Low-disturbance Farming Regenerates Healthy Deep Soil

towards Sustainable Agriculture

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11 **Science for Society**

12 By 2050, how to feed 10 billion people on limited land without destroying the environment

13 has become a global challenge. Prior research has shown that low- disturbance farming

14 enhances biodiversity and natural biological processes in farmland topsoil, which benefit crop

15 production. However, whether long-term low-disturbance farming could regenerate deep-soil

16 health to meet sustainable intensification of crop production remains unclear. We evaluate the

17 impact of low-disturbance practices on 3-meter soil profile after 10-year manipulations and

18 provide an underneath mechanism on how long-term low-disturbance farming regenerate

19 deep healthy soil. We found that low-disturbance farming not only promoted soil nutrient and

20 water holding capacities, restored microbial diversity, richness, and ecological function in the

21 whole 3-m soil profile, but also improved crop production (especially during drought) and

22 potentially reduced energy consumption and greenhouse gas emissions, thus promoting

23 sustainable agriculture and ensuring food and environment security.

Summary

Intensive conventional farming has degraded farmland topsoil and seriously threaten food and environment security globally. Although low-disturbance practices have been widely adapted to restore soil health, whether this measure in a long run can potentially recover the critical deep soil to meet sustainable intensification of crop production are still unclear. Here we compared soil microbiome, physiochemical parameters along 3-m deep soil profiles, and crop yield in Northeast China subjected to ten years of farming practices at 3 levels of disturbance, including conventional tillage (CT), no-tillage without stover mulching (NTNS), and no-tillage with stover mulching (NTSM). We found that low-disturbance practices (NTNS and NTSM) promoted the ability of the deep soil to retain water and nitrogen, regenerated whole-soil microbial diversity and function, and significantly improved corn yield in the drought year. This study implies that the low-disturbance practices could dig deeper for agricultural resource and reduce energy consumption and greenhouse gas emissions, thus regenerating highly efficient, sustainable agriculture.

38   **Graphical Abstract**
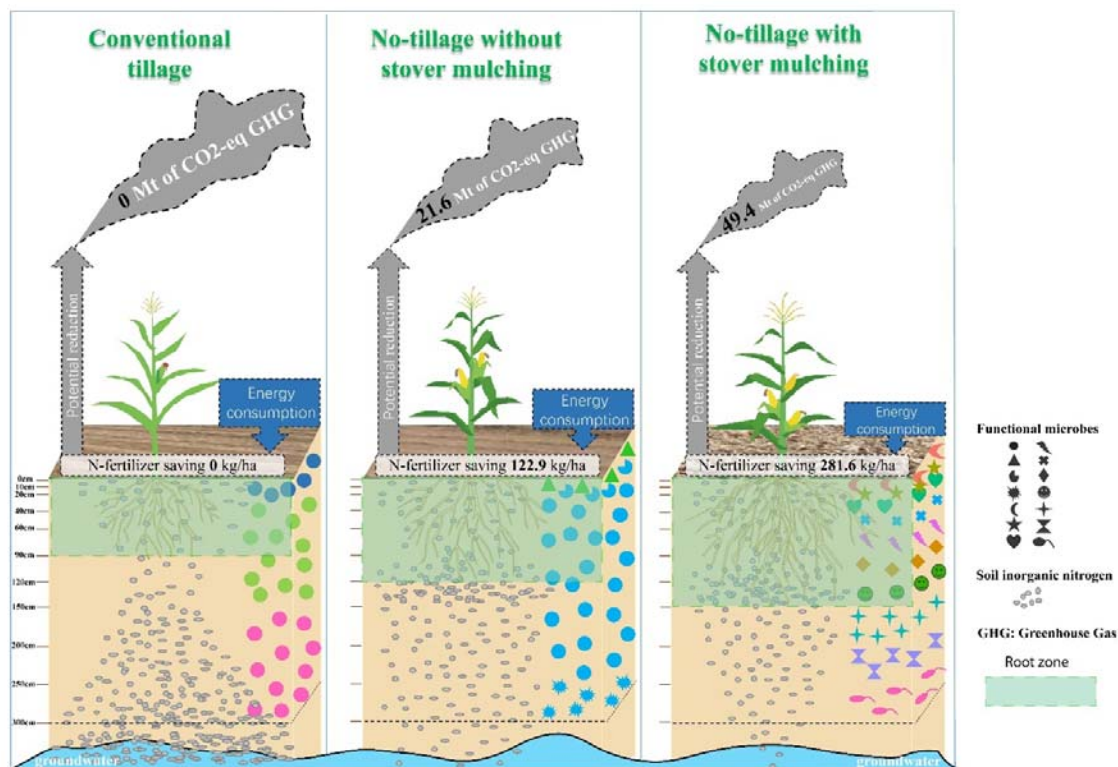
The simplified illustration shows the changes of soil inorganic nitrogen and microbes
in the soil profile and the potential impact on the environment in a low-fertility trial in Northeast China.
Similarity of microbial communities is indicated by the same color. The data in the dashed box indicates the predicted value

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40   **Keywords**: Sustainable agriculture, no-tillage, stover mulching, microbial diversity and

41   function, deep-soil resource.

Introduction

Since the Industrial Revolution, the rate of soil carbon loss has increased dramatically, resulting in a global carbon debt due to agriculture of 116 Pg carbon for the top 2 m of soil[1]. The loss of soil carbon in farmlands has not only changed global climate but also produced catastrophic cascade impacts on global food and environment security, as soil carbon is the cornerstone for healthy and productive soil that will be needed to sustainably feed 10 billion people in 2050 (United Nations, World Population Prospects 2019). It is well known that intensive conventional farms with high energy inputs (chemical fertilizers) and disturbance (e.g. tillage/compaction, burn/remove stover) have caused a series of environmental issues, like water pollution, biodiversity loss, freshwater depletion and climate change[2]. Even worse, increasing the amount of chemical fertilizer is unlikely to continue the increase in quantity and quality of food products worldwide[3], while the options to expand the farmland area at the expense of nature and biodiversity that already under pressure is limited[4]. Moreover, the topsoil disturbance, tillage in particular, prevents root growth into deeper soil[5], thus critically minimizing the exploitation of deep soil profile[6] and reducing crop's nutrient using efficiency and its resilience to drought.

Since the 1970s, low-disturbance practices (e.g. reduced tillage, no-tillage and stover mulching) have been gradually applied to restore soil health and reduce non-point source pollution[7]. Growing evidence shows that no-tillage and stover mulching boosted top-soil organic carbon (SOC)[8,9], increased soil aggregate stability[10] and reduced soil erosion and surface runoff[11]. All these benefits from low-disturbance practices are tied with complex microbial processes that interact with crops' fine roots and drive soil carbon transformation and stabilization[12,13]. Many crops' roots (depending on the species and management) can penetrate over one meter in depth, which means they can potentially forage for nutrient and water in deep soil and impact deep-soil microbes as well[4,6,14]. However, most studies by now

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67   mainly focused on farmland topsoil or soils within 1-m depth [15-17], and soil below 1 meter,

68   which belongs to Earth's Critical zone, was often overlooked. Studies in natural ecosystem

69   indicate roots in deep soils (> 1 m) can not only promote the absorption of deep nutrients and

70   water for plant growth, reduce nutrient losses, but also have effects on deep soil microbial

71   community[4,14,18], although observing and measuring deep roots remain methodological

72   challenging[4,14]. More importantly, microbes inhabiting in the deep soils (> 1 m) may

73   substantially impact long-term carbon sequestration, mineral weathering and crop

74   production[19-21], and play important roles in bridging aboveground vegetation with parent soils

75   and even acts as an essential buffer protecting underground water[22].

76   Recent research shows that corn belts in the U.S.A., western Europe, and China have

77   experienced the most soil carbon loss globally[1]. The corn belt in Northeast China is

78   considered as the "breadbasket" of the country, having the largest grain production and

79   overlapping with the most fertile Mollisol region that sustains 3% of population in the

80   world[23], accounting for over 30% of corn production of China[24]. Here, a 10-year

81   manipulative experiment was conducted at a temperate corn farm in Northeast China,

82   investigating   farming   practices   with   three   levels   of   disturbance:

83   highdisturbance—conventional tillage (CT), low disturbance—no-tillage without stover

84   mulching (NTNS) and lowest disturbance—no-tillage with 100% stover mulching (NTSM).

85   We compared soil physiochemical properties, fine-root associated organic carbon, and

86   microbial communities of the 3-m soil profiles at the end of dormant season after the 10-year

87   manipulation, and multi-year corn yield as well. We aimed at testing whether the lowest

88   disturbance practice—no-tillage with 100% stover mulching as a nature-based solution, close

89   to undisturbed natural ecosystem, would regenerate healthy deep-soil with highly diversified

90   and functional microbes over time toward a highly resilient, sustainable agricultural

91   ecosystem.

Results

Soil properties and corn yield

Soil properties varied significantly among disturbance practices and at different soil depths (Fig. 1 and Table S1). The SOC, TN and C/N ratio substantially decreased from the soil surface to around 150 cm depths and then remained unchanged within 150-300 cm (Fig. 1). The NTSM slightly increased SOC, TN and C/N ratio at 0-20 cm soil layers compared with the NTNS and the CT (Fig. 1 and Table S1). The NTSM and NTNS reduced soil pH in surface and deeper layers (Fig. 1d) and increased soil moisture at surface layers (0-60 cm) (Fig. 1e). In the CT plots, soil NO_3^--N concentration first decreased and then increased remarkably, ranged from 4.19 to 23.32 mg kg^{-1} (Fig. 1i). However, under the NTNS and NTSM treatments, soil NO_3^--N decreased significantly at 0-40 cm then increased to the maximum at 120-150 cm. Interestingly, above 120-150 cm layer, NO_3^--N was significantly higher with low-disturbance practices than conventional tillage, while the soil below 150 cm under low-disturbance practices had much lower NO_3^--N compared to conventional tillage (Fig. 1i). The NTNS plots contained much higher amounts of ammonium than the CT and the NTSM plots (Fig. 1h). Soil salt-extractable organic carbon (SEOC)—a proxy for biotically-derived organic acid declined from the surface to 40-60 cm and then increase to its peak at 60-90 cm under CT, at 90-120 cm under NTNS and at 120-150 cm under NTSM (Fig. 1b). As SEOC is a sensitive indicator of root depth and density[6], we estimate that corn roots reached up to 60-90 cm, 90-120 cm and 120-150 cm under the CT, the NTNS and the NTSM, respectively, which is in line with reported corn root depths (~150 cm) [5,25]. The NTSM increased the SEOC concentration at almost all soil layers compared with the CT and the NTNS (Fig. 1b), in which at the surface and 120-150 cm depth the contents of SEOC with NTSM were twice higher than CT. The increased SEOC in deep soils under NTSM reduced soil pH as shown by a significant negative relationship between SEOC and pH (r=–0.678,

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117     $p<0.05$). The relative contributions of SEOC to SOC (SEOC/SOC) in the NTSM were also

118   always higher than in the CT and NTNS (Fig. 1c). Based on the estimated root depths and

119   soil bulk density, total soil inorganic nitrogen available for the coming growing season in the

120   NTSM and the NTNS was approximate 427.34 and 352.34 kg ha^{-1}, respectively, while only

121   179.63 kg ha^{-1} in conventional tillage.

122   The mean annual corn yield (2013-2016) in the NTSM is 13416.8 kg/ha, which is much

123   higher than the CT and NTNS (Fig. 2), particularly during the drought year of 2015, with

124   only 409.6 mm of rainfall during the growing season (about 100 mm lower than the mean

125   rainfall), while the corn yield in NTSM is 36.4% and 22.3% higher than the CT and NTNS,

126   respectively (Fig. 2).

127   **Microbial diversity, composition, and structure.**

128   The microbial richness (Chao1), observed number of species (Observed-species) and

129   diversity (Shannon-Index) first increased within 0-20 cm and decreased from 20 to 90 cm,

130   then increased hereafter (Fig. 3). The low-disturbance practices significantly increased Chao1,

131   Observed-species and Shannon-Index, particularly in 0-40 cm soil depths (Fig. 3). There

132   were 54 microbial phyla across all soil samples. The dominant phyla (relative abundance > 1%

133   across all soil samples) were Proteobacteria, Actinobacteria, Chloroflexi, Acidobacteria,

134   Nitrospirae, Gemmatimonadetes, Planctomycetes, and these phyla accounted for 60-91% of

135   the total microbial abundances in the whole soil profile (Fig. S1a). Bacteroidetes,

136   Verrucomicrobia, Latescibacteria, Parcubacteria, Firmicutes, Microgenomates and

137   Saccharibacteria were less dominant (relative abundance > 0.1% across all soil samples) but

138   were still found across all soil samples (Fig. S1a). Although no difference in the composition

139   of dominant phyla among treatments was found, there were more non-dominant phyla with

140   higher relative abundance in low disturbance practices than conventional tillage practice (Fig.

141   S1b).

142   Indicator analysis identified 16 and 51 clearly classified genera (relative abundances >

143   0.005%) in the NTNS and the NTSM plots, respectively, while no indicator genera were

144   found in the conventional tillage plots (Fig. 4 and Table S2). The indicator genera in the

145   NTNS plots belonged to Proteobacteria, Actinobacteria, Chloroflexi, Gemmatimonadetes and

146   Planctomycetes, and most of them appeared in the surface soil (0-20 cm) with only 1 genus

147   below 150 cm. Importantly, more extra indicator genera — including Bacteroidetes,

148   Acidobacteria, Deferribacteres, Firmicutes, Verrucomicrobia, Chlorobi and Spirochaetae —

149   existed in the NTSM plots, in which under 150 cm we observed 7 genera (Fig. 4 and Table

150   S2).

151   Microbial community structures were visualized by Non-metric multidimensional scaling

152   (MDS) and tested by PERMANOVA based on Bray–Curtis. The microbial communities

153   among treatments in the root zones were marginally different (PERMANOVA p=0.08);

154   however, below the root zone they differed distinctively (PERMANOVA p=0.02). The

155   disturbance practices influenced the vertical distribution dissimilarity in microbial

156   community structure (Fig. 5). Three clusters — 0-10 cm and 10-20 cm, 20-150 cm and

157   150-300 cm — were observed in the CT plots (PERMANOVA-F=9.57, p=0.0001) (Fig. 5). In

158   the NTNS plots, 0-10 cm formed an independent cluster, while other soil depths showed

159   some separation (e.g. 20-120 cm were separated from 150-300 cm soil depths by axis 1);

160   however, Bray-Curtis distances between adjacent depths were too close to be separated

161   (PERMANOVA-F=8.18, p=0.0001) (Fig. 5). The NTSM treatment clustered 0-10 cm and

162   10-20 cm together, 120-150 cm, 150-200 cm, 200-250 cm and 250-300 cm separately, and the

163   other depths show some separations as well (PERMANOVA-F=11.32, p=0.0001) (Fig. 5).

