ~20 and ~10 cm depth, respectively. These treatments were compared to a no-tillage treatment and two control treatments, both permanent pasture and permanent fallow. Bacterial and fungal

communities collected from the site were not impacted by the spatial location of sampling across the study area but were affected by physicochemical changes associated with tillage induced soil homogenisation and plant presence. Tillage treatment effects on both species richness and composition were more evident for bacterial communities than fungal communities, and were greater at depths <15 cm. Homogenisation of soil and changing land management appears to redistribute both microbiota and nutrients deeper in the soil profile while consequences for soil biogeochemical functioning remain poorly understood.

Introduction

Tillage alters soil porosity, distributes carbon and nitrogen throughout the soil profile, impacts microbial respiration and potentially leads to carbon loss (Singh et al. 2010). More stable aggregate structure in the upper surfaces of non-tilled soils is proposed to improve soil porosity and moderate evaporation, improving soil water conservation (Busari et al. 2015). While increasing the abundance of water storage pores (Pagliai et al. 2004), the lower aeration of non-tilled soils may simultaneously decrease oxygen availability, lowering aerobic turnover in the soil and decreasing gaseous losses (Skiba et al. 2002). Consequently, the use of no-till soil management has been promoted to land managers seeking to reduce soil carbon losses and curb greenhouse gas emissions (Conant et al. 2007).

The impacts of soil management on microbial diversity and functioning are still under investigation. Crop residues and root exudates are the main sources of soil C (Gougoulias et al. 2014) with tillage distributing these C-sources deeper into the soil and altering soil structure. Tillage, therefore affects microbial access to fresh C at depth, releases previously inaccessible C

and changes soil water and gas distribution thus affecting microbial metabolic rates. By contrast, no-till management restricts microbial access to fresh C (by leaving residues at the surface and in the vicinity of roots) and minimises soil disturbance, therefore impacting how soil-C will be processed. Since soil microorganisms have primary control over C flows within the soil and between the soil and atmosphere (Balaine et al. 2016), alterations of soil C distributions by tilling are likely to impact both microbial community composition and functioning.

The impacts of no-till management on soil C stocks are variable compared with conventional tilled systems (Helgason et al. 2014) where C stocks may be far higher (e.g., 1.7 times greater; Wakindiki et al., 2017) due to surface derived plant C being incorporated into the soil. With this in mind, it is conceivable that a moderate degree of tillage or inversion tillage may aid restoration of soil C stocks at deeper levels in the soil profile. However, to confirm the restoration of soil C would first require confirmation that appropriate levels of C exist, appropriate microbial communities are present that can decompose the residues at depth and that other limiting nutrients are made available. Until recently, few studies have examined the impact of different tillage practices on soil microbial community structure, specifically at depths >15 cm (Ceja-Navarro et al. 2010; Navarro-Noya et al. 2013; van Groenigen et al. 2010). Since both bacterial and fungal communities play major roles in soil organic matter cycling, we examined their composition within a long-term (12-year) trial to evaluate the effects of tillage down to a depth of 30 cm. For both communities, we hypothesised that there would be weaker depth-related gradients in community composition in tilled soil, since tillage should homogenise the soil and overshadow any depth-dependant effects. To further explore the role of tillage on depth-related gradients in soil microbial community composition, we chose to compare communities

in untilled soil to communities in soil tilled to depths of either 10 or 20 cm. We also expected fungal communities to be more prone to disturbance from ploughing because of their extensive hyphal networks (Wardle 1995). Therefore, our objectives were to investigate the degree of homogenisation of soil biological and chemical properties to depths of 30 cm, and to identify the main drivers of microbial community responses to tillage intensity.

Methods

Experimental Site and Field Trial Description

Replicated soil samples were taken pre-harvest (09/03/2012) from 15 plots at a long-term tillage trial run by Plant & Food Research, near Lincoln, in the South Island of New Zealand (43°40'S latitude, 172°28'E longitude; mean annual air temperature 11.4 °C, mean annual rainfall 867 mm). The soil underlying this site is a Wakanui silt loam, classified as Udic Dystocrypt according to USDA taxonomy (Soil Survey Staff 1999). Before trial establishment, the site was sheep-grazed, irrigated permanent pasture that had not been cultivated for at least 14 years. Three tillage methods applied in Spring and Autumn seasons were evaluated, these being; No-tillage (Nn): no cultivation, seeds direct drilled; Minimum tillage (Mm): the top 10 cm cultivated using a spring tined implement, followed by secondary cultivation (harrowing and rolling twice); Intensive tillage (Ii): cultivation to ~20 cm using a mouldboard plough, followed by secondary cultivation (one pass with a spring tined implement followed by harrowing and rolling twice). All tillage operations were carried out using standard commercial equipment. Spring-sown main crops rotation included barley, wheat, and peas. They were followed by winter-grazed (sheep) cover crops (oats or forage brassicas). All crops were sown using a Great Plains direct drill. Fertiliser (N and P) were applied to the spring crops to ensure these nutrients were not limiting. Plots representing the original ryegrass-clover pasture were maintained within the trial as a

control. To balance the trial design, these plots were split into subplots; permanent pasture (Pp), and permanent fallow (Pf). The Pp sub-plots were grazed with sheep (typically 10 times per year; 20 sheep per plot). The main fertiliser applied to the Pp plot was superphosphate. The Pf subplots received no fertiliser and had no animal or vehicle trafficking throughout the trial. Herbicide (Glyphosate) was used to maintain the Pf subplots plant free. Management (irrigation, fertiliser, grazing regime) of the Pp plots remained the same as before the trial. All treatments (i.e. Arable crops, Pp and Pf) were irrigated in summer to ensure that water was not limiting to pasture or crop growth. Treatment plots were replicated three times in an incomplete Latin square (i.e. five treatments x three replicate plots = 15 plots; see Fig. 1). The size of individual plots was 28 m x 9 m. Further trial details can be obtained from Fraser et al. (2013). The long term field trial was operated by Plant and Food Research. No additional permits were required for sample collection.

Two types of soil samples were taken from each plot: (1) six surface 25 mm diameter core samples (0 – 7.5 cm) where each sample was analysed separately to confirm the impact of spatial heterogeneity on sample data and (2) eight deeper 5 cm diameter core samples separated into four depth ranges (0 – 7.5 cm, 7.5 – 15 cm, 15 – 25 cm and 25 – 30 cm), which were later composited by depth (Fig. 1). Soil used for chemical and physical analysis was stored at 4 °C prior to use and 2 g aliquots of each sample frozen in Eppendorf tubes for DNA extraction. Soil subsamples were taken from each depth to measure: (1) water content, (2) pH, (3) bulk density/mean weight diameter (MWD), (4) exchangeable acidity, (5) exchangeable aluminium, (6) concentrations of C and N, and (7) microbial biomass C and N.

