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

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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.

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

3

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11

12

13 **Abstract**

14 In arable cropping systems, reduced or conservation tillage practices are linked with improved
15 soil quality, C retention and higher microbial biomass, but most long-term studies rarely focus on
16 depths greater than 15 cm nor allow comparison of microbial community responses to
17 agricultural practices. We investigated microbial community structure in a long-term field trial
18 (12-years, Lincoln, New Zealand) established in a silt-loam soil over four depth ranges down to
19 30 cm. Our objectives were to investigate the degree of homogenisation of soil biological and
20 chemical properties with depth, and to determine the main drivers of microbial community
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22 depth, observed by a homogenisation of microbial community composition within the tilled
23 zone. Tillage treatments were mouldboard plough and disc harrow, impacting soil to ~20 and
24 ~10 cm depth, respectively. These treatments were compared to a no-tillage treatment and two
25 control treatments, both permanent pasture and permanent fallow. Bacterial and fungal

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

33

34 **Introduction**

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

44

45 The impacts of soil management on microbial diversity and functioning are still under
46 investigation. Crop residues and root exudates are the main sources of soil C (Gougoulias et al.
47 2014) with tillage distributing these C-sources deeper into the soil and altering soil structure.
48 Tillage, therefore affects microbial access to fresh C at depth, releases previously inaccessible C

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

55

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

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

77

78 **Methods**

79 **Experimental Site and Field Trial Description**

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

96 control. To balance the trial design, these plots were split into subplots; permanent pasture (Pp),
97 and permanent fallow (Pf). The Pp sub-plots were grazed with sheep (typically 10 times per year;
98 20 sheep per plot). The main fertiliser applied to the Pp plot was superphosphate. The Pf subplots
99 received no fertiliser and had no animal or vehicle trafficking throughout the trial. Herbicide
100 (Glyphosate) was used to maintain the Pf subplots plant free. Management (irrigation, fertiliser,
101 grazing regime) of the Pp plots remained the same as before the trial. All treatments (i.e. Arable
102 crops, Pp and Pf) were irrigated in summer to ensure that water was not limiting to pasture or
103 crop growth. Treatment plots were replicated three times in an incomplete Latin square (i.e. five
104 treatments x three replicate plots = 15 plots; see Fig. 1). The size of individual plots was 28 m x
105 9 m. Further trial details can be obtained from Fraser et al. (2013). The long term field trial was
106 operated by Plant and Food Research. No additional permits were required for sample collection.

107

108

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

118

119 Soil chemical analysis

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

$$130 \quad MWD = \sum_{i=1}^n x_i w_i$$

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

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

138

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

145

146 **Production and manipulation of ARISA data from extracted DNA**

147 *Automated Ribosomal Intergenic Spacer Analysis (ARISA)*

148

149 For each sample (160 in total), DNA was extracted from 0.25 g freeze-dried soil using
150 Powersoil®-htp 96 well DNA isolation kits (MoBio Laboratories Inc., Carlsbad, CA., USA)
151 following the manufacturer's instructions. Automated Ribosomal Integenic Spacer Analysis
152 (ARISA) was then used to evaluate the composition of bacterial and fungal communities in each
153 sample according the method of Lear et al. (2008). This PCR-based method characterises the
154 structure of the microbial community within each sample by recording the length (in base pairs,
155 b.p.) of the intergenic spacer (ITS) regions of the constituent microbes, i.e., between the bacterial
156 16S rRNA and 23S rRNA genes or the fungal 18S rRNA and large ribosomal subunit genes.

157

158 PCR amplification of bacterial ITS regions was undertaken on the extracted DNA using Promega
159 GoTaq® Green DNA polymerase master mix (Invitro Technologies Ltd., Auckland, New
160 Zealand) and the primers SDBact (5'-TGC GGC TGG ATC CCC TCC TT-3') and LDBact (5'-
161 CCG GGT TTC CCC ATT CGG) (Ranjard et al. 2001), with the following amplification
162 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

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

166

167 For the fungi, the PCR primers used were FunNS1 (5'- GAT TGA ATG GCT TAG TGA
168 GG -3') (Martin & Rygiewicz 2005) and 3126T (5'- ATA TGC TTA AGT TCA GCG GGT -3')
169 (Ranjard et al. 2001). PCR amplification used the Phusion® polymerase (NEB, Ipswich, MA,
170 USA) according to the manufacturer's instructions, with the following amplification conditions:
171 (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
172 °C for 20 min. The primer FunNS1 was labelled at the 5'-end with FAM (6-carboxyfluorescein)
173 fluorochrome (IDT, Asia Pacific, Singapore).