164   **Predicted Ecological functions of microbial communities.**

165   According to the results of microbial diversity, composition and structure, the metabolic

166   capabilities of microbial community in the whole 3-m soil profiles were predicted using

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167 Tax4Fun (Fig. S2). Results showed that low-disturbance practices significantly increased the

168 abundance of predicted functions related to carbohydrate metabolism, nucleotide metabolism,

169 glycan biosynthesis and metabolism, lipid metabolism and metabolism related to cofactors

170 and vitamins (Fig. S2a). Moreover, the relative abundances of genes encoding for

171 assimilatory nitrate reduction in low-disturbance practices were higher than that in

172 conventional tillage practice (Fig. S3). The results suggested that in low disturbance practices,

173 microbial community prefer to convert the nitrate/nitrite to ammonia. We then further

174 assessed the impact of stover mulching on functional profiles (Fig. S2b). The extended error

175 bar plot shows that the NTNS enriched the abundance of amino acid metabolism and lipid

176 metabolism, while the NTSM enriched the functions associated to energy metabolism,

177 carbohydrate metabolism, biosynthesis of secondary metabolites, glycan biosynthesis and

178 metabolism as well as metabolism of cofactors and vitamins (Fig. S2b).

179 **Relationships between microbial communities and soil properties**

180 Forward selection in Redundancy analysis (RDA) revealed that soil depth (pseudo-F=48, p=

181 0.002), SOC (pseudo-F=11.5, p= 0.002), SM (pseudo-F=3.4, p= 0.012), soil pH

182 (pseudo-F=2.3, p=0.018) and soil NH_4^+-N (pseudo-F=2.7, p= 0.026) significantly affected the

183 vertical distribution of microbial communities (Fig. S4). Furthermore, the soil properties that

184 regulated the distribution of soil microbes were different under different disturbance practices.

185 Under the CT treatment, soil microbial community was mainly affected by soil NH_4^+-N

186 (pseudo-F=4, p= 0.002) and soil NO_3^--N (pseudo-F=2.3, p= 0.012) that mainly came from

187 applied fertilizer (Fig. 6). The microbial community positively correlated to soil NH_4^+-N in

188 the 0-20 cm soil, to soil NO_3^--N negatively within 20-150 cm, while to soil NO_3^--N positively

189 after 150 cm (Fig. 6). Under the NTNS treatment, soil pH (pseudo-F=3.7, p=0.004)

190 constrained the distribution of the microbial community, in which strong negative

191 correlations occurred in 0-10 cm soil and a positive correlation in 90-150 cm (Fig. 6). Under

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192   the NTSM treatment, soil TN (pseudo-F=11, p=0.002), SM (pseudo-F=2.6, p=0.004) and C/N

193   ratio (pseudo-F=1.8, p=0.016) significantly influenced the soil microbial community

194   separation (Fig. 6). In general, the microbes positively correlated with the soil TN and C/N

195   ratio in the surface soil layers (0-40 cm) and with SM in the middle layers (40-150 cm), while

196   they were mainly influenced by depth in the deeper soil (150-300 cm) (Fig. 6).

Discussion

No-tillage practices promote deep-soil health and corn yield.

199   Plant roots, fine roots in particular, release large amounts of labile organic carbon[26] that are

200   essential for healthy microorganisms in soil. Generally, most studies considered that fine

201   roots are roots < 2mm in diameter, while roots <0.2 mm in diameter can contribute to >50 %

202   of the overall root length and play a major role in releasing root exudates and absorbing

203   nutrients and waters[14]. Currently the measurement of fine roots with diameter less than 0.2

204   mm are still technically difficult and their high turnover rates *in-situ* make the measurement

205   even more complex[14]. To minimize the root-turnover effects to the most degree, we collected

206   soil samples at the end of dormant season, which can likely mirror the long-term legacy

207   effects of our practices. Also instead of detecting fine roots directly, we measured soil

208   salt-extractable organic carbon (SEOC)—a proxy for biotically-derived organic acid, which is

209   a sensitive signal of root density and could be an indicator of root depth[6]. Our results show

210   that no-tillage promotes root growth into deep soil, up to 150 cm in the NTSM. The root

211   exudates with various organic acid and dead roots likely contributed to the lower soil pH in

212   the NTNS and the NTSM, which in turn increased mineral weathering[6] and diversified the

213   microbial communities with multi-ecological functions. The increased fine roots in deeper

214   soil retained more nutrients including nutrients in dead roots and converting nitrate to more

215   stable ammonium (Fig. S3) and also provided labile carbon (Fig. 1b) to remove leaked nitrate

216   through denitrification in deeper soil (below 1.5 m), where higher relative abundance of the

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217   denitrification bacteria (*Pseudomonas* and *Caldithrix*) [27,28] (Fig. 4 and Table. S2) and

218   denitrification genes (Fig. S3) were detected in low disturbance practices — particularly in

219   NTSM. However, shallower roots in the CT treatment can't provide enough labile carbon to

220   remove extra soil NO_3^--N in deep soil, thus causing nitrite accumulation and leaching into

221   deeper soil layers. The amount of inorganic nitrogen accumulated in the root zones under

222   NTSM (427.34 kg ha^{-1}) likely could provide plenty of nitrogen for corn growth in the coming

223   growing season (Fig. 2), based on the removed nitrogen in the grain (~200 kg ha^{-1}).

224   Additionally, in line with many studies that show stover mulching reduces water evaporation

225   and surface runoff and increase soil moisture in top soils[11,29], we found that the soil moisture

226   was significantly higher in the NTSM than in the CT plots. Therefore, no-tillage with stover

227   mulching not only restores soil health by increasing the holding capacities for nutrients and

228   water, thus reducing energy input to farm, but also tended to reduce the risk of nitrate

229   leaching into groundwater. And more importantly, the healthy deep soil in turn raises corn

230   production and promote the crop resistance to drought (Fig. 2). All these are critical to the

231   development of sustainable agriculture and the associated ecosystems.

232   **No-tillage with stover mulching promotes microbial diversity, richness, and ecological**

233   **function contributing to sustainable farming**.

234   Under the CT treatment, tillage heavily disturbed the topsoil and liberated occluded organic

235   materials. Microbes tended to rapidly use available nutrients in the plowed layer (e.g.

236   NH_4^+-N)[30], thereby causing the reduction of microbial metabolic diversity (Fig. S2a). Then,

237   the resistance of the soil to stress or disturbance may also decrease[31]. In deeper soil layers,

238   due to shallower roots, NO_3^--N could quickly move downward and accumulate in deeper soil

239   (Fig. 1i), which not only contaminated the underground water but also limited the activity of

240   non-dominant microbes with important ecological functions, as no indicator genera were

241   identified for each soil depth in CT treatment (Fig. 4 and Table S2). Because the microbial

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242   communities were closely associated with inorganic nitrogen, the microbes under CT were

243   mainly influenced by added chemical fertilizer[32]. Although the dominant microbial

244   communities in CT were similar to those in the NTNS and NTSM, the loss of function

245   resulted from the difference of non-dominant microbes, indicating that the soil under CT had

246   degraded.

247   Under the NTNS treatment, soil pH was the major edaphic factor affecting the microbial

248   community and the indicator genera (Fig. 6 and Table S3). The lower soil pH possibly was

249   caused by deeper roots as shown by higher SEOC that is generally positively related to root

250   density[6]. Soil pH is often observed as a major factor determining the microbial composition

251   and structure in natural ecosystems[33,34], as microbes often show a narrow tolerance to soil pH.

252   In addition, soil pH regulates the availability of nutrient and mitigate ion toxicity[33-35]. Under

253   NTNS, soil pH and depth explained 35% distribution of the microbial community (Fig. 6).

254   We speculated that other edaphic factors (e.g. salinity and iron) directly or indirectly related

255   to soil pH and SEOC also influenced the changes in the microbial community.

256   Under NTSM treatment, TN and C/N significantly correlated with soil microbial

257   community due to the high C/N ratio of stover and roots (Fig. 6). Prior studies have reported

258   that, following maize stover mulching, more organic N, amino acid N, and amino sugar N

259   were observed in soil[36,37], which increased the retention time of nitrogen, hence meeting the

260   nutrient requirement of corn growth and reducing nitrate loss to underground water. The

261   increased available nitrogen, labile carbon and water in deep soil under NTSM can increase

262   the resilience and resistance of maize to disturbances with higher grain production (Fig. 2).

263   Zhang et al.[38] also observed litter-covered soil showed greater resistance to heating and

264   copper addition due to the changes in soil properties and microbial community structure.

265   Resistance to disturbance or stresses is the nature of a healthy soil and is essential for

266   maintaining ecosystem functions, such as decomposing organic matter[38,39]. Under the NTSM

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267 treatment, the microorganisms associated with the degradation of relatively stable carbon

268 compounds, such as Planctomycetes and Verrucomicrobia (Table S4)[40,41] as well as the

269 indicator *Cellulomonas* and *Azospirillum* (Fig. 4 and Table S2) with the function of cellulose

270 decomposition[42,43] were increased. The predicted functional profiles related to energy

271 metabolism (Carbon fixation pathways in prokaryotes), carbohydrate metabolism (TCA cycle,

272 amino sugar, nucleotide sugar, galactose, fructose), biosynthesis of secondary metabolites

273 (Carotenoid and Betalain) and glycan biosynthesis were increased, suggesting a higher

274 metabolic activity and a change in substrate quality (Fig. S2). In addition, stover mulching

275 also increased the ecological filter function of soil depth for selecting microbial communities

276 as more indicator genera of each soil depths were identified under NTSM compared to NTNS

277 and CT practices (Fig. 4 and Table S2). And these indicators residing at different soil depths

278 might enhance the anti-disturbance ability of NTSM. For example, denitrification bacteria

279 *Caldithrix* and *Pseudomonas*[27,28] were the indicator genera of 150-200 cm and 250-300 cm,

280 respectively (Fig. 4 and Table S2), which might explain the low nitrate in the deep soil in

281 NTSM. *Ignavibacteria* and *Spirochaeta,* the indicator genera of deep soil, have the ability to

282 grow under the conditions of strictly anaerobic[44] and severely limited nutrients[45], respectively.

283 Surface indicator genera belonging to Bacteroidetes might have the ability to degrade organic

284 matter that is difficult to decompose[46].

285 **Implications for climate change and food security**.

286 It was observed that about 179.63, 352.34 and 427.34 kg ha^{-1} inorganic N were kept in the

287 root-zone soil in the CT, NTNS and NTSM, respectively. Generally, corn roots reach their

288 maximum depth at the silking stage[47], which is also the time when the heaviest rainfall occurs

289 in northeastern China. We therefore expect that the available N kept in the root zone would be

290 utilized by crops in the coming growing season before the storm leach the nitrogen down to

291 ground water, which means that fertilizer N could be cut to meet crop growth in, at least,

292   Northeast China and also prevent reactive N losses. Since the nitrogen use efficiency (NUE)

293   of maize system under the conventional management is 51% in northeast China (NUE is

294   defined as the efficiency of fertilizer N transferring to harvested crop N)[48]. Then, we

295   conservatively calculate the required fertilizer N in the next year based on two assumptions: 1)

296   the NUE of soil available N in root zone is equal to that NUE of applied fertilizer N, both of

297   them are 50%; 2) the mineralized N during the coming growing season is neglected. Thus, N

298   supply requirement = Fertilizer N×NUE + N in root zone ×NUE + Stover-N, where Stover-N

299   for NTSM is 60 kg ha^{-1}. We estimated the N requirement for each disturbance practice by

300   multiplying grain yield by grain N concentration (1.4%)[48] plus multiplying stover yield by

301   stover N concentration (0.8%)[49]. For CT, NTNS and NTSM, the mean annual corn yields

302   were 10946.74, 12487.81 and 13416.81 kg ha^{-1}, and the stover yields were 966.67, 10083.33

303   and 10833.33 kg ha^{-1}, respectively. Thus, the N requirements were 230.6, 255.5 and 274.5 kg

304   ha^{-1} for CT, NTNS and NTSM, respectively. Therefore, the theoretically conservative

305   amounts of fertilizer N in the coming growing season are 281.6, 158.7 and 1.7 kg ha^{-1} for CT,

306   NTNS and NTSM, respectively. No fertilizer-N is needed to apply without reducing corn

307   yield in the NTSM plot. Compared to CT, the NTNS and NTSM could at least save

308   respectively about 122.9 and 281.6 kg ha^{-1} N-fertilizer. For every kilogram of fertilizer-N

309   produced and used on cropland, up to 87.9 MJ of energy is consumed[50] and 13.5 kg of

310   CO_2-equivalent (eq) (CO_2-eq) is emitted[51]. Hence, totally 24,752.6 MJ of energy

311   consumption could be reduced and 3,801.6 kg CO_2-eq emission could be cut per hectare

312   cornland in Northeast China at least by using NTSM tillage practice. If this could be applied

313   to all maize farmland in Northeast China (13,000,000 ha, Source: China Statistics Yearbook

314   2018), 0.3 EJ of energy could be saved and 49.4 Mt of CO_2-eq could be reduced. Based on

315   the average annual energy consumption for households of China in 2017 (15 EJ, China

316   Statistics Yearbook 2018) and CO_2 emissions (9,839 Mt, Global Carbon Atlas), the NTSM

317 practice in corn farming of Northeast China has the potential to save 2% of household energy

318 and to reduce 0.5% of CO_2 emissions each year in the whole country..

319 Our results, particularly higher SEOC content, microbial diversity, and indicator genera in the

320 NTSM deep soil compared with CT, clearly showed that low-disturbance practices can dig

321 deeper for agricultural resource over time. This indicates that crops, like corn in this study,

322 under appropriate management can ultimately explore nutrients and water from deeper soil,

323 thus not only increasing the volume of soil (almost double in this study) exploited without

324 reclaiming more natural land areas but also reducing nutrient loss into ground water.

325 Meanwhile, the input of labile carbon including SEOC into deeper soil in the NTSM provides

326 essential energy and nutrients to microbes and gradually shape highly diversified and

327 functional microbial communities in the deep soil over time, and hence improve the

328 self-sustaining ability of farmland in the face of climate change. The improvements of

329 microbial communities in the deep soil (1-3 m) at the end of dormant season in our study

330 provide evidence for the first time that a nature-based management in farmland is conducive

331 to deep-soil health for sustainable farm in a long run. Although many scientists have realized

332 the importance of deep rooting for sustainable intensification of crop production[4,14], the

333 deep-root studies are still rare due to technological bottleneck. We did a literature review on

334 whether no-tillage and straw mulching extend root depth in other crops (Fig. S5) as roots are

335 the driver for these changes. However, we can't find root-depth studies including both

336 no-tillage and straw mulching. In these studies, no-tillage as a conservative management

337 increases root depth by 23 cm, 39 cm and 14 cm in corn, wheat, and sunflower, respectively.