Soil chemical analysis

Gravimetric soil moisture content was determined by the mass difference before and after drying at 105°C for 16 h. The pH of each sample was determined using a glass electrode at 1:2 field moist sample to water ratio (Hendershot et al. 2008). Bulk density (< 4 mm) was calculated from the weight of field-moist soil of known volume, corrected for its stone and moisture contents. Aggregate stability or mean weight diameter (MWD) was determined by first separating 2-4 mm aggregates from whole soil by sieving, and then air-drying them at 25˚C before aggregate stability determination using a wet-sieving method (Kemper & Rosenau 1986). The air-dried 2−4 mm aggregates (50 g) were sieved underwater for 20 minutes on a nest of sieves (2.0, 1.0 and 0.5 mm diameter). The soil remaining on each sieve was weighed after oven drying at 105˚C. The aggregate stability was expressed as a mean weight diameter (MWD):

$$MWD = \sum_{i=1}^{n} x_i w_i$$

where x_i is the mean diameter of adjacent sieves and w_i is the proportion of the total sample retained on a sieve.

Exchangeable acidity (Exch. Acid.) and aluminium (Exch. Al.) was determined by extraction using 1 *M* KCl. The amount of H^+ and Al^{3+} in the extracts was determined by titration as described by Sims (1996). Total carbon (C) and nitrogen (N) contents were determined by the Dumas dry combustion method at 950°C using a Truspec C/N analyzer (LECO, St. Joseph, Michigan, USA).

Microbial biomass C (MBC) and N (MBN) were determined by chloroform fumigation-extraction as described by Sparling & West (1988). Pre- and post-fumigation extracts were analysed for organic C by combustion catalytic oxidation using a TOC-V_{CSH} analyzer (Shimadzu Corporation, Kyoto, Japan) and for organic N by the persulfate oxidation method described by Cabrera & Beare (1993). Physicochemical data collected from the site are provided in Supplementary File 1.

Production and manipulation of ARISA data from extracted DNA

Automated Ribosomal Intergenic Spacer Analysis (ARISA)

For each sample (160 in total), DNA was extracted from 0.25 g freeze-dried soil using Powersoil®-htp 96 well DNA isolation kits (MoBio Laboratories Inc., Carlsbad, CA., USA) following the manufacturer's instructions. Automated Ribosomal Integenic Spacer Analysis (ARISA) was then used to evaluate the composition of bacterial and fungal communities in each sample according the method of Lear et al. (2008). This PCR-based method characterises the structure of the microbial community within each sample by recording the length (in base pairs, b.p.) of the intergenic spacer (ITS) regions of the constituent microbes, i.e., between the bacterial 16S rRNA and 23S rRNA genes or the fungal 18S rRNA and large ribosomal subunit genes.

PCR amplification of bacterial ITS regions was undertaken on the extracted DNA using Promega GoTaq® Green DNA polymerase master mix (Invitro Technologies Ltd., Auckland, New Zealand) and the primers SDBact (5'-TGC GGC TGG ATC CCC TCC TT-3') and LDBact (5'-CCG GGT TTC CCC ATT CGG) (Ranjard et al. 2001), with the following amplification conditions: (i) 95 °C for 5 min; (ii) 30 cycles of 95 °C for 30 s, 61.5 °C for 30 s, 72 °C for 90 s

and then (iii) 72 °C for 10 min. The primer SDBact was labelled at the 5'-end with HEX (6-carboxyhexafluorescein) fluorochrome (Invitrogen Molecular Probes, New Zealand) to enable analysis by ARISA.

For the fungi, the PCR primers used were FunNS1 (5'- GAT TGA ATG GCT TAG TGA GG -3') (Martin & Rygiewicz 2005) and 3126T (5'- ATA TGC TTA AGT TCA GCG GGT -3') (Ranjard et al. 2001). PCR amplification used the Phusion® polymerase (NEB, Ipswich, MA, USA) according to the manufacturer's instructions, with the following amplification conditions: (i) 98 °C for 2 min; (ii) 35 cycles of 98 °C for 10 s, 55 °C for 30 s, 72 °C for 45 s and then (iii) 72 °C for 20 min. The primer FunNS1 was labelled at the 5'-end with FAM (6-carboxyfluorescein) fluorochrome (IDT, Asia Pacific, Singapore).

Products were each purified (Zymo DNA clean and Concentrator kit; Ngaio Diagnostics Ltd., Nelson, New Zealand) and DNA concentration (ng μl^{-1}) individually determined using a Nanodrop ND-100 spectrophotometer (NanoDrop Technologies, Rockland, DE). Appropriate volumes of cleaned PCR product (diluted with ultrapure H_2O if necessary) providing a final DNA mass of 5 to 10 ng were then transferred to a 96-well sequencing plate and dried in a speedvac for 2 h at 60 ºC. The dry sample was resuspended in 15 µl Hi-Di deionised formamide and Genescan LIZ-1200 internal size standard (ABI Ltd.). The sample was heated (5 min, 95 ºC) and analysis was carried out on an ABI 3130XL genetic analyser with POP7 chemistry and a 36 cm array (ABI Ltd.).

Quantitative Analysis of ARISA Data

GENEMAPPER software (v. 3.7; ABI Ltd) was used to assign a fragment length (in nucleotide base pairs) to ARISA peaks, via comparison with the standard ladder (LIZ1200; ABI Ltd.). To include the maximum number of peaks whilst excluding background fluorescence, only peaks with a fluorescence value of 50 U or greater were analysed. As the 16S-23S region is thought to range between ~140 and 1530 bp (Fisher & Triplett 1999), fragments <150 bp were excluded from analysis. No samples contained fragments > 1000 bp. The same size (bp) parameters were used for the fungi as these samples also did not contain any fragments > 1000 bp. The total area under the curve was normalised (to 100) to remove differences in profiles caused by different initial DNA template quantities, and peak size was rounded to the nearest whole number. Each ARISA sample therefore consisted of 851 operational taxonomic groupings of bacteria or fungi, which represent the length of the intergenic spacer region of constituent microbes (in bp), thereby providing an informative profile of the bacterial and fungal community composition within each sample. OTU tables are available provided in Supplemental Files S2-S3.

