

1 **Low-disturbance farming regenerates healthy critical zone**

2 **towards sustainable agriculture**

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15 **Classification**

16 Biological Sciences: Agricultural Sciences;

17 Physical Sciences: Environmental Sciences.

18 **Keywords:** Sustainable agriculture, no-tillage, stover mulching, microbial diversity and
19 function, deep soil.

20 **Author Contributions**

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

24 **Abstract**

25 Intensive conventional farming has degraded soil quality in farmlands and other
 26 ecosystems globally. Although low-disturbance practices have been widely adapted to
 27 restore soil health and save energy, the underlying mechanisms associated with farm
 28 sustainability are still unclear. Here, we compared soil microbiome, physiochemical
 29 parameters along 3-m deep soil profiles, and crop yield in Northeast China subjected
 30 to ten years of farming practices at 3 levels of disturbance, including conventional
 31 tillage (CT), no-tillage without stover mulching (NTNS), and no-tillage with stover
 32 mulching (NTSM). We found that low-disturbance practices (NTNS and NTSM)
 33 promoted the ability of the soil to retain water and nitrogen, regenerated whole-soil
 34 microbial diversity and function, and significantly improved corn yield at the drought
 35 year. This study implies that the NTSM practice could cut fertilizer-N input by 281.6
 36 kg/ha to corn farmland in, at least, Northeast China and may potentially reduce
 37 China's total greenhouse gas emissions by 1.6% and save about 6.7% households
 38 energy while without reducing corn production.

39 **Significance Statement**

40 Intensive conventional farming with high-energy input that has vitally degraded
 41 soils in farmlands. Low-disturbance practices (no-tillage and straw return) as
 42 sustainable ways have been broadly applied, however, little has been done to evaluate
 43 the impact on the soils beyond 1-m depth, a major part of Critical Zone in
 44 agro-ecosystem. Our results show that low disturbance practices not only promoted
 45 soil nutrient and water holding capacities, restored microbial diversity, richness, and
 46 ecological function in the whole 3-m soil profile, but also improved crop production
 47 and potentially reduced energy consumption and cut greenhouse gas emissions, thus
 48 contributing to sustainable farming.

49 **Introduction**

50 Since the Industrial Revolution, the rate of soil carbon loss has increased
 51 dramatically, resulting in a global carbon debt due to agriculture of 116 Pg carbon for
 52 the top 2 m of soil(1). The loss of carbon in farmlands has not only changed global
 53 climate but also produced catastrophic cascade impacts on global food security, as soil
 54 carbon is the cornerstone for healthy and productive soil that will be needed to feed
 55 10 billion people in 2050 (United Nations, World Population Prospects 2019). It is
 56 well known that intensive conventional farms with high energy inputs and disturbance
 57 (e.g. chemical fertilizers, tillage/compaction, burn/remove stover) have caused soil
 58 carbon loss with a series of environmental issues(2). Even worse, increasing the
 59 amount of chemical fertilizer is unlikely to continue the increase in quantity and
 60 quality of food products worldwide(3). Moreover, tillage particularly prevents root
 61 growth into deeper soil(4), thus affecting mineral weathering in deep soil(5) and
 62 reducing crop resilience to drought.

63 Since the 1970s, low-disturbance conservative practices (e.g. reduced tillage,
 64 no-tillage and stover mulching) have been gradually applied to restore soil health and
 65 reduce non-point source pollution(6). Growing evidence shows that no-tillage and
 66 stover mulching boosted top-soil organic carbon (SOC)(7-9), increased soil
 67 aggregate(10) and reduced soil erosion and surface runoff(11). All these benefits from
 68 low-disturbance practices are tied with complex microbial processes that interact with
 69 crops and drive soil carbon transformation and stabilization(12, 13). However, most
 70 studies only focused on topsoil or soils within 1-m depth(14-16). Soil below 1 meter,
 71 which belongs to Earth's Critical zone, was often overlooked despite that some crops
 72 have roots over 1 meter deep and microbes in the deep soils (> 1 m) may substantially
 73 impact long-term carbon sequestration, mineral weathering and crop

74 production(17-19). Furthermore, deep roots influence material cycles in surficial soil
 75 and microbes inhabiting in the deep soils, which plays important roles in bridging
 76 aboveground vegetation with parent soils and even acts as an essential buffer
 77 protecting underground water(20). To completely evaluate the impact of
 78 low-disturbance practices on agro-ecosystem, an urgent need is to test the changes
 79 and functions of microbial communities in deep soil(21). Since microbial activities in
 80 deep soil are generally limited by the availability of labile carbon, we hypothesized
 81 that low-disturbance practices are conducive to deeper root growth, which could
 82 provide labile carbon and nutrients(4) and accelerate mineral weathering(5), thus
 83 substantially influencing the microbial composition in the deep soils and in turn the
 84 sustainability of whole ecosystem.