338 While some studies also show no effects or even reduce root depth[52,53], the possible reason is

339 the legacy effects of tillage, for example, long-term no-tillage leads to soil compaction, and

340 the soil system might be still in its transition stage. Coupled with our results, therefore,

341 low-disturbance practices as a nature-based agricultural management likely can develop a

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342   deeper root system to explore more resource in deep soil to sustain food production.

343   **Conclusion**

344   According to ecological theory[54,55], microbial community assembly in the CT treatment

345   was mainly based on deterministic processes and significantly influenced by environmental

346   stress and fertilizer nitrogen. Stover mulching might alter these processes through deeper

347   roots affecting the vertical heterogeneity in resource availability[5]. When energy resources are

348   richer in the soil, environmental stress tend to alleviate[56], and higher biodiversity was caused

349   due to more stochastic processes introduced in community assembly[57]. In view of the

350   importance of microbial community assembly in predicting ecosystem service functions, our

351   results provide underneath evidence that lowest-disturbance practice—no-tillage with stover

352   mulching promotes deep-soil health to cope with environmental stress through increasing

353   water and nutrient holding capacity, microbial richness, microbial diversity and ecological

354   functions, building up sustainable intensification of crop production. Therefore, no-tillage

355   with stover mulching is recommendable to be applied more generally to other crops globally

356   and might provide a nature-based measure to ensure the global food demand while without

357   threatening environmental security and reclaiming more lands.

358   **Experimental Procedures**

359   **Site description and soil sampling.**

360   The field experiment was established in 2007 at the Lishu Conservation Tillage Research and

361   Development Station of the Chinese Academy of Sciences in Jilin province, Northeast China

362   (43.19° N, 124.14° E). The region has a humid continental climate with a mean annual

363   temperature of 6.9 °C and the mean annual precipitation of 614 mm. The soils are classified

364   in the Mollisol order (Black Soil in Chinese Soil Classification) with a clay loam texture[58].

365   The site has been continuously planted with maize since 2007. We set up an experiment by a

366   randomized complete block design with four replicates and five treatments. Each plot area

367   was $261m^2$ ($8.7 \times 30m$). The five treatments included conventional tillage (moldboard plowing

368   to a depth around 30 cm and removed the stover), no-tillage (no soil disturbance and direct

369   seeding), and no-tillage with three-level stover mulching (33%, 67% and 100% newly

370   produced maize stover were evenly spread over the soil surface each fall). For each treatment,

371   slow-release fertilizer was applied at one time when sowing, which was equal to 240 kg/ha N;

372   47 kg/ha P; 90 kg/ha K. The rainfall data were obtained from local meteorological

373   administration. The grain yield was estimated by manually harvesting 20 m^2 area, randomly

374   taken from each plot.

375   In this experiment, in order to reduce the damage to the plots and reduce costs, 3 plots

376   were randomly taken from each treatment including conventional tillage (CT), no-tillage

377   without stover mulching (NTNS), no-tillage with 100% stover coverage (NTSM) as three

378   comparative practices. In April 2017, triplicate soil cores (0-300 cm) were collected from

379   each plot at the end of dormant season. After removing surface stover, we took soil cores by a

380   stainless-steel hand auger and sliced each into ten layers: 0-10 cm, 10-20 cm, 20-40 cm,

381   40-60 cm, 60-90 cm, 90-120 cm, 120-150 cm, 150-200 cm, 200-250 cm, 250-300 cm. In total,

382   90 soil samples were collected and transported to the laboratory within 3 hours, then passed

383   through a 2-mm sieve. All visible roots, crop residues and stones were removed. Each soil

384   sample was divided into three subsamples: one subsample for DNA extraction and soil

385   salt-extractable organic carbon (SEOC) measurement that was immediately placed into a

386   polyethylene plastic bag and stored at -80 °C, one for chemical measurements including

387   ammonium nitrogen (NH_4^+-N) and nitrate nitrogen (NO_3^--N) (within one day), and the

388   remaining one was air dried for other soil physicochemical properties.

389   **Soil properties**

390   Soil total nitrogen (TN) content was measured by an Element analyzer Vario EL III

18

391 (Elementar Analysensysteme GmbH, Hanau, Germany). Soil organic carbon (SOC) was

392 converted from soil organic matter that was measured by potassium dichromate oxidation [59].

393 Soil pH was measured in deionized free-CO_2 water (1:2.5 w/v). Gravimetric soil moisture

394 was determined by oven-drying fresh soil to a constant weight at 105 °C. Soil NH_4^+-N and

395 NO_3^--N were extracted from fresh soil by 2 M KCl and measured by a continuous flow

396 analytical system (AA3, SEAI, Germany). To reflect soil soluble, exchangeable,

397 mineral-bound OC, SEOC was extracted from the frozen soil samples with 0.5 M K_2SO_4 (1:5

398 w/v)[60,61].

DNA extraction, PCR amplification and pyrosequencing

400 Soil DNA was extracted from the frozen soil samples (0.5 g wet weight) by using MoBio

401 PowerSoil DNA isolation kit (MoBio Laboratories, Carlsbad, CA, USA) following the

402 instructions of the manufacturer. The quality of DNA was determined by 1% agarose gel

403 electrophoresis. The V3–V4 region of the bacterial 16S rRNA gene was amplified by PCR

404 using the primers 338F and 806R with barcode for Illumina MiSeq sequencing. PCR was

405 performed in a total volume of 50 μl containing 30 ng DNA as a template, 20 mol of each

406 primer, 10mM dNTPs, 5□l 10× Pyrobest buffer and 0.3 U of Pyrobest polymerase (Takara

407 Code: DR005A). Each sample was amplified for three replicates. The PCR products from the

408 same sample were pooled, checked by 2% agarose gel electrophoresis and were then purified

409 using AxyPrepDNA agarose purification kit (AXYGEN). Finally, purified PCR products

410 were sequenced on an Illumina MiSeq platform PE300 sequencer (Illumina, USA).

411 The raw sequence data were further analyzed by the following protocol. Low-quality

412 sequences with an average quality score of less than 20 were filtered by employing

413 Trimmomatic [62]. The FLASH software was used to merge overlapping ends and treat them as

414 single-end reads [63]. The non-amplified region sequences, chimeras and shorter tags were also

415 removed using Usearch and Mothur [64]. The resulting high-quality sequences were clustered

19

416   into Operational Taxonomic Units (OTUs) at 97% sequence similarity using Usearch

417   (Version 8.1.1861 http://www.drive5.com/usearch/). OTUs were then classified against the

418   Silva (Release119 http://www.arb-silva.de) database and the taxonomic information of each

419   OTU representative sequence was annotated using the RDP Classifier [65]. A total of 3,255,693

420   high-quality reads were obtained from all soil samples, which were clustered into 9,573

421   unique OTUs at a 97% sequence similarity. The Good's coverage of all the samples ranged

422   from 0.93 to 0.98, which indicates an adequate level of sequencing to identify the majority of

423   diversity in the samples.

424   **Statistical analyses**

425   Soil properties were analyzed and plotted using Sigmaplot 12.5 software. Alpha

426   diversity indices were calculated in Qiime (version v.1.8) and used to reflect the diversity and

427   richness of the microbial community in different samples. The relative abundances of

428   individual phyla in different samples were computed by R packages. The indicator analysis

429   based on genera-specific to each soil depth was conducted using indicspecies package of R

430   with 9999 permutations, and the P-values were corrected for multiple testing using qvalue

431   package of R[66]. Functional profiles of the microbial community were predicted by Tax4fun

432   (an open-source package in R) [67] and further statistical analysis was conducted by STAMP

433   using Welch's t-test [68]. Non-metric multidimensional scaling (MDS) was performed by

434   "vegan" package of R to describe differences in microbial community structure among

435   samples. Permutational multivariate analysis of variance (PERMANOVA) was employed on

436   Bray-Curtis distances to test the differences in soil microbial communities among various

437   sample groups. The redundancy analysis (RDA, Canoco 5 software) were conducted to

438   identify the correlations between microbial community composition and environmental

439   variables. ANOVA were conducted by SPSS Version 22. Percentage data were transformed

440   using arcsine square root function before ANOVA test. All statistical tests were significant at

441   p ≤ 0.05.

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Author Contributions

H.X., XZ and C.L. designed the experiment, F.D. did field and lab measurements, F.D., H.W. and C.L analyzed data and wrote the manuscript, and all the authors discussed results and commented on the manuscript.

Declaration of Interests

The authors declare no competing interests.

Sequence availability

All sequencing data that support the findings of this study have been deposited in the National Center for Biotechnology Information (https://www.ncbi.nlm.nih.gov/), in the Sequence Read Archive (SRA) database (BioProject number: PRJNA488172).

Supporting information

Supporting information may be found online in the Supporting Information section at the end of the article.

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635

636   **Figure legends**

637   **Figure 1.** Soil properties (mean± SE, n = 3) along soil depth under different practices. SOC =

638   soil organic carbon, SEOC = salt-extractable organic carbon, SEOC/SOC = ratio of SEOC to

639   SOC, SM = soil moisture, TN = total nitrogen content, C/N = ratio of SOC to TN, NH_4^+-N =

640   ammonium nitrogen, NO_3^--N = nitrate nitrogen, Inorganic N = NH_4^+-N + NO_3^--N.

641   **Figure 2.** Corn yield (line+ symbol) and annual rainfall during growing and dormant seasons

642   (bar) under different disturbance practices during 2013-2016. Error bars indicate standard

643   errors (n = 3 or 4), different letters indicate significant differences at $P < 0.05$.

644   **Figure 3.** Microbial richness (Chao1), observed number of species (Observed_species) and

645   diversity (Shannon_Index) in the CT (conventional tillage), NTNS (no-tillage without stover

646   mulching) and NTSM (no-tillage with stover mulching) plots. Error bars indicate standard

647   deviation (n = 3).

648   **Figure 4.** Indicator genera significantly ($q < 0.1$) associated with tillage practices. The size of

649   each circle represents the indicator value of a specific genus in the different soil depths. The

650   color indicates the relative abundance of each indicator genus. Taxonomic information,

651   indicator values, P-values, and q-values of all indicator genera are given in Table S2. Zero

652   indicator genera were identified in CT treatment.

653   **Figure 5.** Non-metric multidimensional scaling (MDS) ordination of soil microbial

654   community structures based on Bray-Curtis distances among soil depths at different

655   agricultural disturbance practices. Permutational multivariate analysis of variance

656   (PERMANOVA) revealed that the overall microbial community structures among soil depth

657   were significantly different at each disturbance practice. Circles, triangles and squares

658   represent CT (conventional tillage), NTNS (no-tillage without stover mulching) and NTSM

659   (no-tillage with stover mulching), respectively.

660   **Figure 6.** Redundancy analysis (RDA) of the soil microbial community originating from

661   microbial phyla constrained by soil properties under different agricultural practices. Only soil

662   variables that significantly explained variability in microbial community structure in the

663   forward selection procedure were selected to the ordination (arrows). TN, total nitrogen

664   content; C/N, a ratio of carbon to nitrogen content; NH_4^+-N, ammonium nitrogen; NO_3^--N,

665   nitrate nitrogen；SM, soil moisture. Circles, triangles and squares represent CT (conventional

666   tillage), NTNS (no-tillage without stover mulching) and NTSM (no-tillage with stover

667   mulching), respectively.

668

669   **Figure 1**
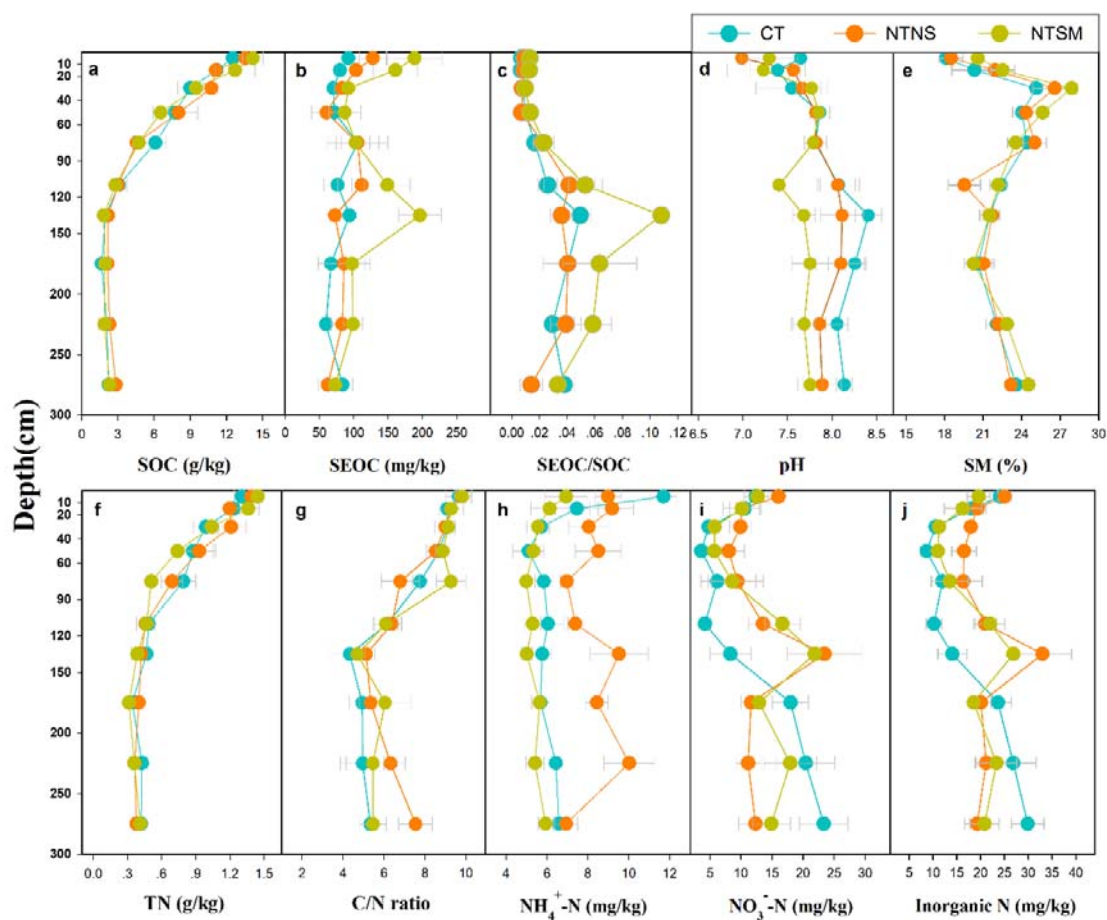
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672   **Figure 2**
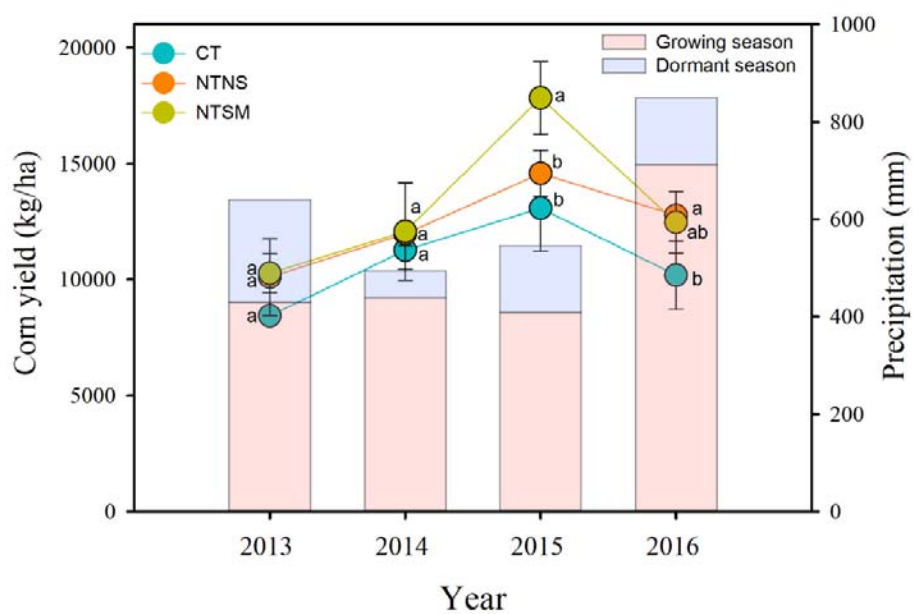
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675   **Figure 3**