To visualise multivariate patterns in the soil microbial community structure among samples, nonmetric multidimensional scaling (nMDS) was done using the Bray Curtis measure. Rather than using multivariate analysis of variance MANOVA to test the data, which assumes normal distributions, and implicitly Euclidean distances, we chose to use permutational MANOVA (or PERMANOVA; Anderson, 2011) with the data assigned to the factors Treatment (Pf, Pf, Ii, Nn and Mm) and Depth (0.7.5, 7.5-15, 15-25 and 35-30 cm). MVDISP was used to compare the extent of multivariate data dispersion across these groups. These multivariate analyses were performed using the PRIMER v.6 computer program (Clarke & Gorley 2006) with the additional add-on package PERMANOVA+ (Anderson et al. 2008).

209

210 We used the aov function in R version 2.14 (R Core Team 2012) to perform analyses of variance
211 on soil chemical data using a two-way layout (treatment; depth), with interaction terms.
212 Canonical redundancy analysis (RDA) and was used to summarise variation in the bacterial and
213 fungal community data that could be explained by our set of explanatory variables (e.g., pH, soil
214 water content). Variance partitioning was then performed using the function varpart.MEM in R,
215 following Borcard et al. (2011) to describe and partition variation in community composition
216 between two sets of explanatory variables: soil chemical properties and geographic location.

217

218 **Results and Discussion**

219 Analysis of the surface soil samples (0 – 7.5 cm) showed significant variation in
220 microbial community composition among treatments (Fig. 2, PERMANOVA all $P < 0.001$).
221 Bacterial and fungal composition from the five treatments differed significantly irrespective of
222 whether the data remained untransformed or was log(X+1) transformed to remove computational
223 bias derived from dominant OTUs (operational taxonomic units – broadly representing
224 'unknown' phyla). These results confirm soil management practices impact the composition of
225 both bacterial and fungal communities, supporting the findings of other recent studies (Busari et
226 al. 2015; Ceja-Navarro et al. 2010; Mathew et al. 2012).

227

228 The treatment differences among the bacterial community data were more pronounced
229 than those of the fungal data, as the former formed more distinct clusters on an nMDS plot (Fig.
230 2). The removal of the Pp and Pf data from the analysis further improved the separation of the
231 cropped tillage treatments and reduced the 2D-Stress goodness of fit statistic to 0.16 and 0.15 for

bacteria and fungi respectively, improving the certainty of the visual nMDS solution (Cox & Cox 1992). All pairwise PERMANOVA comparisons among treatments were significant for bacteria, but only three (Pp-Ii, Pf-Nn, and Mm-Pf) were significant for fungi (i.e., PERMANOVA P all < 0.001) suggesting that bacterial communities in the surface soil (0-7.5 cm) were more sensitive to tillage treatment than fungal communities. This tillage treatment effect may be because bacteria tend to dominate in soils that are intensively managed, where they drive decomposition and nutrient cycles (Garcia-Orenes et al. 2013). The greatest pairwise Bray-Curtis distances between data representing any two treatments for both taxa were between the various no-till treatments, i.e., between Pf and Pp for bacteria and between Pf and Nn for fungi. Since greater average Bray-Curtis distances among data indicate greater differences in overall community composition, these findings confirm that tillage as a disturbance drives microbial community composition to a lesser degree than other management effects, such as the presence of permanent pasture, grazing or vegetation removal. We suggest tillage treatment differences have less effect because for both bacterial and fungal communities, average community similarity (i.e., average Bray-Curtis distances were least) comparing the treatments Mm and Nn. For samples taken across depths, two-way ANOVA showed that all soil chemical properties, except concentrations of exchangeable aluminium and acidity, differed significantly by treatment depth (Table 1 and Supplementary Figure S1). With the exception of soil water content, the greatest difference among treatments was again between the non-till control treatments Pp and Pf, and the biggest difference among depths was between 0 – 7.5 cm and 15 – 25 cm, noting that chemical data was never obtained from the deepest (25-30 cm) samples. Previous research at this field site has indicated that crop productivity is not influenced by tillage, neither is nutrient input (pers. comm., Denis Curtin, 2017). However, tillage introduced a degree of homogenisation that was

255 reflected in the depth profiles of soil chemical properties and nutrient distribution. Soil chemical
256 attributes varied little with depth under intensive tillage, which is of relevance since variation in
257 nutrient inputs and soil depth are suggested to be important drivers of microbial community
258 change (Jangid et al. 2008; Jeffery et al. 2007).
259
260 NMDS and PERMANOVA showed that bacterial community composition, like soil
261 chemical properties, varied predictably with depth and tillage treatment. Treatment effects were
262 greatest among the shallowest soil samples (≤ 15 cm) compared with deeper soil, with these data
263 points being separated further apart on the nMDS plot as compared to samples collected at
264 greater depth (Fig. 3a). Multivariate dispersion index values (i.e. mean Bray Curtis dissimilarities
265 among samples within groups) confirmed greater variation in bacterial community composition
266 comparing samples collected at shallower depth (MVDISP = 1.3, 1.2, 0.8 and 0.8 for samples
267 collected from 0-7.5, 7.5-15, 15-25 and 25-30 cm, respectively, where greater values indicate
268 greater multivariate data dispersion within the group). Overall, PERMANOVA only showed
269 significant pairwise treatment effects to a depth of 25 cm. These results are consistent with our
270 hypothesis that the tillage effects on microbial communities would decline or weaken with depth.
271
272 Fungal community composition changed with increasing sample depth but unlike the
273 bacterial community data, no consistent pattern is observable aside from some separation
274 between sample data from tillage treatments that were cropped versus non-cropped Pp and Pf
275 treatments (Fig. 3b). MVDISP calculations showed that the multivariate dispersion of samples
276 was lowest for those taken at 0-7.5 or 25-30 cm and therefore the fungal communities did not
277 show the same patterns of decreasing variation among treatments with depth (MVDISP = 0.8,

1.1, 1.5 and 0.7 for samples collected from 0-7.5, 7.5-15, 15-25 and 25-30 cm, respectively). A number of reasons can be proposed to explain this finding. First, since most soil-dwelling fungi are aerobic (Gruninger et al. 2014) is it commonly observed that they form weak depth related gradients in composition compared to bacteria, which have a far greater diversity of metabolic traits related to respiration (Richardson 2000). Additionally, being larger organisms, the biomass of single multicellular fungi is likely to be represented at multiple soil depths (Genney et al. 2006), thereby exhibiting weaker depth-related gradients in composition across small spatial scales. However, it remains possible that the apparent difference in bacterial and fungal community treatment responses is also impacted by the choice of DNA fragments amplified, which can influence both the number and composition of OTUs detected in a community (Kumar et al. 2011). To address this issue, it may be desirable in future studies to assess variation in both bacterial and fungal community composition using a range of genetic markers, analysed by either DNA fingerprinting (Adair et al. 2013) or sequencing methods (Hermans et al. 2017).