174

175 Products were each purified (Zymo DNA clean and Concentrator kit; Ngaio Diagnostics
176 Ltd., Nelson, New Zealand) and DNA concentration ($\text{ng } \mu\text{l}^{-1}$) individually determined using a
177 Nanodrop ND-100 spectrophotometer (NanoDrop Technologies, Rockland, DE). Appropriate
178 volumes of cleaned PCR product (diluted with ultrapure H₂O if necessary) providing a final
179 DNA mass of 5 to 10 ng were then transferred to a 96-well sequencing plate and dried in a
180 speedvac for 2 h at 60 °C. The dry sample was resuspended in 15 μl Hi-Di deionised formamide
181 and Genescan LIZ-1200 internal size standard (ABI Ltd.). The sample was heated (5 min, 95 °C)
182 and analysis was carried out on an ABI 3130XL genetic analyser with POP7 chemistry and a 36
183 cm array (ABI Ltd.).

184

185 Quantitative Analysis of ARISA Data

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

199

200 To visualise multivariate patterns in the soil microbial community structure among samples,
201 nonmetric multidimensional scaling (nMDS) was done using the Bray Curtis measure. Rather
202 than using multivariate analysis of variance MANOVA to test the data, which assumes normal
203 distributions, and implicitly Euclidean distances, we chose to use permutational MANOVA (or
204 PERMANOVA; Anderson, 2011) with the data assigned to the factors Treatment (Pf, Pf, Ii, Nn
205 and Mm) and Depth (0.7.5, 7.5-15, 15-25 and 35-30 cm). MVDISP was used to compare the
206 extent of multivariate data dispersion across these groups. These multivariate analyses were
207 performed using the PRIMER v.6 computer program (Clarke & Gorley 2006) with the additional
208 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

232 bacteria and fungi respectively, improving the certainty of the visual nMDS solution (Cox & Cox
233 1992). All pairwise PERMANOVA comparisons among treatments were significant for bacteria,
234 but only three (Pp-Ii, Pf-Nn, and Mm-Pf) were significant for fungi (i.e., PERMANOVA P all <
235 0.001) suggesting that bacterial communities in the surface soil (0-7.5 cm) were more sensitive
236 to tillage treatment than fungal communities. This tillage treatment effect may be because
237 bacteria tend to dominate in soils that are intensively managed, where they drive decomposition
238 and nutrient cycles (Garcia-Orenes et al. 2013). The greatest pairwise Bray-Curtis distances
239 between data representing any two treatments for both taxa were between the various no-till
240 treatments, i.e., between Pf and Pp for bacteria and between Pf and Nn for fungi. Since greater
241 average Bray-Curtis distances among data indicate greater differences in overall community
242 composition, these findings confirm that tillage as a disturbance drives microbial community
243 composition to a lesser degree than other management effects, such as the presence of permanent
244 pasture, grazing or vegetation removal. We suggest tillage treatment differences have less effect
245 because for both bacterial and fungal communities, average community similarity (i.e., average
246 Bray-Curtis distances were least) comparing the treatments Mm and Nn. For samples taken
247 across depths, two-way ANOVA showed that all soil chemical properties, except concentrations
248 of exchangeable aluminium and acidity, differed significantly by treatment depth (Table 1 and
249 Supplementary Figure S1). With the exception of soil water content, the greatest difference
250 among treatments was again between the non-till control treatments Pp and Pf, and the biggest
251 difference among depths was between 0 – 7.5 cm and 15 – 25 cm, noting that chemical data was
252 never obtained from the deepest (25-30 cm) samples. Previous research at this field site has
253 indicated that crop productivity is not influenced by tillage, neither is nutrient input (pers.
254 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,

278 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
279 number of reasons can be proposed to explain this finding. First, since most soil-dwelling fungi
280 are aerobic (Gruninger et al. 2014) is it commonly observed that they form weak depth related
281 gradients in composition compared to bacteria, which have a far greater diversity of metabolic
282 traits related to respiration (Richardson 2000). Additionally, being larger organisms, the biomass
283 of single multicellular fungi is likely to be represented at multiple soil depths (Genney et al.
284 2006), thereby exhibiting weaker depth-related gradients in composition across small spatial
285 scales. However, it remains possible that the apparent difference in bacterial and fungal
286 community treatment responses is also impacted by the choice of DNA fragments amplified,
287 which can influence both the number and composition of OTUs detected in a community (Kumar
288 et al. 2011). To address this issue, it may be desirable in future studies to assess variation in both
289 bacterial and fungal community composition using a range of genetic markers, analysed by either
290 DNA fingerprinting (Adair et al. 2013) or sequencing methods (Hermans et al. 2017).