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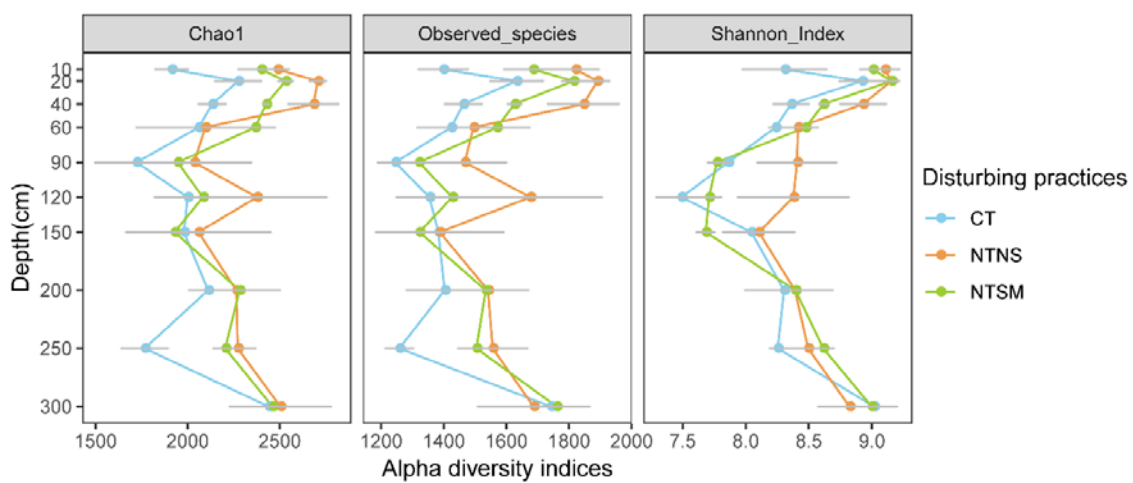
678   **Figure 4**
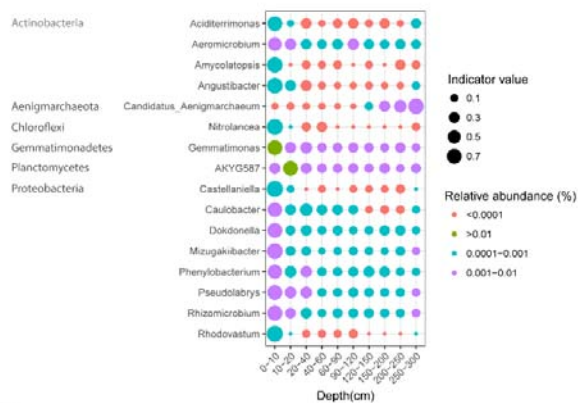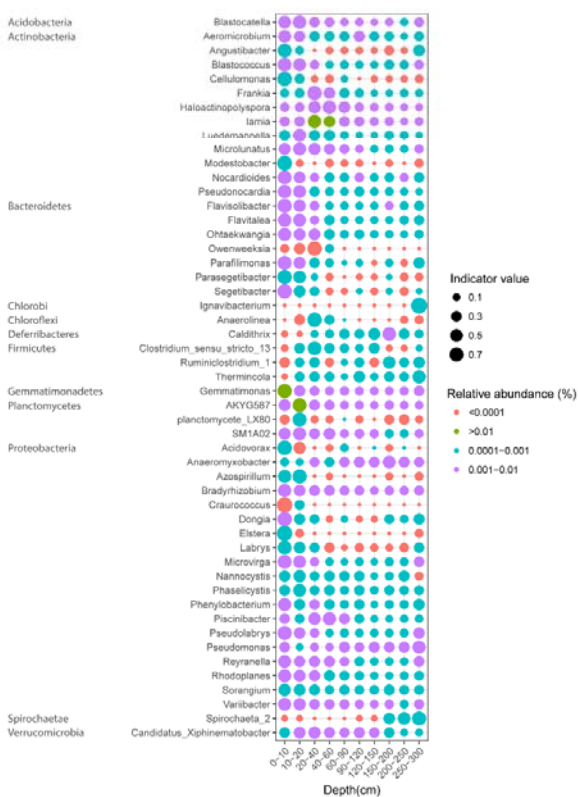
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681   **Figure 5**
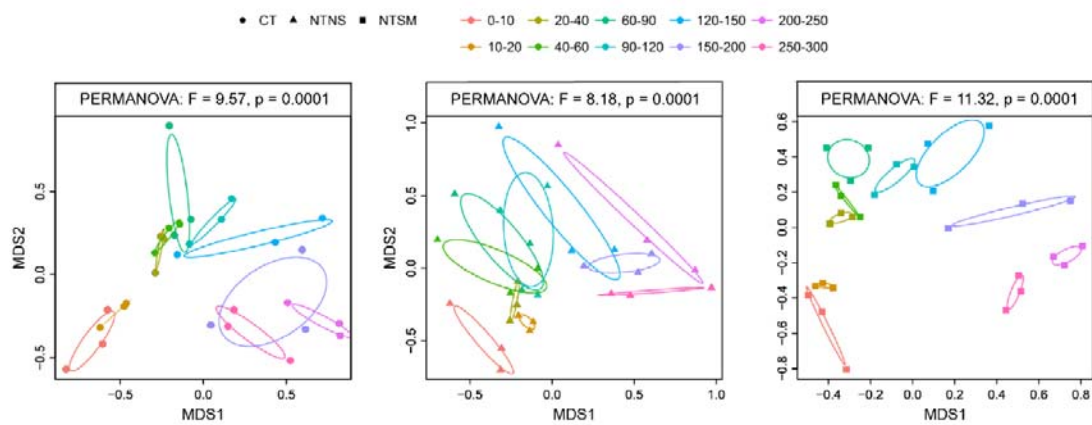
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684 **Figure 6**
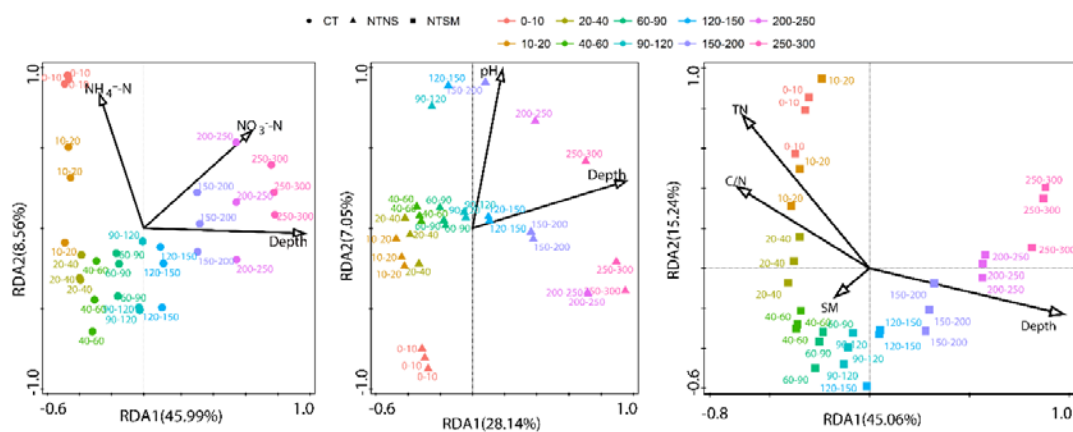
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1   # Supplemental Information

2   # Low-disturbance Farming Regenerates Healthy Deep Soil

3   # towards Sustainable Agriculture

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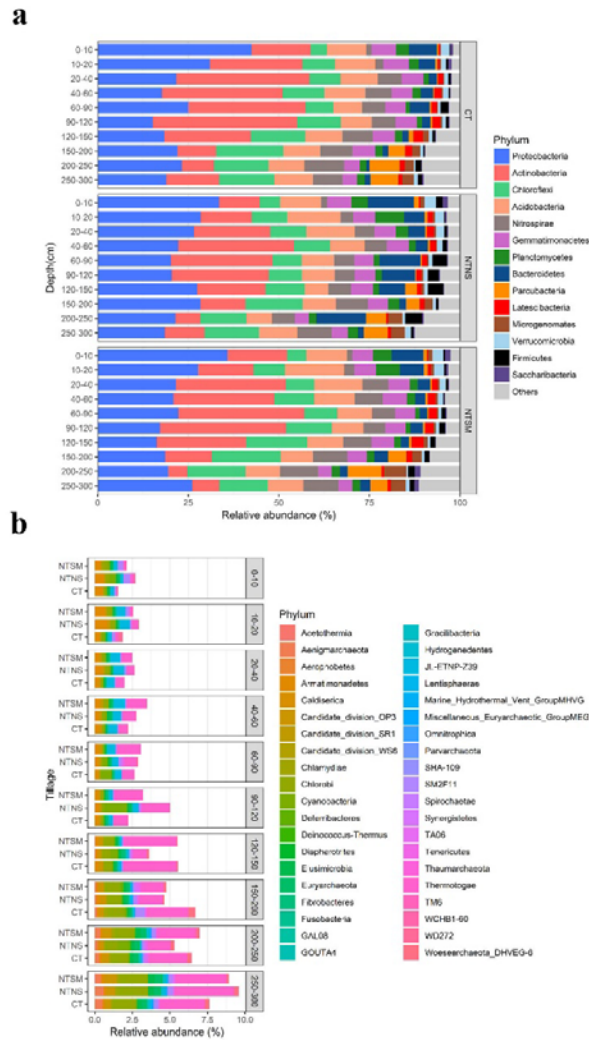
Figure S1. The relative abundance of bacterial community composition at the phylum level. **a** Only the bacterial phyla with the relative abundance > 0.1% across all soil samples were shown. **b** "Others" in the (**a**) panel represents the sum of bacterial phyla that individual relative abundance < 0.1% across all soil samples were shown. Abbreviations: CT (conventional tillage), NTNS (no-tillage without stover mulching) and NTSM (no-tillage with stover mulching).

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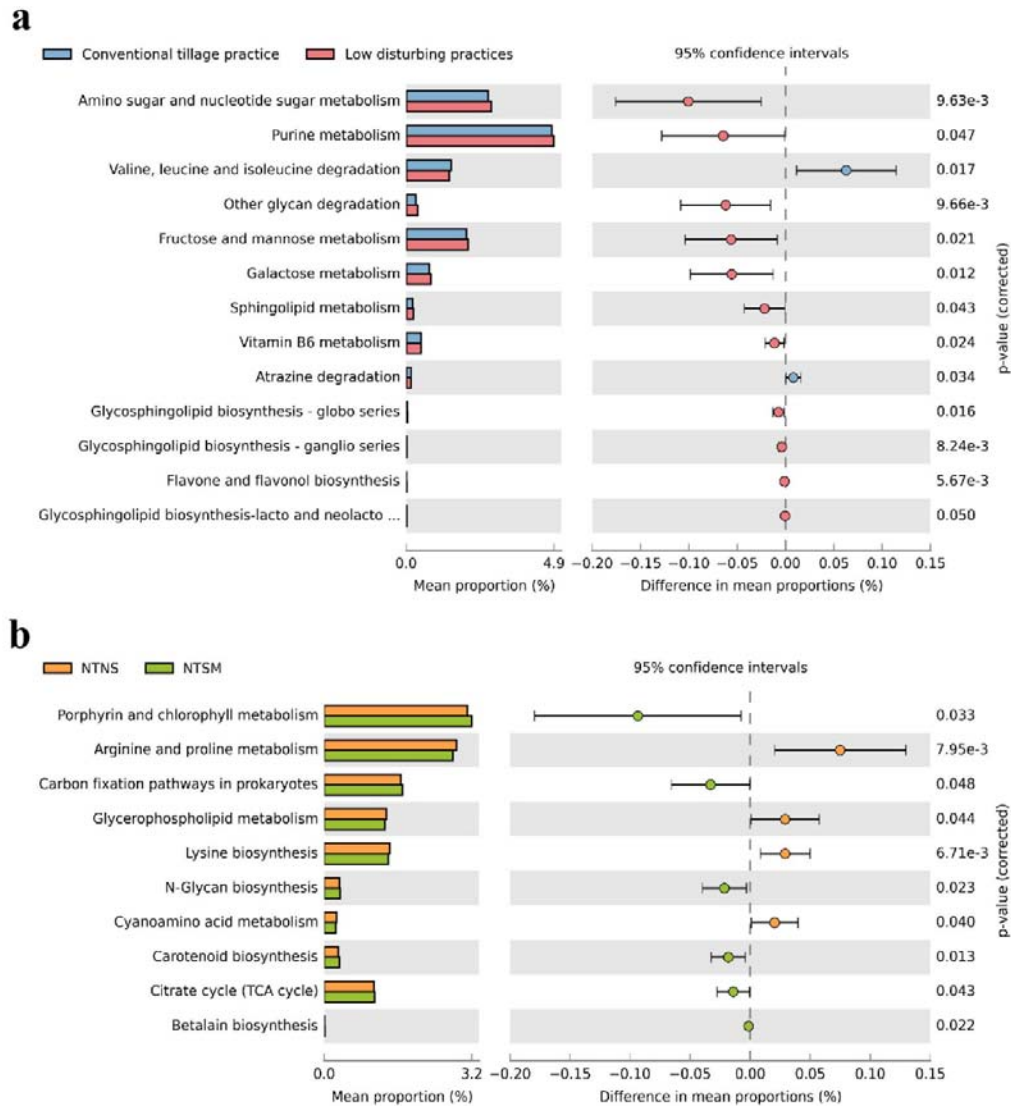
Figure S2. Extended error bar plots showing significant differences of 16S rRNA gene-predicted functional profiles obtained with Tax4Fun. **a** difference between mean proportions of conventional practice and low disturbance practices; **b** differences between mean proportions of NTNS (no-tillage without stover mulching) and NTSM (no-tillage with 100% stover mulching).