Canonical redundancy analysis (RDA) was used to describe and partition variation in community composition between two sets of explanatory variables: soil chemical properties and geographic location. Soil chemical properties alone explained 25% and 22% of the variation in bacterial and fungal community composition, respectively; whereas, spatial location could explain only 2% or 3% of the variation in bacterial or fungal composition, respectively. This confirms that within this field trial microbial communities are responding more to soil chemical properties rather than to spatial location in the field or plot.

Two-way ANOVA confirmed that relative bacterial OTU richness (variety or number of OTUs) in the 0 – 7.5 cm depth was greater than at lower depths (Fig. 4; $P < 0.001$), but did not differ among treatments. In contrast, fungal richness did not significantly differ by depth or treatment perhaps also explaining why variance partitioning showed that soil chemical properties explained 31% of the bacterial richness but only 5% of the variation in fungal richness. Depth × treatment interactions were not significant for the OTU richness of either taxon. The general lack of effect of either sample depth or treatment on microbial community richness was not unexpected, however. DNA fingerprinting methods do not provide species level diversity estimates and are not suitable to report absolute measures of community richness (Fierer 2007). Additionally, the metabolic complexity of microbial life means that high levels of diversity are commonly observed even in environments that are commonly perceived as being extreme for life, such as high temperature, highly acidic and polluted environments (Du et al. 2009; Savage et al. 2016), or in deep sediments for example (Lehman et al. 2001).

Overall, our study confirms tillage has significant impacts for both the biology and chemistry of soil. Previous studies examining soil carbon and nitrogen concentrations have suggested no significant difference, or even lower soil carbon concentrations under reduced tillage systems (Powleson et al. 2014). However, tillage practices have the potential to impact not only total carbon and nitrogen stocks, but their distribution in the soil. Here, as observed by others (Du et al. 2009; Zhao et al. 2015), we confirm concentrations of soil and microbial biomass carbon and nitrogen were reduced in the surface soil by tillage, whereas they were greater at depth, indicating the transfer of biomass to lower soil layers by mechanical tillage. Although the ARISA methodology lacks in-depth precision below perhaps order level (Gobet et

al. 2014), the method was sufficient to indicate that bacterial community composition is more responsive to tillage treatment differences than fungi. Tillage affected the composition, but not the richness, of soil microbial communities. Changes in community composition with depth appeared to be related to tillage intensity with the deeper mouldboard plough (0-20 cm) acting to homogenise soil nutrients and microbial communities throughout the soil depth affected by this disturbance. Moderate tillage with disc harrow (0-10 cm) and the no-till treatments behaved similarly to each other, exhibiting a higher degree of community variation with depth. We confirm the significant impact of tillage on soil microbial community composition, perhaps resulting from the homogenisation of local soil chemical characteristics. Soil microorganisms are known to impact agricultural production, for example by controlling nutrient availability and by mediation of plant stress tolerance (de Souza et al. 2015; Ferrara et al. 2012; Zahran 1999). Further studies, perhaps also investigating plant biomass yield and quality, are now required to confirm the impact of tillage related changes in soil microbial community composition for plant health and production potential.

References

Adair K, Wratten S, D., and Lear G. 2013. Soil phosphorous depletion and shifts in plant communties change bacterial community structure in a long-term grassland managemnt trial. *Environmental Microbiology Reports* 5:404-413.

Anderson MJ. 2001. A new method for non-parametric multivariate analysis of varience. *Austral Ecology* 26:32-46.

Anderson MJ, Gorley RN, and Clarke KR. 2008. *PERMANOVA+ for PRIMER: guide to software and statistical methods. PRIMER-E Ltd., Plymouth, United Kingdom*.

Balaine N, Clough TJ, Beare MH, Thomas SM, and Meenken ED. 2016. Soil gas diffusivity controls N_2O and N_2 emissions and their ratio. *Soil Science Society of America Journal* 80:529-540.

Borcard D, Gillert F, and Legendre P. 2011. *Numerical Ecology with R*. New York: Springer.

352 Busari MA, Kukal SS, Kaur A, Bhatt R, and Dulazi AA. 2015. Conservation tillage impacts in
353 soil, crop and the environment. *International Soil and Water Conservation Research*
354 3:119-129.
355 Cabrera ML, and Beare MH. 1993. Alkaline persulfate oxidation for determining total nitrogen
356 in microbal biomass extracts. *Soil Science Society of America Journal* 57:1007-1012.
357 Ceja-Navarro JA, Rivera-Orduna FN, Patino-Zuniga L, Vila-Sanjurjo A, Crossa J, Govaerts B,
358 and Dendooven L. 2010. Phylogenetic and multivariate analyses to determine the effects
359 of different tillage and residue management practices on soil bacterial communities.
360 *Applied and Environmental Microbiology* 76:3685-3691.
361 Clarke KR, and Gorley RN. 2006. PRIMER v6:User Manual/Tutorial. PRIMER_E, Plymouth,
362 UK.
363 Conant RT, Easter M, Paustian K, Swan A, and Williams S. 2007. Impacts of periodic tillage on
364 soil C stocks: A synthesis. *Soil and Tillage Research* 95:1-10.
365 Cox MAA, and Cox TF. 1992. Interpretation of stress in nonmetric multidimensional scaling.
366 *Statistica Applicata* 44:611-628.
367 de Souza R, A. A, and Passaglia LMP. 2015. Plant growth-promoting bacteria as inoculants in
368 agricultural soils. *Genetics and Molecular Biology* 38:1415-1457.
369 Du Z, Ren T, and Hu C. 2009. Tillage and residue removal effects on soil and nitreogen storage
370 in the North China Plain. *Soil Science Society of America Journal* 74:196-202.
371 Ferrara FIS, Oliveira ZM, Gonzales HHS, Floh EIS, and Barbosa HR. 2012. Endophytic and
372 rhizospheric enterobacteria isolated from sugar cane have differnt potentials for
373 producing plant growth-promoting substances. *Plant and Soil* 353:409-417.
374 Fierer N. 2007. Tilting at windmills: a response to a recent critique of terminal restriction
375 fragment length polymorphism data. *Applied and Environmental Microbiology* 73:8041-
376 8042.
377 Fisher MM, and Triplett EW. 1999. Automated approach for ribosomal intergenic spacer
378 analysis of microbial diversity of microbial diversity and its applications to freshwater
379 bacterial communities. *Applied and Environmental Microbiology* 65:4630-4636.
380 Fraser PM, Curtin D, Harrison-Kirk T, Meenken ED, Beare MH, Tabley F, Gillespie RN, and
381 Francis GS. 2013. Winter nitrate leaching under different tillage and winter cover crop
382 management practices. *Soil Science Society of America Journal* 77.
383 Garcia-Orenes F, Morugan-Coronado A, Zornoza R, and Scow K. 2013. Changes in soil
384 microbial community structure infleunced by agricultural management practices in a
385 Mediterranean agro-ecosystem. *PLoS ONE* 8:e80522.
386 Genney DR, Anderson IC, and Alexander IJ. 2006. Fine-scale distribution of pine
387 ectomycorrhizas and their extramatrical mycelium. *New Phytologist* 170:381-390.
388 Gobet A, Boetius A, and Ramette A. 2014. Ecological coherence of diversity patterns derived
389 from classical fingerprinting and Next Generation Sequencing techniques. *Environmental*
390 *Microbiology* 16:2672-2681.
391 Gougoulias C, Clark JM, and Shaw LJ. 2014. The role of soil microbes in the global carbon
392 cycle: tracking the below-ground microbial processing of plant-derived carbon for
393 manipulating carbon dynamics in agricultural systems. *Journal of the Science of Food*
394 *and Agriculture* 94:2362-2371.
395 Gruninger RJ, Puniya AK, Callaghan TM, Edwards JE, Youssef N, Dagar SS, Fliegerova K,
396 Griffith GW, Forster R, Tsang A, McAllister T, and Elshahed MS. 2014. Anaerobic fungi