291

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

299

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

313

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

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

337

338

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340

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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, 15 – 25 cm and 25 – 30 cm, before the soil from each depth was composited, providing an additional 60 samples for analysis.

A

Rep 3

Rep 2

Rep 1

Pf
Pp
Mm
-
li
-

li
-
-
-
-
-

Nn
-
li
-
-
-

25 m

-
-
-
-
Nn
-
-
-

Nn
-
Mm
-
-
-
Pf
Pp

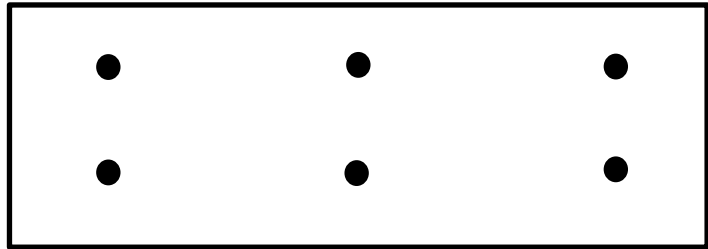
-
-
Pf
Pp
Mm
-
-
-

9 m

28 m

9 m

B



C

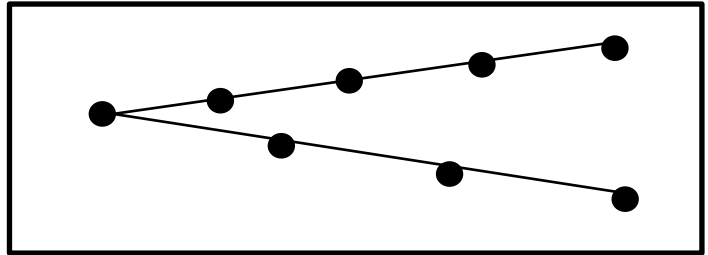
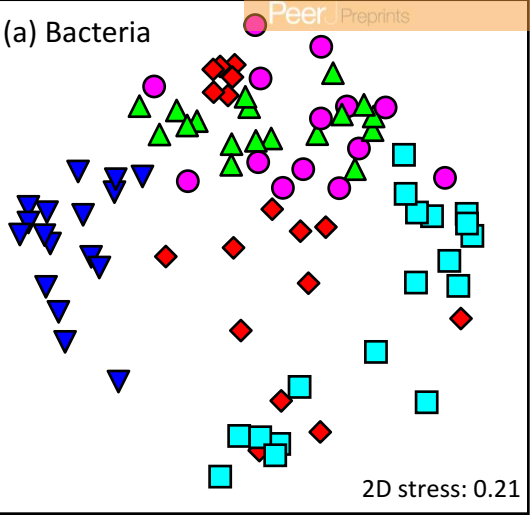


Figure 2(on next page)

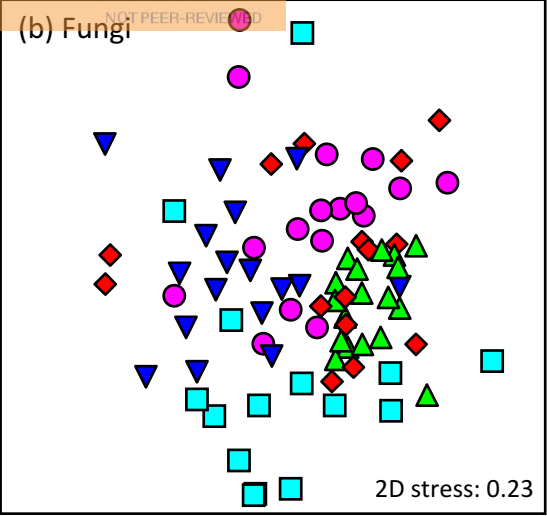
Non-metric multi-dimensional scaling plots of (a) bacteria; and, (b) fungi grouped according to treatments

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 ($p < 0.001$).

(a) Bacteria



(b) Fungi



- ▼ Permanent pasture, no-till
- Permanent fallow, no-till
- ◆ Cropping, intensive till
- ▲ Cropping, moderate till
- Cropping, no-till

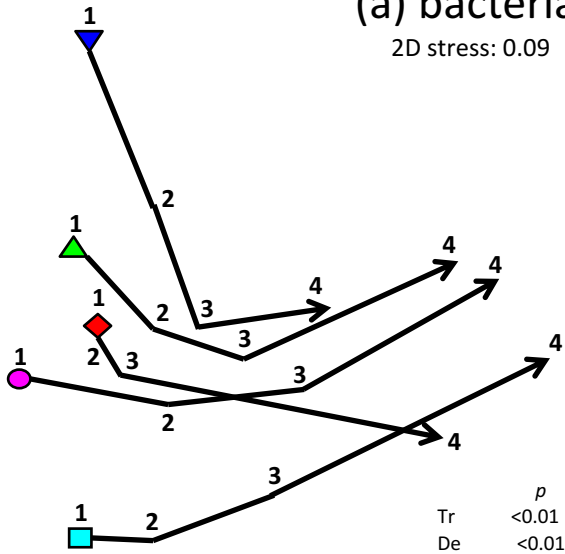
Figure 3(on next page)

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.