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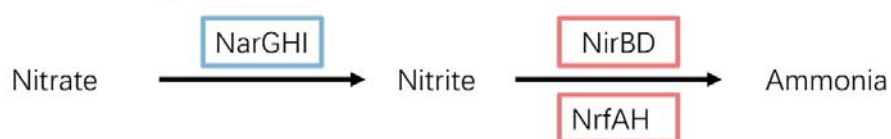
Figure S3. The denitrification and nitrification genes that influenced by different tillage practices. Genes in red rectangles means higher abundance in low disturbing practices; Genes in blue rectangles means higher abundance in conventional tillage practice.

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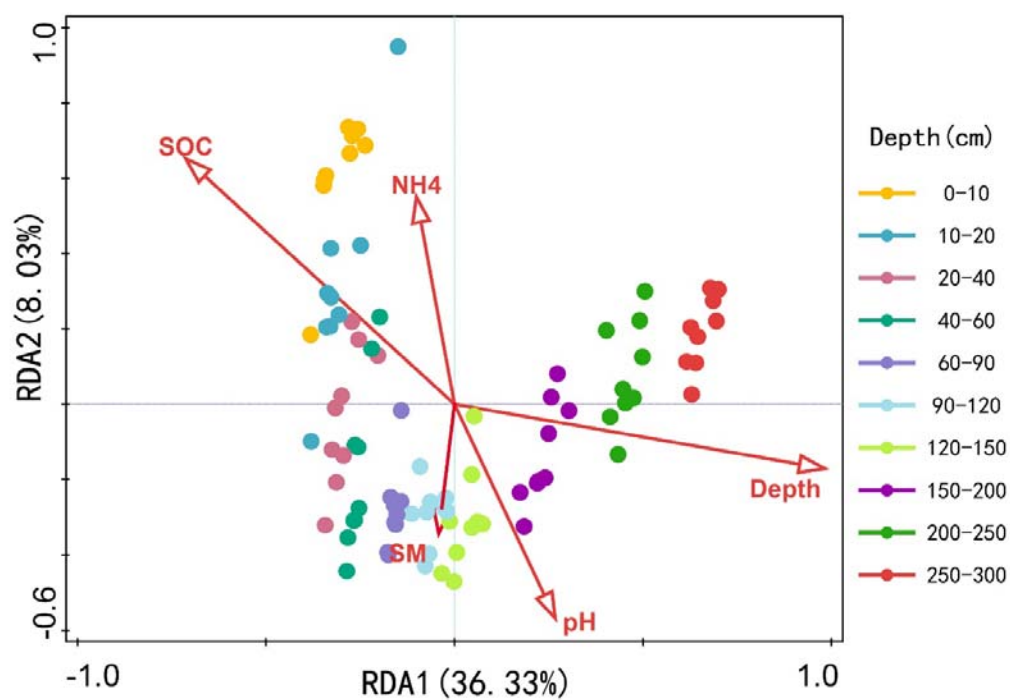
Figure S4. Redundancy analysis (RDA) of soil microbial community originating from microbial phyla constrained by soil properties among soil depths. Only soil variables that significantly explained variability in microbial community structure in the forward selection procedure were selected to the ordination (arrows). Abbreviations: SOC, soil organic carbon; NH4, ammonium nitrogen; SM, soil moisture.
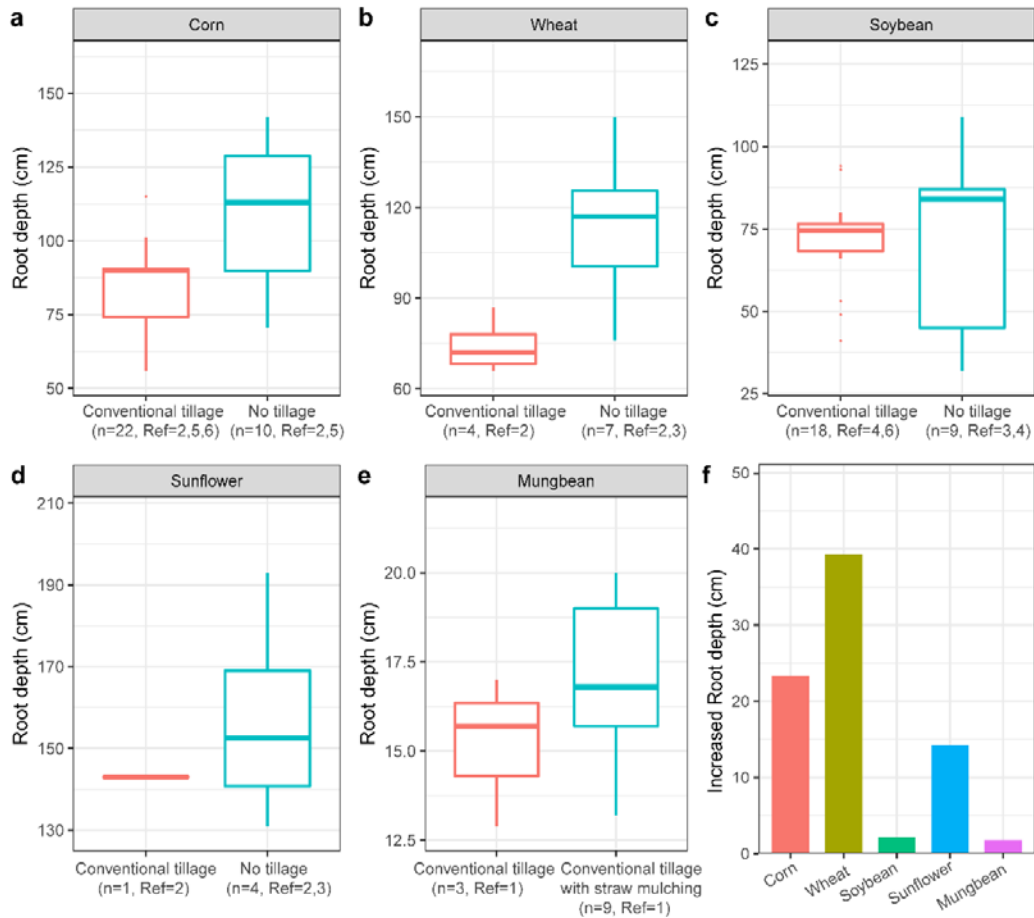
Figure S5. Root depths of different crops under different tillage practices. a, corn; b, wheat; c, soybean; d, sunflower; e, mungbean; f, the average root depth of crops under one tillage practice minus the average root depth under another tillage practice. n, numbers of observations; Ref, corresponding references number; Totally 87 observations were collected from following references: 1, Bunna et al., 2011; 2, Kemper et al., 2011; 3, Merrill et al., 2002; 4, Turman et al., 1995; 5, Dwyer et al., 1996; 6, Dwyer et al., 1988. See details in Table S5.

Table S1. Soil properties at different soil depths among disturbance practices.

Soil Properties	Tillage	Soil depth (cm)									
		0-10	10-20	20-40	40-60	60-90	90-120	120-150	150-200	200-250	250-300
SOC	CT	12.53±0.53a	11.18±0.40a	9.00±1.83a	7.72±3.16a	6.12±1.49a	3.06±1.10a	1.98±0.61a	1.66±0.16a	2.08±0.49a	2.23±0.10a
	NTNS	13.56±0.24a	11.12±0.47a	10.76±0.99a	8.03±2.80a	4.56±0.45a	3.01±1.27a	2.19±0.71a	2.14±0.28a	2.31±0.57a	2.82±0.40a
	NTSM	14.19±1.46a	12.72±2.88a	9.48±0.28a	6.55±0.28a	4.73±0.91a	2.80±0.17a	1.83±0.51a	1.94±0.86a	1.89±0.82a	2.31±0.78a
		A	B	C	D	E	F	F	F	F	F
SEOC	CT	91.96±3.52a	80.04±13.92a	70.49±7.75a	71.80±29.67a	105.57±53.39a	76.38±35.40a	93.72±11.15a	66.86±5.74a	59.60±10.62a	83.62±25.8a
	NTNS	127.96±33.79ab	103.30±50.78a	82.94±18.61a	60.26±37.99a	105.71±76.22a	111.66±60.32a	72.59±11.21a	85.71±64.89a	83.35±27.37a	62.26±19.6a
	NTSM	188.67±68.99b	160.78±56.729a	92.00±3.789a	86.77±40.429a	102.67±36.07a	149.09±58.04a	196.49±53.90b	97.68±31.36a	98.79±24.44a	73.03±14.1a
		A	ABC	BC	C	ABC	ABC	AB	BC	BC	C
SEOC/SOC	CT	0.0073±0.0003a	0.0072±0.0012a	0.0081±0.0022a	0.0098±0.0048a	0.0167±0.0054a	0.026±0.012a	0.0496±0.0129a	0.0406±0.006a	0.0294±0.0066a	0.0379±0.013a
	NTNS	0.0095±0.0027ab	0.0092±0.0041a	0.0077±0.0011a	0.0072±0.0025a	0.0223±0.0141a	0.0416±0.0318a	0.036±0.0139a	0.0405±0.0311a	0.0392±0.0191a	0.0211±0.008a
	NTSM	0.013±0.0037c	0.0126±0.0039a	0.0097±0.0002a	0.0134±0.0068a	0.0232±0.013a	0.0532±0.0209a	0.1081±0.0081b	0.0636±0.0465a	0.0587±0.0234a	0.0333±0.007a
		A	A	A	A	AB	CD	E	D	CD	BC
pH	CT	7.65±0.10a	7.39±0.53a	7.55±0.70a	7.86±0.19a	7.81±0.22a	8.07±0.40a	8.41±0.25a	8.26±0.19a	8.06±0.22a	8.14±0.15a
	NTNS	6.99±0.06a	7.57±0.08a	7.67±0.13a	7.82±0.04a	7.82±0.05a	8.06±0.34a	8.11±0.42ab	8.10±0.49a	7.86±0.55a	7.89±0.37a
	NTSM	7.30±0.77a	7.23±0.71a	7.77±0.18a	7.85±0.06a	7.79±0.03a	7.41±0.04b	7.68±0.22b	7.75±0.36a	7.69±0.22a	7.75±0.24a
		A	AB	BC	CD	CD	CD	D	D	CD	CD
SM	CT	18.14±0.75a	20.32±3.08a	25.14±0.72a	24.03±0.52a	24.40±1.44a	22.38±0.77a	21.51±1.33a	20.60±1.82a	22.07±1.48a	23.57±0.53
	NTNS	18.50±1.63ab	21.93±0.28a	26.58±3.00a	24.34±1.82a	25.02±1.56a	19.53±2.18a	21.71±0.39a	21.02±1.46a	22.17±0.80a	23.17±0.64

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	NTSM	20.58±0.64b A	22.53±1.62a BC	27.90±0.27a E	25.64±0.72a D	23.52±1.06a D	22.17±1.12a BC	21.50±0.78a BC	20.26±0.41a B	22.87±0.56a C	24.53±0.79a D
TN	CT	1.31±0.12a	1.23±0.03a	0.99±0.20a	0.88±0.31a	0.79±0.19a	0.49±0.09a	0.47±0.05a	0.34±0.06a	0.43±0.08a	0.42±0.04a
	NTNS	1.39±0.07a	1.20±0.05a	1.21±0.22a	0.93±0.25a	0.69±0.16a	0.46±0.14a	0.42±0.08a	0.40±0.05a	0.36±0.07a	0.38±0.06a
	NTSM	1.44±0.08a	1.36±0.17a	1.04±0.09a	0.74±0.03a	0.51±0.07a	0.47±0.07a	0.39±0.09a	0.31±0.03a	0.36±0.05a	0.41±0.06a
		A	B	C	D	E	F	F	F	F	F
C/N	CT	9.65±1.10a	9.09±0.14a	9.08±0.44a	8.70±0.50a	7.76±0.64a	6.16±1.23a	4.33±1.66a	4.94±1.11a	4.96±1.40a	5.34±0.33a
	NTNS	9.77±0.43a	9.27±0.32a	8.99±0.86a	8.53±0.80a	6.80±1.59a	6.39±0.77a	5.14±0.71a	5.36±0.30a	6.33±0.56a	7.54±1.43a
	NTSM	9.82±0.64a	9.28±1.06a	9.14±0.48a	8.87±0.60a	9.27±1.24a	6.09±0.99a	4.71±0.65a	6.05±2.16a	5.46±2.76a	5.46±1.12a
		A	A	A	AB	B	C	C	CD	CD	D
NH_4^+-N	CT	11.69±1.10b	7.47±1.81a	5.72±0.55a	5.11±1.33a	5.84±0.76a	6.05±1.32ab	5.77±0.80a	5.68±0.74a	6.44±0.26a	6.59±0.89a
	NTNS	8.99±1.08ab	9.18±1.84a	8.04±1.67b	8.52±1.97b	6.97±0.52b	7.39±0.64b	9.53±2.44b	8.44±0.96b	10.02±2.14b	6.94±0.99a
	NTSM	6.94±1.78a	6.13±1.59a	5.57±0.97a	5.34±0.59a	4.99±0.08a	5.3±0.68a	5.02±0.41a	5.64±0.08a	5.42±0.76a	5.93±0.63a
		A	B	BCD	BCD	D	CD	BCD	BCD	BC	BCD
NO_3^--N	CT	12.39±3.33a	10.56±4.17a	4.80±0.93a	3.61±0.86a	6.16±4.51a	4.19±1.69a	8.30±5.65a	17.98±5.06a	20.43±8.04a	23.32±6.79a
	NTNS	16.04±1.61a	10.17±1.65a	9.93±2.97b	8.03±4.36a	9.43±7.15a	13.55±4.16b	23.45±10.34b	11.63±2.74a	11.15±3.46a	12.35±4.71a
	NTSM	12.67±3.35a	10.15±5.26a	5.74±1.34a	5.71±2.04a	8.57±6.69a	16.68±4.89b	21.90±1.24ab	12.92±1.50a	17.93±7.36a	14.91±5.30a
		AB	BCD	CD	D	CD	BC	A	AB	A	A
Inorganic N	CT	24.08±2.24a	18.02±4.51a	10.53±1.09a	8.71±2.16a	12.01±4.05a	10.24±2.84a	14.07±5.26a	23.67±4.77a	26.87±8.30a	29.91±5.91a
	NTNS	25.03±2.40a	19.35±3.07a	17.97±1.37b	16.55±4.47b	16.40±6.79a	20.95±4.16a	32.97±10.59b	20.08±3.65a	21.17±4.06a	19.29±4.60a
	NTSM	19.61±4.23a	16.28±6.79a	11.31±0.65a	11.05±1.50ab	13.56±6.69a	21.99±5.33b	26.91±1.65ab	18.56±1.57a	23.34±7.40a	20.84±5.28a
		A	BC	CD	D	CD	BC	A	AB	A	A

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48   Different letters indicate significant differences at $P<0.05$. Abbreviations: SOC = soil organic carbon; SEOC = salt-extractable organic

49   carbon; SEOC/SOC = ratio of SEOC to SOC; SM = soil moisture; TN = total nitrogen content; C/N = ratio of SOC to TN; NH_4^+-N = ammonium nitrogen;

50   NO_3^--N = nitrate nitrogen ; Inorganic N = NH_4^+-N + NO_3^--N.

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52 Table S2. Taxonomic information, indicator values, P-values and q-values of indicator genera associated with tillage practices.