(phylum *Neocallimastigomycota*): advances in understanding their taxonomy, life cycle, ecology, role and biotechnological potential. *FEMS Microbiology Ecology* 90:1-17.

Helgason BL, Gregorich EG, Janzen HH, Ellert BH, Lorenz N, and Dick RP. 2014. Long-term microbial retention of residue C is site-specific and depends on residue placement. *Soil Biology & Biochemistry* 68:231-240. 10.1016/j.soilbio.2013.10.002

Hendershot WH, Lalande H, and duquette M. 2008. Soil reaction and exchangeable activity. In: Carter MR, and Gregorich EG, eds. *Soil Sampling Methods and Analysis*. Boca Raton, FL, USA: CRC Press, 173.

Hermans SM, Buckley HL, Case BS, Curran-Cournane F, Taylor M, and Lear G. 2017. Bacteria as emerging indicators of soil condition. *Applied and Environmental Microbiology* 83:e02826-02816.

Jangid K, Williams MA, Franzluebbers AJ, Sanderlin JS, Reeves JH, Jenkins MB, Endale DM, Coleman DC, and Whitman WB. 2008. Relative impacts of land-use, management intensity and fertilization upon soil microbial community structure in agricultural systems. *Soil Biology & Biochemistry* 40:2843-2853. 10.1016/j.soilbio.2008.07.030

Jeffery S, Harris JA, Rickson RJ, and Ritz K. 2007. Microbial community phenotypic profiles change markedly with depth within the first centimetre of the arable soil surface. *Soil Biology & Biochemistry* 39:1226-1229.

Kemper WD, and Rosenau RC. 1986. Aggregate stabilty and size distribution. In: Klute A, ed. *Methods of Soil Analysis, Part 2 Chemical and Mineralogical Methods, 2nd Edition*. Madison, WI: Soil Science Society of America, 491-515.

Kumar PS, Brooker MR, Dowd SE, and Camerlengo T. 2011. Target region selection is a critical determinant of community fingerprints generated by 16S pyrosequencing. *PLoS ONE* 6:e20956.

Lear G, Anderson MJ, Smith JP, Boxen K, and Lewis GD. 2008. Spatial and temporal heterogeneity of the bacterial communties in stream epilithic biofilms. *FEMS Microbiology Ecology* 65:463-473.

Lehman RM, Roberto FF, Earley D, Bruhn DF, Brink SE, O'Connell SP, Delwiche ME, and Colwell FS. 2001. Attached and unattached bacterial communities in a 120-meter corehole in an acidic, crystaline rock aquifer. *Applied and Environmental Microbiology* 67:2095-2106.

Martin KJ, and Rygiewicz PT. 2005. Fungal-specific PCR primers developed for analysis of the ITS region of environmental DNA extracts. *BMC Microbiology* 5:28.

Mathew RP, Feng Y, Githinji L, Ankumah R, and Balkcom KS. 2012. Impact of no-tillage and conventional tillage systems on soil microbial communities. *Applied and Environmental Soil Science* article ID 548620.

Navarro-Noya YE, Gomez-Acata S, Montoya-Ciriaco N, Rojas-Valdez A, Suarez-Arriaga MC, Valenzuela-Encinas C, Jimenez-Bueno N, Verhulst N, Govaerts B, and Dendooven L. 2013. Relative impacts of tillage, residue management and crop-rotation on soil bacterial communities in a semi-arid agroecosytem. *Soil Biology & Biochemistry* 65:86-95.

Pagliai M, Vignozzi N, and Pellegrini S. 2004. Soil structure and the effect of management practices. *Soil and Tillage Research* 79:131-143.

Powleson DS, Stirling CM, Jat ML, Gerard BG, Palm CA, Sanchez PA, and Cassman KG. 2014. Limited potential of no-till agriculture for climate change mitigation. *Nature Climate Change* 4:678-683.

R Core Team. 2012. R: A language and environment for statistical computing. R foundation for statistical computing, Vienna, Austria. http://www.r-project.org/.

Ranjard L, Poly F, Lata JC, Mougal C, Thioulouse J, and Nazaret S. 2001. Characterization of bacterial and fungal soil communities by automated ribosomal intergenic spacer analysis fingerprints: biological and methodological variability. *Applied and Environmental Microbiology* 67:4479-4487.

Richardson DJ. 2000. Bacterial respiration: a flexible process for a changing environment. *Microbiology* 146:511-571.

Savage AM, Hills J, Driscoll K, Fergus DJ, Grunden AM, and Dunn RR. 2016. Microbial diversity of extreme habitats in human homes. *PeerJ* 4:e2376.

Sims JT. 1996. Lime Requirement. In: Sparks DL, ed. *Methods of Soil Analysis Part 2: Chemical Properties (3rd Ed)*. Madison, WI: Soil Science Society of Americal Journal, 491-515.

Singh BK, Bardgett RD, Smith P, and Reay DS. 2010. Microorganisms and climate change: terrestrial feedbacks and mitigation options. *Nature Reviews Microbiology* 8:779-790.

Skiba U, van Dijk S, and Ball BC. 2002. The influence of tillage on NO and N_2O fluxes under spring and winter barley. *Soil Use and Management* 18:340-345.

Soil Survey Staff. 1999. Soil taxonomy: A basic system of soil classification for making and interpreting soil surveys. 2nd edition. National Resources Conservation Service. U.S. Department of Agriculture Handbook 436.