(a) bacteria

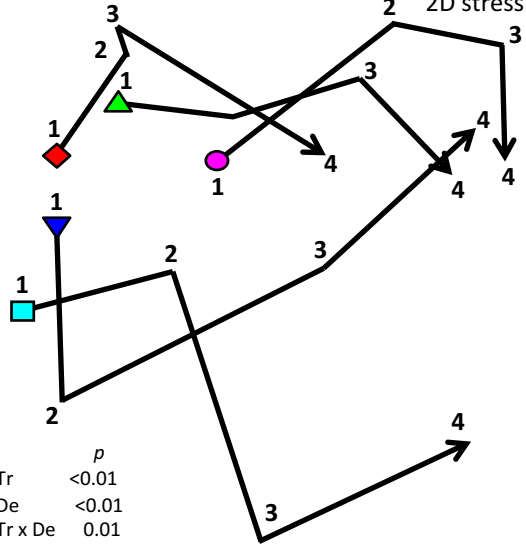
2D stress: 0.09



	<i>p</i>
Tr	<0.01
De	<0.01
Tr x De	<0.01

(b) fungi

2D stress: 0.16



	<i>p</i>
Tr	<0.01
De	<0.01
Tr x De	0.01

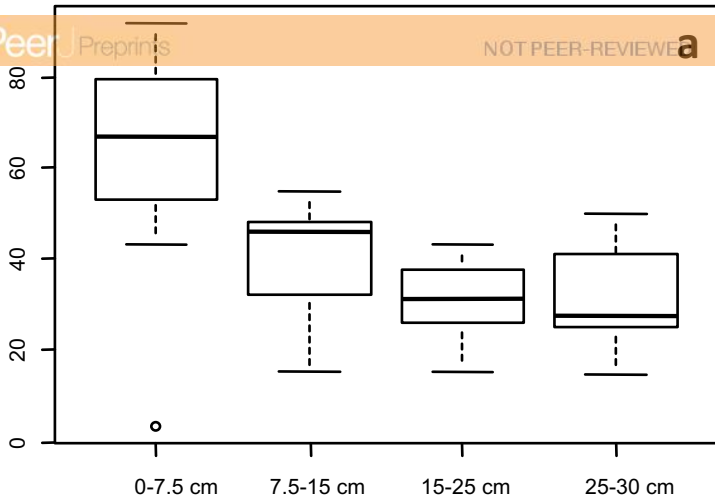
- ▼ Permanent pasture, no-till
- Permanent fallow, no-till
- ◆ Cropping, intensive till
- ▲ Cropping, moderate till
- Cropping, no-till

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.

Bacterial richness



Fungal richness

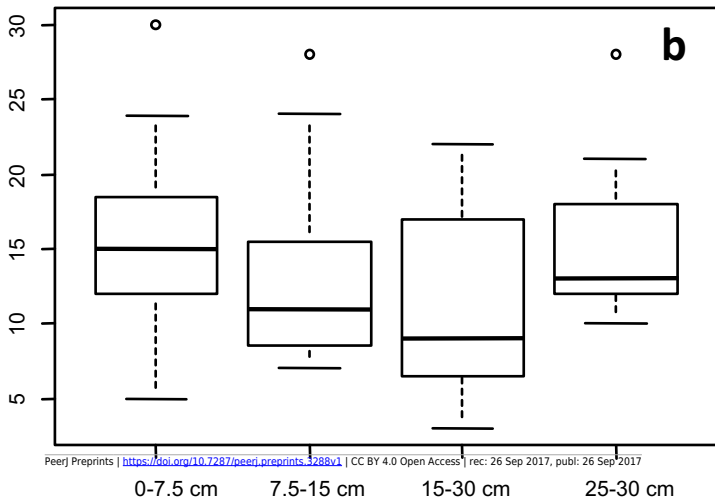
**b**

Table 1 (on next page)

Mean values (\pm 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, li = 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 <i>P</i>	Depth <i>P</i>	Interaction <i>P</i>	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

1

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

3