Land use	Phylum	genera	Soil Depth (cm)										p.value	qvalues
			0-10	10-20	20-40	40-60	60-90	90-120	120-150	150-200	200-250	250-300		
CT	0 indicator genera													
NTNS	Actinobacteria	Aciditerrimonas	0.712	0.067	0.283	0.067	0.189	0.267	0.115	0.249	0.067	0.231	0.0018	0.08426
NTNS	Actinobacteria	Angustibacter	0.816	0.295	0.295	0.098	0.070	0.070	0.098	0.070	0.070	0.120	0.0018	0.08426
NTNS	Actinobacteria	Aeromicrobium	0.572	0.341	0.274	0.210	0.280	0.348	0.243	0.194	0.262	0.206	0.0018	0.08426
NTNS	Actinobacteria	Amycolatopsis	0.803	0.000	0.147	0.104	0.147	0.000	0.104	0.000	0.254	0.147	0.0018	0.08426
NTNS	Chloroflexi	Nitrolancea	0.879	0.000	0.174	0.246	0.000	0.000	0.000	0.000	0.000	0.123	0.0018	0.08426
NTNS	Gemmatimonadetes	Gemmatimonas	0.712	0.315	0.363	0.178	0.190	0.231	0.190	0.152	0.253	0.135	0.0013	0.08426
NTNS	Proteobacteria	Caulobacter	0.673	0.276	0.434	0.265	0.262	0.200	0.085	0.147	0.135	0.110	0.0034	0.0975
NTNS	Proteobacteria	Phenylobacterium	0.643	0.359	0.342	0.189	0.184	0.236	0.305	0.285	0.154	0.130	0.0018	0.08426
NTNS	Proteobacteria	Rhizomicrobium	0.747	0.280	0.294	0.145	0.227	0.221	0.234	0.183	0.173	0.150	0.0018	0.08426
NTNS	Proteobacteria	Pseudolabrys	0.717	0.357	0.342	0.165	0.165	0.219	0.193	0.161	0.177	0.128	0.0018	0.08426
NTNS	Proteobacteria	Rhodovastum	0.890	0.000	0.118	0.118	0.118	0.167	0.000	0.000	0.000	0.000	0.0018	0.08426
NTNS	Proteobacteria	Castellaniella	0.903	0.094	0.000	0.094	0.000	0.094	0.094	0.094	0.187	0.000	0.0018	0.08426

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	Phylum	Genus												
NTNS	Proteobacteria	Dokdonella	0.725	0.209	0.289	0.162	0.223	0.132	0.153	0.256	0.248	0.121	0.0018	0.08426
NTNS	Proteobacteria	Mizugakiibacter	0.857	0.226	0.210	0.072	0.127	0.160	0.139	0.098	0.208	0.116	0.0018	0.08426
NTNS	Planctomycetes	AKYG587	0.306	0.709	0.301	0.200	0.193	0.237	0.178	0.285	0.125	0.223	0.0027	0.08426
NTNS	Aenigmarchaeota	Candidatus_Aenigmarchaeum	0.067	0.097	0.082	0.074	0.047	0.030	0.129	0.308	0.432	0.794	0.0026	0.08426
NTSM	Actinobacteria	Blastococcus	0.660	0.541	0.208	0.131	0.172	0.147	0.125	0.249	0.189	0.216	0.0115	0.09926
NTSM	Actinobacteria	Modestobacter	0.800	0.115	0.000	0.163	0.115	0.115	0.000	0.115	0.000	0.200	0.003	0.09403
NTSM	Actinobacteria	Angustibacter	0.699	0.176	0.000	0.125	0.125	0.125	0.088	0.249	0.088	0.431	0.0093	0.09454
NTSM	Actinobacteria	Cellulomonas	0.778	0.265	0.094	0.162	0.094	0.000	0.094	0.094	0.094	0.162	0.003	0.09403
NTSM	Actinobacteria	Aeromicrobium	0.546	0.381	0.288	0.275	0.263	0.376	0.249	0.231	0.102	0.249	0.003	0.09403
NTSM	Actinobacteria	Nocardioides	0.664	0.427	0.197	0.245	0.214	0.214	0.167	0.224	0.183	0.274	0.0063	0.09403
NTSM	Actinobacteria	Pseudonocardia	0.630	0.542	0.270	0.183	0.146	0.258	0.149	0.168	0.127	0.101	0.0063	0.09403
NTSM	Bacteroidetes	Ohtaekwangia	0.583	0.441	0.415	0.317	0.172	0.241	0.146	0.147	0.113	0.195	0.0086	0.09403
NTSM	Bacteroidetes	Flavisolibacter	0.717	0.481	0.226	0.147	0.150	0.129	0.133	0.160	0.180	0.260	0.003	0.09403
NTSM	Bacteroidetes	Flavitalea	0.618	0.552	0.202	0.295	0.128	0.140	0.128	0.128	0.214	0.268	0.0079	0.09403
NTSM	Bacteroidetes	Parafilimonas	0.687	0.467	0.294	0.175	0.029	0.101	0.041	0.133	0.101	0.377	0.0072	0.09403

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NTSM	Bacteroidetes	Parasegetibacter	0.742	0.540	0.089	0.125	0.000	0.072	0.072	0.051	0.162	0.145	0.003	0.09403
NTSM	Bacteroidetes	Segetibacter	0.749	0.432	0.237	0.112	0.079	0.056	0.125	0.079	0.193	0.056	0.0074	0.09403
NTSM	Gemmatimonadetes	Gemmatimonas	0.730	0.367	0.238	0.184	0.202	0.172	0.162	0.167	0.182	0.290	0.005	0.09403
NTSM	Proteobacteria	Phenylobacterium	0.573	0.357	0.286	0.295	0.190	0.290	0.104	0.195	0.195	0.380	0.0083	0.09403
NTSM	Proteobacteria	Bradyrhizobium	0.590	0.411	0.326	0.306	0.241	0.235	0.154	0.212	0.198	0.249	0.003	0.09403
NTSM	Proteobacteria	Rhodoplanes	0.566	0.440	0.368	0.346	0.252	0.218	0.133	0.126	0.168	0.241	0.0054	0.09403
NTSM	Proteobacteria	Microvirga	0.672	0.536	0.199	0.144	0.102	0.158	0.102	0.188	0.171	0.299	0.0109	0.0984
NTSM	Proteobacteria	Labrys	0.673	0.423	0.257	0.275	0.056	0.194	0.137	0.112	0.217	0.291	0.0063	0.09403
NTSM	Proteobacteria	Pseudolabrys	0.706	0.391	0.219	0.136	0.185	0.157	0.149	0.160	0.175	0.380	0.0052	0.09403
NTSM	Proteobacteria	Variibacter	0.571	0.486	0.299	0.277	0.258	0.193	0.159	0.206	0.175	0.264	0.0059	0.09403
NTSM	Proteobacteria	Craurococcus	0.882	0.272	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.009	0.09454
NTSM	Proteobacteria	Dongia	0.740	0.355	0.237	0.084	0.084	0.068	0.076	0.244	0.153	0.360	0.0096	0.09454
NTSM	Proteobacteria	Elstera	0.894	0.183	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.183	0.0072	0.09403
NTSM	Proteobacteria	Reyranella	0.551	0.373	0.364	0.355	0.234	0.248	0.154	0.163	0.151	0.329	0.0073	0.09403
NTSM	Proteobacteria	Acidovorax	0.775	0.490	0.000	0.115	0.163	0.000	0.000	0.115	0.000	0.000	0.0063	0.09403

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NTSM	Proteobacteria	Sorangium	0.491	0.417	0.341	0.209	0.287	0.231	0.231	0.333	0.251	0.251	0.0059	0.09403
NTSM	Acidobacteria	Blastocatella	0.545	0.623	0.295	0.242	0.151	0.163	0.108	0.156	0.140	0.252	0.0114	0.09926
NTSM	Actinobacteria	Luedemannella	0.372	0.608	0.386	0.399	0.175	0.101	0.101	0.238	0.189	0.101	0.0031	0.09403
NTSM	Actinobacteria	Microlunatus	0.433	0.559	0.459	0.341	0.241	0.135	0.125	0.163	0.130	0.193	0.0031	0.09403
NTSM	Planctomycetes	AKYG587	0.431	0.680	0.330	0.265	0.163	0.182	0.137	0.213	0.157	0.153	0.0031	0.09403
NTSM	Planctomycetes	SM1A02	0.449	0.568	0.318	0.406	0.173	0.223	0.189	0.183	0.169	0.179	0.0099	0.09458
NTSM	Planctomycetes	planctomycete_LX80	0.252	0.690	0.126	0.126	0.000	0.126	0.000	0.252	0.309	0.126	0.0054	0.09403
NTSM	Proteobacteria	Azospirillum	0.591	0.731	0.000	0.088	0.000	0.000	0.000	0.125	0.000	0.176	0.0079	0.09403
NTSM	Proteobacteria	Nannocystis	0.376	0.492	0.242	0.329	0.303	0.316	0.105	0.197	0.365	0.183	0.0054	0.09403
NTSM	Proteobacteria	Phaselicystis	0.340	0.555	0.248	0.328	0.203	0.304	0.175	0.263	0.226	0.248	0.0049	0.09403
NTSM	Deferribacteres	Caldithrix	0.079	0.046	0.125	0.202	0.312	0.257	0.444	0.646	0.336	0.194	0.0047	0.09403
NTSM	Firmicutes	Ruminiclostridium_1	0.302	0.220	0.302	0.191	0.078	0.246	0.220	0.505	0.246	0.405	0.0068	0.09403
NTSM	Proteobacteria	Anaeromyxobacter	0.185	0.123	0.251	0.254	0.293	0.352	0.333	0.540	0.368	0.275	0.0098	0.09454
NTSM	Actinobacteria	Iamia	0.282	0.306	0.609	0.400	0.317	0.268	0.189	0.207	0.118	0.177	0.0026	0.09403
NTSM	Actinobacteria	Frankia	0.186	0.237	0.747	0.403	0.271	0.197	0.093	0.107	0.161	0.140	0.0026	0.09403

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NTSM	Bacteroidetes	Owenweeksia	0.192	0.385	0.745	0.192	0.000	0.000	0.000	0.000	0.000	0.000	0.0102	0.09559
NTSM	Chloroflexi	Anaerolinea	0.000	0.333	0.745	0.272	0.000	0.000	0.000	0.000	0.136	0.192	0.0082	0.09403
NTSM	Firmicutes	Clostridium_sensu_stricto_13	0.108	0.324	0.675	0.296	0.216	0.153	0.350	0.076	0.076	0.076	0.0071	0.09403
NTSM	Verrucomicrobia	Candidatus_Xiphinematobacter	0.311	0.423	0.472	0.334	0.251	0.353	0.292	0.292	0.108	0.117	0.0094	0.09454
NTSM	Chlorobi	Ignavibacterium	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1.000	0.0035	0.09403
NTSM	Firmicutes	Thermincola	0.055	0.257	0.300	0.251	0.077	0.414	0.134	0.300	0.232	0.593	0.0068	0.09403
NTSM	Proteobacteria	Pseudomonas	0.312	0.101	0.164	0.143	0.343	0.235	0.344	0.314	0.346	0.579	0.01	0.09462
NTSM	Spirochaetae	Spirochaeta_2	0.058	0.058	0.000	0.000	0.000	0.058	0.058	0.404	0.535	0.714	0.0104	0.09654
NTSM	Actinobacteria	Haloactinopolyspora	0.203	0.224	0.498	0.601	0.353	0.204	0.162	0.163	0.115	0.116	0.0048	0.09403
NTSM	Proteobacteria	Piscinibacter	0.298	0.237	0.499	0.586	0.351	0.240	0.117	0.113	0.148	0.161	0.0114	0.09926