Sparling GP, and West AW. 1988. A direct extraction method to estimate soil microbial C: calibration in situ using microbial respiration and 14-C labelled cells. *Soil Biology & Biochemistry* 20:337-343.

van Groenigen K-J, Bloem J, Baath E, Boeckx P, Rousk J, Bode S, Forristal D, and Jones MB. 2010. Abundance, production and stabilization of microbial biomass under conventional and reduced tillage. *Soil Biology and Biochemistry* 42:48-55.

Wakindiki IIC, and Njeru SK. 2017. Organic carbon associated with tillage-induced aggregates of soil quartz-dominated dominated loamy soils in a semi-arid region of South Africa. *South African Journal of Plant and Soil* 34:239-242.

Wardle DA. 1995. Impacts of disturbance on detritus food webs in agro-ecosystems of contrasting tillage and weed management practices. *Advances in Ecological Research* 26:105-185.

Zahran HH. 1999. *Rhizobium*-legume sybmiosis and nitrogen fixation under severe conditions and in an arid climate. *Microbiology and Molecular Reviews* 63:968-989.

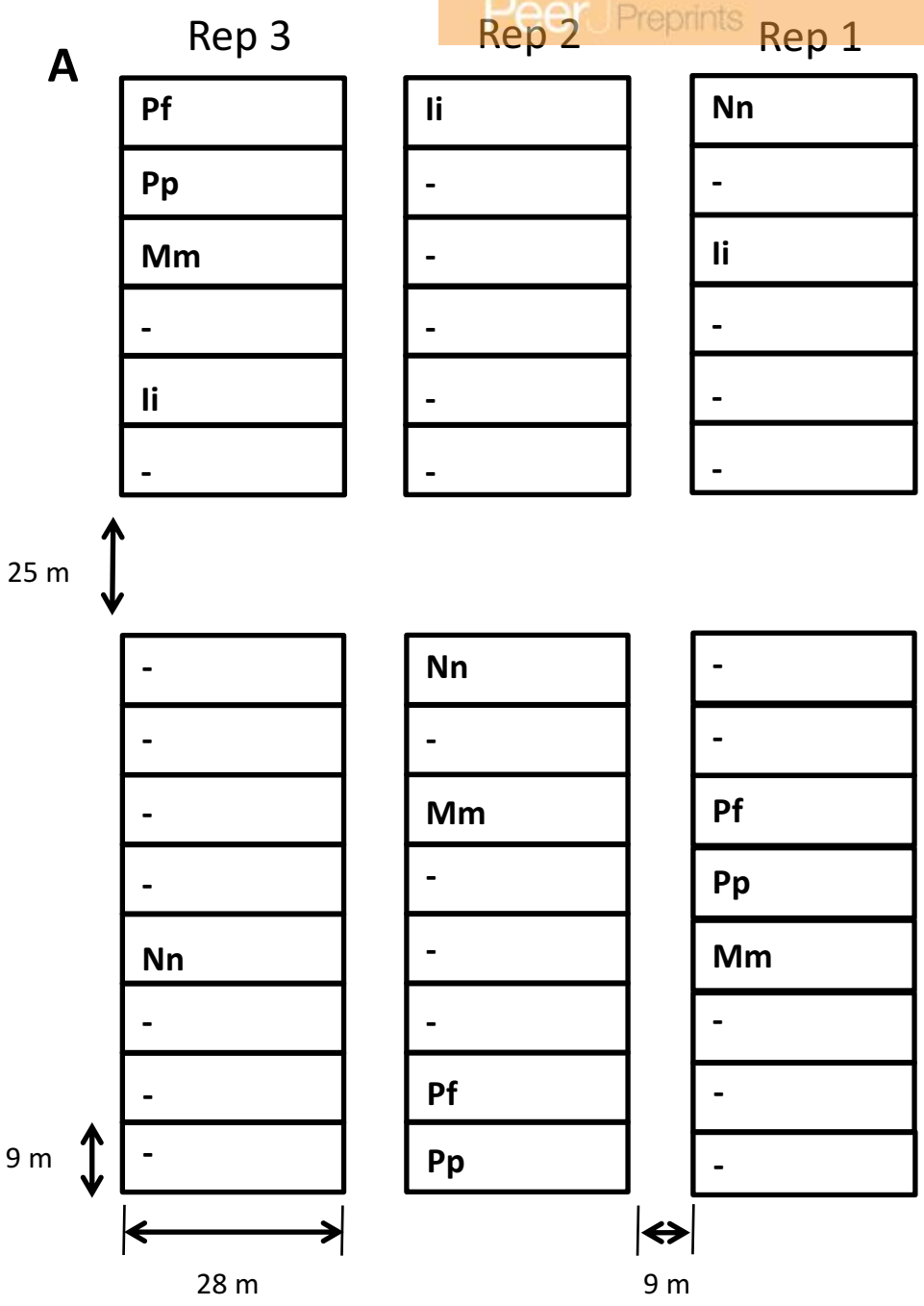
Zhao X, Xue J-F, Zhang X-Q, Kong F-L, Chen F, Lal R, and Zhang J-L. 2015. Stratification and storage of soil organic carbon and nitrogen as affected by tillage practices in the North China Plain. *PLoS ONE* 10:0128873.

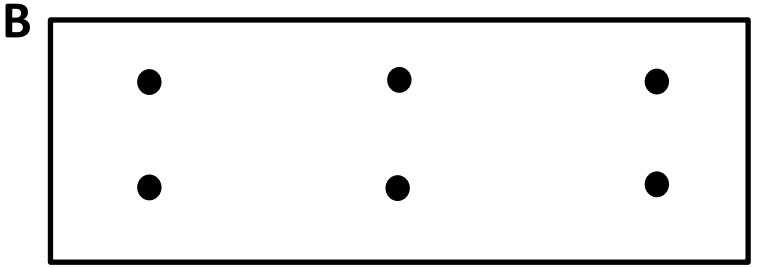
480

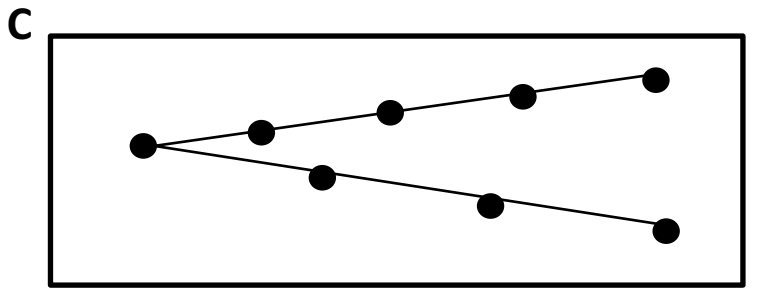
Figure 1 (on next page)

Map of the field plot trial (located: Lat. 43°40’03”S, Long. 172°28’05”E) and soil sampling strategy.