53

54   Table. S3 Spearman correlations between indicator genera and soil properties.

Land use	Phylum	genera	Depth	SOC	SEOC	SEOC/SOC	pH	SM	TN	C/N	NO_3^--N	NH_4^+-N
CT	NA											
NTNS	Actinobacteria	Aciditerrimonas	-0.222	0.192	0.314	0.067	-0.113	-0.263	0.148	0.209	0.079	0.022
NTNS	Actinobacteria	Angustibacter	-0.554**	0.463**	0.203	-0.515**	-0.516**	-0.205	0.475**	0.461*	0.099	0.137
NTNS	Actinobacteria	Aeromicrobium	-0.536**	0.271	0.311	-0.135	-0.485**	-0.206	0.29	0.246	0.131	0.261
NTNS	Actinobacteria	Amycolatopsis	-0.241	0.242	0.109	-0.282	-0.422*	-0.171	0.226	0.29	0.297	0.12
NTNS	Chloroflexi	Nitrolancea	-0.458*	0.513**	0.04	-0.397*	-0.43*	-0.088	0.439*	0.543**	0.213	0.141
NTNS	Gemmatimonadetes	Gemmatimonas	-0.597**	0.402*	0.376*	-0.165	-0.38*	-0.317	0.39*	0.357	0.128	0.31
NTNS	Proteobacteria	Caulobacter	-0.696**	0.671**	0.231	-0.508**	-0.453*	-0.087	0.659**	0.648**	0.14	-0.018
NTNS	Proteobacteria	Phenylobacterium	-0.675**	0.4*	0.103	-0.341	-0.456*	-0.284	0.521**	0.357	0.303	0.34
NTNS	Proteobacteria	Rhizomicrobium	-0.51**	0.277	0.145	-0.25	-0.294	-0.29	0.288	0.333	0.357	0.251
NTNS	Proteobacteria	Pseudolabrys	-0.594**	0.39*	0.347	-0.287	-0.471**	-0.264	0.402*	0.409*	0.222	0.301
NTNS	Proteobacteria	Rhodovastum	-0.509**	0.456*	-0.042	-0.384*	-0.423*	-0.197	0.466**	0.384*	0.374*	-0.059
NTNS	Proteobacteria	Castellaniella	-0.249	0.103	0.209	0.011	-0.307	-0.485**	0.098	0.127	-0.046	0.387*
NTNS	Proteobacteria	Dokdonella	-0.437*	0.337	0.24	-0.206	-0.552**	-0.06	0.367*	0.304	-0.017	0.283
NTNS	Proteobacteria	Mizugakiibacter	-0.436*	0.248	0.313	-0.203	-0.465**	-0.192	0.212	0.352	0.142	0.249
NTNS	Planctomycetes	AKYG587	-0.444*	0.354	-0.128	-0.422*	-0.386*	0.099	0.344	0.378*	0.069	0.069
NTNS	Aenigmarchaeota	Candidatus_Aenigmarchaeum	0.57**	-0.388*	-0.24	0.068	-0.062	0.127	-0.483**	-0.173	-0.091	0.068
NTSM	Actinobacteria	Blastococcus	-0.383*	0.425*	0.148	-0.343	-0.144	-0.194	0.433*	0.319	-0.135	0.221
NTSM	Actinobacteria	Modestobacter	-0.343	0.334	0.193	-0.253	-0.254	-0.204	0.385*	0.251	-0.134	0.148
NTSM	Actinobacteria	Angustibacter	-0.039	0.17	0.245	0.015	0.059	-0.269	0.125	0.018	-0.045	0.418*
NTSM	Actinobacteria	Cellulomonas	-0.417*	0.477**	0.022	-0.463**	-0.014	-0.214	0.499**	0.361	-0.013	0.299
NTSM	Actinobacteria	Aeromicrobium	-0.67**	0.634**	0.459*	-0.391*	-0.447*	-0.197	0.615**	0.473**	-0.016	0.116
NTSM	Actinobacteria	Nocardioides	-0.466**	0.523**	0.171	-0.42*	-0.239	-0.111	0.539**	0.291	-0.051	0.373*
NTSM	Actinobacteria	Pseudonocardia	-0.7**	0.61**	0.272	-0.496**	-0.125	-0.18	0.631**	0.486**	-0.254	-0.001

NTSM	Bacteroidetes	Ohtaekwangia	−0.821**	0.786**	0.125	−0.726**	−0.183	0.101	0.812**	0.601**	−0.43*	0.03
NTSM	Bacteroidetes	Flavisolibacter	−0.453*	0.524**	0.18	−0.428*	−0.276	−0.08	0.578**	0.416*	−0.01	0.213
NTSM	Bacteroidetes	Flavitalea	−0.469**	0.563**	0.023	−0.558**	−0.163	0.02	0.653**	0.403*	−0.173	0.247
NTSM	Bacteroidetes	Parafilimonas	−0.409*	0.519**	0.122	−0.451*	−0.218	−0.046	0.501**	0.335	−0.146	0.464**
NTSM	Bacteroidetes	Parasegetibacter	−0.441*	0.449*	0.177	−0.363*	−0.269	−0.234	0.478**	0.286	−0.028	0.112
NTSM	Bacteroidetes	Segetibacter	−0.591**	0.47**	0.324	−0.32	−0.175	−0.092	0.535**	0.404*	−0.216	0.288
NTSM	Gemmatimonadetes	Gemmatimonas	−0.467**	0.619**	−0.08	−0.605**	−0.262	0.027	0.658**	0.452*	−0.032	0.291
NTSM	Proteobacteria	Phenylobacterium	−0.427*	0.589**	−0.163	−0.631**	−0.246	0.158	0.598**	0.392*	−0.129	0.346
NTSM	Proteobacteria	Bradyrhizobium	−0.785**	0.809**	0.139	−0.719**	−0.175	0.104	0.819**	0.669**	−0.446*	0.153
NTSM	Proteobacteria	Rhodoplanes	−0.778**	0.825**	0.069	−0.774**	−0.234	0.181	0.904**	0.66**	−0.427*	0.109
NTSM	Proteobacteria	Microvirga	−0.403*	0.493**	0.186	−0.378*	−0.193	−0.082	0.576**	0.263	−0.114	0.252
NTSM	Proteobacteria	Labrys	−0.47**	0.5**	0.182	−0.427*	−0.254	0.022	0.549**	0.277	−0.195	0.269
NTSM	Proteobacteria	Pseudolabrys	−0.403*	0.476**	0.077	−0.418*	−0.249	−0.105	0.578**	0.287	−0.018	0.214
NTSM	Proteobacteria	Variibacter	−0.743**	0.801**	0.062	−0.737**	−0.147	0.138	0.793**	0.681**	−0.439*	0.115
NTSM	Proteobacteria	Craurococcus	−0.58**	0.531**	0.288	−0.401*	−0.148	−0.281	0.53**	0.362*	−0.118	0.308
NTSM	Proteobacteria	Dongia	−0.305	0.365*	0.031	−0.345	−0.239	−0.265	0.35	0.28	0.006	0.335
NTSM	Proteobacteria	Elstera	−0.406*	0.493**	0.301	−0.317	−0.428*	−0.384*	0.507**	0.407*	0.166	0.416*
NTSM	Proteobacteria	Reyranella	−0.694**	0.775**	−0.023	−0.767**	−0.254	0.178	0.813**	0.614**	−0.317	0.111
NTSM	Proteobacteria	Acidovorax	−0.639**	0.62**	0.39*	−0.388*	−0.311	−0.352	0.571**	0.521**	−0.222	0.258
NTSM	Proteobacteria	Sorangium	−0.521**	0.465**	0.164	−0.372*	−0.229	−0.244	0.499**	0.305	−0.182	0.351
NTSM	Acidobacteria	Blastocatella	−0.67**	0.749**	0.053	−0.711**	−0.145	0.152	0.793**	0.561**	−0.41*	0.218
NTSM	Actinobacteria	Luedemannella	−0.647**	0.543**	0.011	−0.559**	0.128	0.211	0.602**	0.431*	−0.535**	0.028
NTSM	Actinobacteria	Microlunatus	−0.8**	0.838**	−0.004	−0.828**	0.005	0.254	0.835**	0.728**	−0.523**	0.057
NTSM	Planctomycetes	AKYG587	−0.642**	0.609**	0.088	−0.583**	−0.033	0.111	0.581**	0.472**	−0.495**	−0.014
NTSM	Planctomycetes	SM1A02	−0.796**	0.783**	0.165	−0.711**	−0.151	0.176	0.752**	0.62**	−0.391*	0.175
NTSM	Planctomycetes	planctomycete_LX80	−0.254	0.223	0.194	−0.087	−0.195	−0.12	0.255	0.133	−0.067	0.312
NTSM	Proteobacteria	Azospirillum	−0.464**	0.481**	0.236	−0.341	−0.124	−0.245	0.488**	0.288	−0.154	0.28

53

NTSM	Proteobacteria	Nannocystis	-0.466**	0.5**	0.081	-0.436*	-0.301	-0.171	0.494**	0.388*	-0.034	-0.017
NTSM	Proteobacteria	Phaselicystis	-0.368*	0.385*	-0.032	-0.399*	-0.12	-0.087	0.349	0.319	0.04	0.086
NTSM	Deferribacteres	Caldithrix	0.642**	-0.706**	-0.057	0.705**	0.185	-0.36	-0.786**	-0.525**	0.371*	-0.112
NTSM	Firmicutes	Ruminiclostridium_1	0.315	-0.257	-0.123	0.222	0.04	-0.36	-0.305	-0.268	0.054	-0.065
NTSM	Proteobacteria	Anaeromyxobacter	0.634**	-0.702**	-0.207	0.604**	-0.006	-0.299	-0.736**	-0.541**	0.496**	-0.15
NTSM	Actinobacteria	Iamia	-0.656**	0.577**	-0.124	-0.672**	0.183	0.527**	0.591**	0.503**	-0.665**	-0.211
NTSM	Actinobacteria	Frankia	-0.59**	0.605**	-0.179	-0.707**	-0.058	0.487**	0.589**	0.619**	-0.448*	-0.152
NTSM	Bacteroidetes	Owenweeksia	-0.545**	0.574**	-0.024	-0.593**	0.036	0.324	0.569**	0.482**	-0.399*	-0.018
NTSM	Chloroflexi	Anaerolinea	-0.247	0.321	-0.333	-0.515**	0.208	0.618**	0.312	0.225	-0.407*	0.103
NTSM	Firmicutes	Clostridium_sensu_stricto_13	-0.361	0.261	0.127	-0.248	0.162	0.421*	0.311	0.263	-0.219	-0.185
NTSM	Verrucomicrobia	Candidatus_Xiphinematobacter	-0.674**	0.568**	0.384*	-0.411*	0.021	0.165	0.522**	0.435*	-0.408*	0.002
NTSM	Chlorobi	Ignavibacterium	0.521**	-0.241	-0.401*	0.097	0.112	0.249	-0.201	-0.305	0.146	0.224
NTSM	Firmicutes	Thermincola	0.33	-0.195	-0.268	0.075	0.089	0.287	-0.168	-0.312	-0.092	-0.138
NTSM	Proteobacteria	Pseudomonas	0.621**	-0.539**	-0.171	0.451*	0.068	-0.169	-0.523**	-0.478**	0.386*	0.007
NTSM	Spirochaetae	Spirochaeta_2	0.73**	-0.577**	-0.306	0.458*	0.083	-0.159	-0.649**	-0.512**	0.403*	0.336
NTSM	Actinobacteria	Haloactinopolyspora	-0.611**	0.54**	-0.117	-0.632**	0.181	0.431*	0.524**	0.571**	-0.575**	-0.278
NTSM	Proteobacteria	Piscinibacter	-0.664**	0.671**	-0.105	-0.717**	0.069	0.49**	0.653**	0.668**	-0.616**	-0.135

55 SOC = soil organic carbon; SEOC = salt-extractable organic carbon; SEOC/SOC = ratio of SEOC to SOC; SM = soil moisture; TN = total nitrogen

56 content; C/N = ratio of SOC to TN; NH_4^+-N = ammonium nitrogen; NO_3^--N = nitrate nitrogen. * means p< 0.05,** means p< 0.01, *** means p<

57 0.0001. Red number means significant negative correlation and blue number means significant positive correlation.

58

59   Table S4. The relative abundances of dominant phyla at different depths within three disturbance practices.

Phylum	Tillage	Soil depth(cm)									
		0-10	10-20	20-40	40-60	60-90	90-120	120-150	150-200	200-250	250-300
Acidobacteria	CT	10.97±2.42a	11.25±2.25a	10.30±3.12a	11.24±1.46a	7.94±2.63a	8.53±0.56a	10.26±1.81b	10.21±1.42a	9.94±0.62a	10.62±0.55a
	NTNS	11.25±2.39a	14.72±1.73a	13.33±1.27a	9.66±0.89a	8.90±2.61a	9.06±2.03a	6.46±2.11a	9.16±2.28a	7.02±3.10a	10.62±1.37a
	NTSM	11.22±4.10a BC	16.39±3.96a D	13.29±1.20a CD	11.23±0.86a ABC	9.58±0.83a A	8.71±0.82a A	9.95±0.67b A	8.89±0.19a AB	9.45±0.92a A	9.94±0.53a ABC
Actinobacteria	CT	16.31±4.30a	25.59±10.25a	36.75±8.40a	33.29±7.31a	32.40±14.77a	39.85±4.54a	23.87±13.38a	10.73±4.61a	8.79±3.48a	14.59±6.34a
	NTNS	11.21±1.05a	14.14±4.86a	21.10±3.69a	31.95±1.76a	28.07±7.97a	26.81±10.20a	18.93±8.40a	12.45±5.72a	6.86±0.71a	10.98±4.99a
	NTSM	16.45±4.70a BC	15.22±0.99a CD	30.34±1.66a E	27.92±1.27a E	34.74±4.81a E	34.84±3.96a E	24.63±4.89a B	12.89±8.00a AB	5.37±0.42a A	7.51±0.78a F
Bacteroidetes	CT	7.71±2.10a	4.59±1.00a	2.20±0.94a	3.35±1.15a	5.57±6.84a	2.36±1.63a	1.77±1.08a	1.57±1.10a	0.78±0.20a	1.58±0.32a
	NTNS	12.72±4.35a	5.61±1.35a	4.58±2.47a	3.44±1.27a	11.30±14.44a	9.12±10.07a	7.25±5.20a	1.88±0.16a	13.68±20.79a	1.55±0.55a
	NTSM	8.69±0.52a C	6.62±1.02a BC	3.45±1.07a AB	2.88±0.31a AB	2.15±0.74a AB	3.51±1.46a AB	2.29±1.58a AB	3.79±2.59a AB	2.13±1.68a AB	2.73±1.29a A
Chloroflexi	CT	4.44±0.65a	8.87±1.22a	8.56±1.97a	11.52±0.99a	7.58±3.70a	12.06±2.62a	14.99±4.28ab	18.48±4.36a	15.02±1.82a	15.25±1.81a
	NTNS	5.70±0.90a	9.75±0.79a	9.99±1.50a	9.91±2.04a	8.11±3.30a	9.14±2.01a	10.80±2.25a	15.81±1.92a	12.75±7.97a	15.01±2.04a
	NTSM	5.30±0.83a A	8.63±1.11a BC	7.82±2.38a BC	10.88±1.07a C	9.11±1.05a B	12.61±2.34a C	16.89±1.40b D	19.00±1.07a E	16.04±2.28a D	13.26±2.22a D
Firmicutes	CT	0.22±0.04a	0.65±0.25a	0.68±0.23a	0.41±0.15a	2.13±2.74a	0.84±0.34a	0.69±0.31a	0.39±0.31a	1.54±1.63a	1.18±0.50a
	NTNS	1.77±2.43a	0.38±0.10a	0.77±0.63a	1.14±1.43a	3.96±5.65a	3.14±3.38a	4.24±5.72a	0.62±0.42a	4.71±5.95a	0.60±0.38a
	NTSM	0.38±0.11a A	0.48±0.12a A	0.64±0.24a AB	0.38±0.06a A	1.18±0.82a AB	1.50±0.36a AB	1.42±0.52a AB	1.23±0.59a AB	1.75±1.65a B	1.31±0.59a AB
Gemmatimonadetes	CT	6.72±0.07a	7.11±0.33a	6.03±0.86a	5.72±0.40a	5.63±1.79a	5.89±0.98a	6.15±0.27a	6.29±1.04a	4.42±0.97a	3.99±0.54a
	NTNS	6.65±1.75a	6.11±1.08a	5.85±0.48a	6.13±0.75a	5.34±2.21a	5.72±1.53a	6.08±1.74a	5.62±1.19a	3.79±1.23a	4.43±0.46a