(A) Map of study site. Tillage treatments are (Pp) permanent pasture, (Pf) permanent fallow, (Ii) intensive tillage to 20 cm, (Mm) minimum tillage to 10 cm and (Nn) no-till. Plots labelled (-) represent a variety of treatments not investigated in the present study. All 15 plots (5 treatments x 3 replicates) were sampled twice on the same day. (B) During the first sampling event six samples were collected from each plot from a depth of 0 – 7.5 cm to provide a total of 90 samples. (C) During the second sampling event, eight sample cores were collected from each plot and cores separated into depths of 0 – 7.5 cm, 7.5 – 15 cm, 150 – 25 cm and 25 – 30 cm, before the soil from each depth was composited, providing an additional 60 samples for analysis.





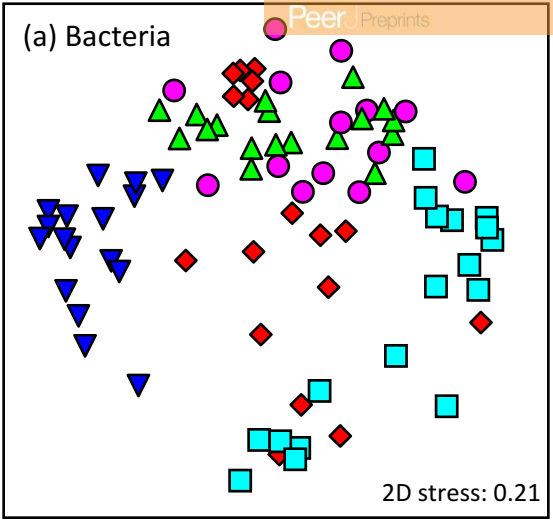


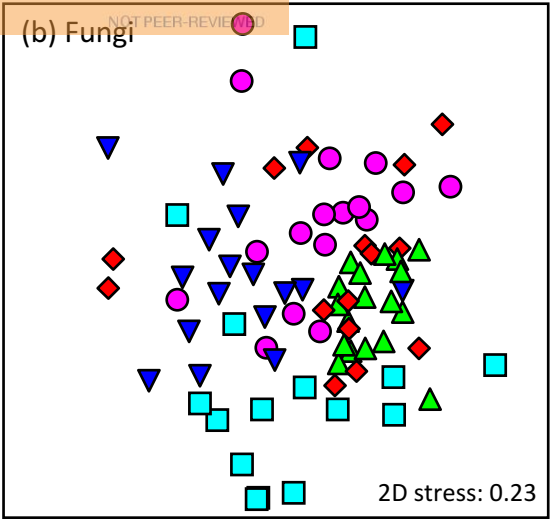
PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.3288v1 | CC BY 4.0 Open Access | rec: 26 Sep 2017, publ: 26 Sep 2017

Figure 2(on next page)

Non-metric multi-dimensional scaling plots of (a) bacteria; and, (b) fungi grouped according to treatments

Impact of tillage treatment on soil microbial community composition. Plots are non-metric multi-dimensional scaling plots of (a) bacterial; and, (b) fungal community data grouped according to treatments: (triangle-down) Permanent pasture, (square) Permanent fallow, (diamond) Intensive tillage, (triangle-up) Moderate tillage, (circle) No-till. The scaling is based on a Bray-Curtis similarity matrix of ARISA profiles. All data are from soil samples of 0 – 7.5 cm depth. 2D stress values are 0.21 and 0.23 for bacterial and fungal data, respectively. PERMANOVA revealed significant treatment effects for both bacterial and fungal communities ($p < 0.001$).





Permanent pasture, no-till
Permanent fallow, no-till
Cropping, intensive till
Cropping, moderate till
Cropping, no-till

Figure 3(on next page)

Non-metric multi-dimensional scaling plots of (a) bacteria; and, (b) fungi grouped according to treatment and sampling depth.

Impact of crop management on microbial community composition measured at different soil depths. Plots are non-metric multi-dimensional scaling plots of (a) bacterial; and, (b) fungal community data grouped according to treatments (triangle-down) Permanent pasture, (square) Permanent fallow, (diamond) Intensive tillage, (triangle-up) Moderate tillage, (circle) No-till. The scaling is based on a Bray-Curtis similarity matrix of ARISA profiles. The trajectory shows the movement of data points related to depth (1) 0 – 7.5 cm, (2) 7.5 – 15 cm, (3) 15 – 25 cm, (4) 25 – 30 cm for average data from each treatment. 2D stress values are 0.09 and 0.16 for bacterial and fungal data, respectively. The significance (PERMANOVA p values) of differences related to treatment (Tr), sample depth (De) and their interaction (Tr x De) are shown on each plot.