	NTSM	5.68±0.12a A	6.10±0.37a A	5.87±0.24a A	6.67±0.72a A	5.58±1.05a A	5.62±1.21a A	6.26±0.15a A	5.29±0.59a A	3.76±0.50a B	3.83±0.73a B
Latescibacteria	CT	0.47±0.35a	0.70±0.15a	1.50±0.66a	1.92±0.16a	1.58±1.17a	2.14±0.81a	2.62±0.99a	1.92±1.03a	1.37±0.34b	1.05±0.04a
	NTNS	0.47±0.05a	1.52±0.13b	2.00±0.78a	1.70±0.43a	1.53±0.81a	1.43±0.19a	1.25±0.72a	1.41±0.28a	0.58±0.33a	0.96±0.18a
	NTSM	0.39±0.16a A	0.93±0.22a BC	1.73±0.31a D	2.25±0.41a D	2.25±0.71a D	2.38±1.08a D	3.32±2.31a D	1.50±0.56a CD	0.82±0.16ab B	1.03±0.39a BC
Microgenomates	CT	0.15±0.13a	0.34±0.27a	0.25±0.15a	0.42±0.29a	0.18±0.07a	0.50±0.41a	1.65±1.45a	2.28±1.79a	2.57±0.85a	3.15±0.33a
	NTNS	1.11±1.19a	0.56±0.27a	0.54±0.10a	0.25±0.14a	0.25±0.17a	0.47±0.34a	0.75±0.70a	2.18±0.53a	3.93±3.67a	3.77±1.33a
	NTSM	1.17±0.83a AB	0.85±0.21a AB	0.48±0.20a AB	0.60±0.12a AB	0.40±0.14a A	0.60±0.32a AB	1.10±0.71a B	2.70±0.77a C	6.04±3.86a D	4.01±0.49a D
Nitrospirae	CT	1.45±0.42a	2.12±0.10a	6.56±2.39a	7.27±2.40a	6.45±1.95a	6.53±0.93a	8.37±2.48a	8.95±1.14a	10.94±2.01a	8.23±1.64a
	NTNS	1.68±0.42a	3.49±0.30b	5.44±2.79a	5.98±1.79a	5.58±2.52a	5.49±2.08a	6.30±2.51a	8.77±1.39a	6.37±3.74a	9.44±1.77a
	NTSM	1.50±0.12a A	2.79±0.59ab B	7.18±0.76a C	7.72±0.87a C	6.33±1.32a C	5.99±0.69a C	7.86±1.61a D	9.55±1.86a D	10.50±0.77a D	9.66±2.27a D
Parcubacteria	CT	0.41±0.21a	0.50±0.06a	0.26±0.11a	0.40±0.13a	0.42±0.14a	0.55±0.10a	1.34±0.86a	4.58±4.11a	8.40±0.86a	7.68±2.24a
	NTNS	1.38±0.89a	0.82±0.30ab	0.52±0.09b	0.49±0.22a	0.40±0.24a	0.38±0.21a	3.23±4.78a	3.71±2.12a	5.57±4.43a	6.60±0.70a
	NTSM	1.04±0.14a AB	1.06±0.30b AB	0.43±0.15ab A	0.53±0.22a AB	0.38±0.02a A	0.56±0.16a AB	0.68±0.29a B	5.07±3.23a C	9.37±1.25a D	4.75±1.92a D
Planctomycetes	CT	3.59±2.72a	2.71±0.83a	1.51±0.30a	2.31±0.92a	1.09±0.49a	1.41±0.56a	1.99±0.25a	2.01±0.34a	1.76±0.21a	2.13±0.31a
	NTNS	4.52±2.72a	7.95±2.24b	3.48±1.18b	1.93±0.52a	1.62±0.82a	1.83±1.39a	1.66±0.89a	3.09±0.39b	2.16±1.47a	2.86±0.96a
	NTSM	5.14±0.41a C	6.50±1.04b D	2.19±0.52ab BC	2.27±0.52a BC	1.18±0.45a A	1.28±0.34a AB	1.74±0.45a ABC	2.15±0.34a C	2.28±0.42a ABC	2.19±0.77a C
Proteobacteria	CT	42.48±8.42a	31.01±7.20a	21.70±4.94a	17.84±2.00a	25.04±13.62a	15.23±0.20a	18.41±1.18a	22.01±2.86a	23.32±6.01a	18.93±3.75a
	NTNS	33.50±2.43a	28.43±0.73a	26.56±6.21a	22.26±4.25a	20.17±2.38a	20.42±3.04b	27.44±4.76b	28.36±11.14a	21.46±0.93a	18.58±0.86a
	NTSM	35.85±1.47a D	27.79±1.87a C	21.62±2.49a B	20.94±0.10a AB	22.32±6.42a AB	17.21±0.65ab A	16.33±0.95a AB	18.61±1.27a B	19.48±2.98a AB	26.09±2.82b AB

Saccharibacteria	CT	0.70±0.33a	0.91±1.14a	0.20±0.14a	0.13±0.06a	0.23±0.14a	0.22±0.09a	0.32±0.23a	0.21±0.12a	0.20±0.12a	0.42±0.22a
	NTNS	1.45±0.58a	0.27±0.15a	0.28±0.11a	0.32±0.25a	0.25±0.12a	0.34±0.38a	0.25±0.02a	0.10±0.04a	0.45±0.26a	0.42±0.16a
	NTSM	1.59±1.39a	0.65±0.28a	0.26±0.11a	0.23±0.11a	0.25±0.09a	0.28±0.08a	0.11±0.08a	0.19±0.01a	1.67±1.82a	1.30±1.14a
		E	BCD	AB	AB	AB	ABC	AB	A	CD	D
Verrucomicrobia	CT	2.40±1.80a	1.34±0.21a	1.16±0.11a	1.56±0.64a	0.81±0.10a	1.04±0.24a	1.01±0.35a	0.84±0.08a	0.46±0.16a	1.18±0.50a
	NTNS	3.22±1.20a	2.52±0.34b	2.28±0.40b	1.55±0.39a	1.17±0.62a	1.15±0.57a	0.90±0.36a	0.93±0.29a	0.68±0.37a	1.69±0.61a
	NTSM	3.00±0.56a	2.74±0.41b	1.67±0.33ab	1.42±0.09a	0.83±0.06a	0.98±0.10a	0.74±0.31a	0.83±0.25a	0.49±0.03a	1.10±0.61a
		E	D	CD	C	B	B	AB	AB	A	BC

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61 Different letters indicate significant differences at P<0.05.

62 Table S5 Rooting depths of crops under different tillage managements. Only crops with more than two tillage managements are retained.

ID	Reference	Crop	Tillage Practices	Straw mulching	Root depth (cm)	Location	Date
1	Bunna et al., 2011	Mungbean	Conventional	No	15.7	Takeo, Cambodia	2010, 1
		Mungbean	Conventional	Yes	17	Takeo, Cambodia	2010, 1
		Mungbean	Conventional	Yes	16	Takeo, Cambodia	2010, 1
		Mungbean	Conventional	Yes	16.8	Takeo, Cambodia	2010, 1
		Mungbean	Conventional	No	17	Kampong Thom, Cambodia	2009, 12
		Mungbean	Conventional	Yes	20	Kampong Thom, Cambodia	2009, 12
		Mungbean	Conventional	Yes	19	Kampong Thom, Cambodia	2009, 12
		Mungbean	Conventional	Yes	20	Kampong Thom, Cambodia	2009, 12
		Mungbean	Conventional	No	12.9	CARDI in Cambodia	2010, 2
		Mungbean	Conventional	Yes	13.2	CARDI in Cambodia	2010, 2
		Mungbean	Conventional	Yes	14.9	CARDI in Cambodia	2010, 2
		Mungbean	Conventional	Yes	15.7	CARDI in Cambodia	2010, 2
2	Kemper et al.,2011	Wheat	No-tillage	No	112	Mandan, ND	1988
		Wheat	Conventional	No	87	Mandan, ND	1988
		Wheat	No-tillage	No	89	Mandan, ND	1989
		Wheat	Conventional	No	69	Mandan, ND	1989
		Wheat	No-tillage	No	76	Mandan, ND	1990
		Wheat	Conventional	No	66	Mandan, ND	1990
		Sunflower	No-tillage	No	193	Mandan, ND	1992
		Sunflower	Conventional	No	143	Mandan, ND	1992
		Wheat	No-tillage	No	150	Oshkosh, WI	2008
		Wheat	Conventional	No	75	Oshkosh, WI	2008
		Corn	No-tillage	No	142	Oshkosh, WI	2009
		Corn	Conventional	No	74	Oshkosh, WI	2009

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		Corn	No-tillage	No	104	Omro, WI	2009
		Corn	Conventional	No	56	Omro, WI	2009
		Corn	No-tillage	No	122	Oshkosh, WI	2009
		Corn	Conventional	No	64	Oshkosh, WI	2009
		Corn	No-tillage	No	131	Oshkosh, WI	2009
		Corn	Conventional	No	67	Oshkosh, WI	2009
		Corn	Conventional	No	58	Wisconsin	2009
		Corn	Conventional	No	69	Wisconsin	2009
		Corn	No-tillage	No	122	Wisconsin	2009
		Corn	No-tillage	No	132	Wisconsin	2009
3	Merrill et al.,2002	Sunflower	No-tillage	No	161	Morton County, ND	1995
		Sunflower	No-tillage	No	131	Morton County, ND	1996
		Sunflower	No-tillage	No	144	Morton County, ND	1997
		Wheat	No-tillage	No	126	Morton County, ND	1995
		Wheat	No-tillage	No	125	Morton County, ND	1996
		Wheat	No-tillage	No	117	Morton County, ND	1997
		Soybean	No-tillage	No	109	Morton County, ND	1995
		Soybean	No-tillage	No	103	Morton County, ND	1996
		Soybean	No-tillage	No	86	Morton County, ND	1997
4	Turman et al.,1995	Soybean	Conventional	No	80	Portageville,MO, USA	1992, 14 May
		Soybean	Conventional	No	66	Portageville,MO, USA	1992, 15 June
		Soybean	Conventional	No	93	Portageville,MO, USA	1992, 7 July
		Soybean	Conventional	No	49	Portageville,MO, USA	1993, 12 May
		Soybean	Conventional	No	41	Portageville,MO, USA	1993, 2 June
		Soybean	Conventional	No	53	Portageville,MO, USA	1993, 21 June
		Soybean	No-tillage	No	76	Portageville,MO, USA	1992, 14 May

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		Soybean	No-tillage	No	87	Portageville,MO, USA	1992, 15 June
		Soybean	No-tillage	No	84	Portageville,MO, USA	1992, 7 July
		Soybean	No-tillage	No	45	Portageville,MO, USA	1993, 12 May
		Soybean	No-tillage	No	32	Portageville,MO, USA	1993, 2 June
		Soybean	No-tillage	No	40	Portageville,MO, USA	1993, 21 June
5	Dwyer et al.,1996	Corn	Conventional	No	80.6	Ottawa, Ontario	1988
		Corn	Conventional	No	101.3	Ottawa, Ontario	1989
		Corn	No-tillage	No	70.6	Ottawa, Ontario	1988
		Corn	No-tillage	No	91.3	Ottawa, Ontario	1989
		Corn	Conventional	No	90.6	Ottawa, Ontario	NA(Clay loam)
		Corn	Conventional	No	91.3	Ottawa, Ontario	NA(Sany loam)
		Corn	No-tillage	No	89.4	Ottawa, Ontario	NA(Clay loam)
		Corn	No-tillage	No	72.5	Ottawa, Ontario	NA(Sany loam)
6	Dwyer et al.,1988	Corn	Conventional	No	90	Ottawa, Ontario	1982
		Corn	Conventional	No	90	Ottawa, Ontario	1982
		Corn	Conventional	No	90	Ottawa, Ontario	1982
		Corn	Conventional	No	90	Ottawa, Ontario	1982
		Corn	Conventional	No	115	Ottawa, Ontario	1983
		Corn	Conventional	No	98	Ottawa, Ontario	1983
		Corn	Conventional	No	90	Ottawa, Ontario	1983
		Corn	Conventional	No	99	Ottawa, Ontario	1983
		Corn	Conventional	No	90	Ottawa, Ontario	1984
		Corn	Conventional	No	86	Ottawa, Ontario	1984
		Corn	Conventional	No	75	Ottawa, Ontario	1984
		Corn	Conventional	No	90	Ottawa, Ontario	1984
		Soybean	Conventional	No	75	Ottawa, Ontario	1982
		Soybean	Conventional	No	80	Ottawa, Ontario	1982

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Soybean	Conventional	No	69	Ottawa, Ontario	1982
Soybean	Conventional	No	75	Ottawa, Ontario	1982
Soybean	Conventional	No	75	Ottawa, Ontario	1983
Soybean	Conventional	No	75	Ottawa, Ontario	1983
Soybean	Conventional	No	74	Ottawa, Ontario	1983
Soybean	Conventional	No	94	Ottawa, Ontario	1983
Soybean	Conventional	No	70	Ottawa, Ontario	1984
Soybean	Conventional	No	77	Ottawa, Ontario	1984
Soybean	Conventional	No	68	Ottawa, Ontario	1984
Soybean	Conventional	No	70	Ottawa, Ontario	1984

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