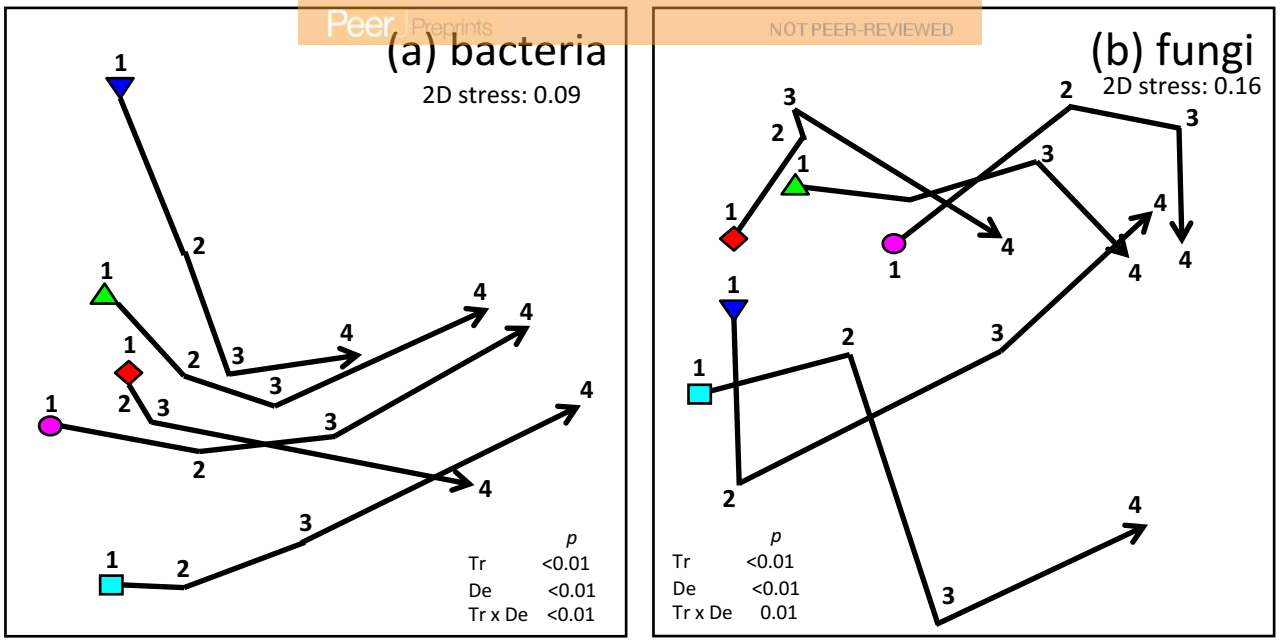
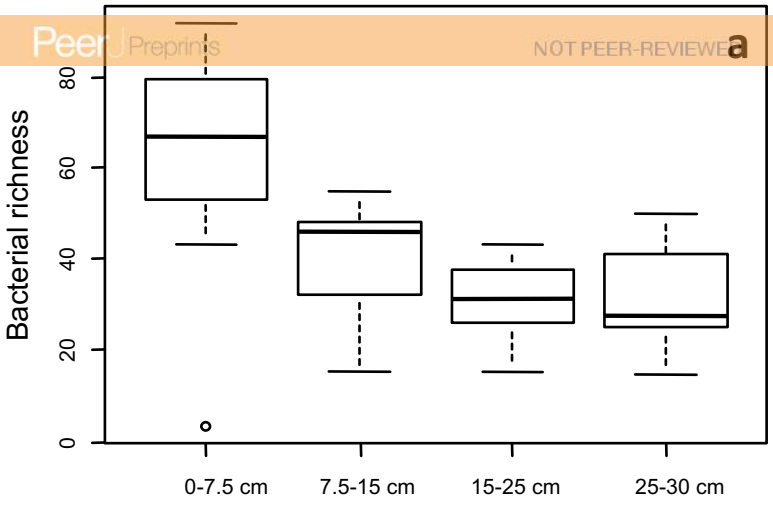
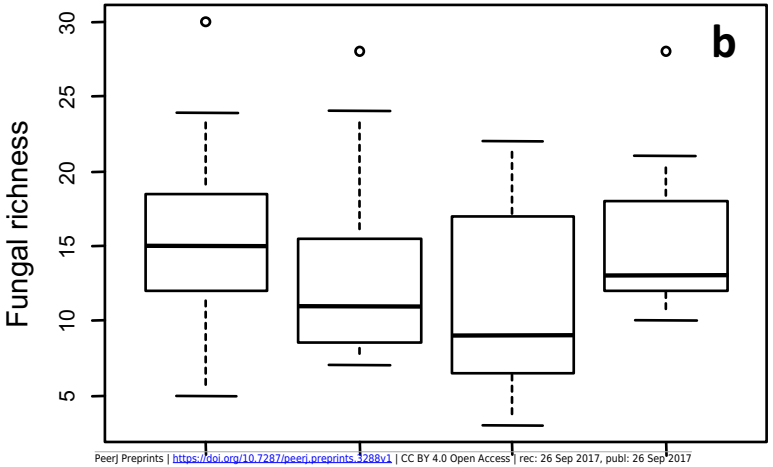


Figure 4(on next page)

Taxon richness of (a) bacteria and (b) fungi as a function of soil depth.

Median values are represented by a thick line in each box, and whiskers represent 1.5 x the interquartile range.





Peerj Preprints | https://doi.org/10.7287/peerj.preprints.3288v1 | CC BY 4.0 Open Access | rec: 26 Sep 2017, publ: 26 Sep 2017

Table 1(on next page)

Mean values (± S.E.) for soil chemistry variables and significance from two-way ANOVA (N = 45 for all comparisons; soil chemistry data were not generated for 25-30 cm] samples).

Different letters (a, b, c, d) indicate significantly different treatment effects using Tukey's Honestly significant difference multiple comparison tests. Treatments are as follows: Pp = Permanent pasture, Pf = Permanent fallow, Ii = Intensive tillage, Mm = Moderate tillage, Nn, = No-till. Depths are as follows: t = top (0-7.5 cm), m = middle (7.5-15 cm) and b = bottom (15-25 cm).

Variable	Unit	Mean	± S.E.	Treatment *P*	Depth *P*	Interaction *P*	Treatment rank*	Depth rank*
Total C	g kg^{-1}	21.9	± 0.09	<0.001	<0.001	<0.001	^{a}Pp>^{b}Mm>^{b}Nn>^{b}Ii>^{c}Pf	^{a}t>^{b}m>^{c}b
Total N	g kg^{-1}	1.90	± 0.01	<0.001	<0.001	<0.001	^{a}Pp>^{b}Mm>^{b}Nn>^{b}Ii>^{c}Pf	^{a}t>^{b}m>^{c}b
MWD	mm	1.30	± 0.09	<0.001	<0.001	0.015	^{a}Pp>^{b}Nn>^{b}Mm>^{c}Ii>^{d}Pf	^{a}t>^{b}m>^{c}b
MBC	µg g^{-1}	386.91	± 32.9	<0.001	<0.001	<0.001	^{a}Pp>^{b}Mm>^{b}Ii>^{b}Nn>^{c}Pf	^{a}t>^{b}m>^{c}b
MBN	µg g^{-1}	59.17	± 4.70	<0.001	<0.001	<0.001	^{a}Pp>^{b}Mm>^{b}Ii>^{b}Nn>^{c}Pf	^{a}t>^{b}m>^{c}b
pH		5.43	± 0.06	<0.001	<0.001	0.003	^{a}Pp>^{b}Mm>^{b}Nn>^{b}Ii>^{c}Pf	^{a}m>^{a}t>^{b}b
Moisture	%	21.03	± 0.44	<0.001	<0.001	<0.001	^{a}Nn>^{a}Ii>^{a}Mm>^{a}Pf>^{b}Pp	^{a}t>^{b}m>^{c}b
Exch. acid	$cmol_c kg^{-1}$	0.53	± 0.05	<0.001	0.822	0.217	^{a}Pf>^{bc}Ii>^{bc}Nn>^{bc}Mm>^{c}Pp	^{a}b>^{a}m>^{a}t
Exch. al	$cmol_c kg^{-1}$	0.34	± 0.04	<0.001	0.267	0.178	^{a}Pf>^{bc}Ii>^{bc}Nn>^{bc}Mm>^{c}Pp	^{a}b>^{a}m>^{a}t

*Means for each level of treatment and depth were ranked from highest to lowest