85 Recent research shows that corn belts in the U.S.A., western Europe, and China
 86 have experienced the most soil carbon loss globally(1). The corn belt in Northeast
 87 China is considered as the “breadbasket” of the country, having the largest grain
 88 production and overlapping with the most fertile Mollisol region that sustains 3% of
 89 population in the world(22), accounting for over 30% of corn production of China(23).
 90 Here, a 10-year manipulative experiment was conducted at a temperate corn farm in
 91 Northeast China, investigating farming practices with three levels of disturbance:
 92 high-disturbance—conventional tillage (CT), low disturbance—no-tillage without
 93 stover mulching (NTNS) and no-tillage with 100% stover mulching (NTSM). We
 94 compared corn yield, soil properties and microbial communities of the 3-m soil
 95 profiles at the end of 10 years. We aimed at testing our main hypothesis that the
 96 lowest disturbance practice—no-tillage with 100% stover mulching, similar to
 97 undisturbed natural ecosystem, would regenerate microbial diversity and function
 98 toward a high-resilient natural ecosystem.

99 **Results**

100 **Soil properties and corn yield.** Soil properties varied significantly among
 101 disturbance practices and at different soil depths (SI Appendix, Table S1). The SOC,
 102 TN and C/N ratio substantially decreased from the soil surface to around 150 cm
 103 depths and then remained unchanged within 150-300 cm (Fig. 1). The NTSM slightly
 104 increased SOC, TN and C/N ratio at 0-20 cm soil layers compared with the NTNS and
 105 the CT (Fig. 1 and SI Appendix, Table S1). The no-tillage practices of NTSM and
 106 NTNS reduced soil pH in surface and deeper layers (Fig. 1d) and increased soil
 107 moisture at surface layers (0-60 cm) (Fig. 1e). In the CT plots, soil NO_3^- -N
 108 concentration first decreased and then increased remarkably, ranged from 4.19 to
 109 23.32 mg kg^{-1} (Fig. 1i). However, under the NTNS and NTSM treatments, soil
 110 NO_3^- -N decreased significantly at 0-40 cm then increased to the maximum at 120-150
 111 cm depth. Interestingly, above 120-150 cm layer, NO_3^- -N was significantly higher
 112 with low-disturbance practices than conventional tillage, while the soil below 150 cm
 113 under low-disturbance practices had much lower NO_3^- -N compared to conventional
 114 tillage (Fig. 1i). The NTNS plots contained much higher amounts of ammonium than
 115 the CT and the NTSM plots (Fig. 1h). Soil salt-extractable organic carbon (SEOC), as
 116 an organic acid proxy, is positively associated with root density and can be an
 117 indicator of root depth in deep soil(5). The SEOC declined from the surface to 40-60
 118 cm and then increase to its peak at 60-90 cm under CT, at 90-120 cm under NTNS
 119 and at 120-150 cm under NTSM (Fig. 1b). Hence, we estimate that corn roots reached
 120 up to 60-90 cm, 90-120 cm and 120-150 cm under the CT, the NTNS and the NTSM,
 121 respectively, which is in line with reported corn root depths (~150 cm)(4, 24). The
 122 NTSM increased the SEOC concentration at almost all soil layers compared with the
 123 CT and the NTNS (Fig. 1b), in which at the surface and 120-150 cm depth the

124 contents of SEOC with NTSM were twice higher than CT. The increased SEOC in
125 deep soils under NTSM reduced soil pH as shown by a significant negative
126 relationship between SEOC and pH ($r=0.678$, $p<0.05$). The relative contributions of
127 SEOC to SOC (SEOC/SOC) in the NTSM were also always higher than in the CT and
128 the NTNS (Fig. 1c). Based on the estimated root depths, total soil inorganic nitrogen
129 available for the coming growing season in the NTSM and the NTNS was 427.34 and
130 352.34 kg ha⁻¹, respectively, while only 179.63 kg ha⁻¹ in conventional tillage.

131 The mean annual corn yield (2013-2016) in the NTSM is 13416.8 kg/ha, which is
132 much higher than the CT and NTNS (Fig. 2), particularly during the drought year of
133 2015, with only 409.6 mm of rainfall during the growing season (about 100 mm lower
134 than the mean rainfall), while the corn yield in NTSM is 36.4% and 22.3% higher
135 than the CT and NTNS, respectively (Fig. 2).

136 **Microbial diversity, composition, and structure.** The microbial richness (Chao1),
137 observed number of species (Observed-species) and diversity (Shannon-Index) first
138 increased within 0-20 cm and decreased from 20 to 90 cm, then increased hereafter
139 (Fig. 3). The low-disturbance practices significantly increased Chao1,
140 Observed-species and Shannon-Index, particularly in 0-40 cm soil depths (Fig. 3).
141 There were 54 microbial phyla across all soil samples. The dominant phyla (relative
142 abundance > 1% across all soil samples) were Proteobacteria, Actinobacteria,
143 Chloroflexi, Acidobacteria, Nitrospirae, Gemmatimonadetes, Planctomycetes, and
144 these phyla accounted for 60-91% of the total microbial abundances in the whole soil
145 profile (SI Appendix, Fig. S1a). Bacteroidetes, Verrucomicrobia, Latescibacteria,
146 Parcubacteria, Firmicutes, Microgenomates and Saccharibacteria were less dominant
147 (relative abundance > 0.1% across all soil samples) but were still found across all soil
148 samples (SI Appendix, Fig. S1a). Although no difference in the composition of

dominant phyla among treatments were found, there are more non-dominant phyla with higher relative abundance in low disturbance practices than conventional tillage practice (SI Appendix, Fig. S1b).

Indicator analysis identified 16 and 51 clearly classified genera (relative abundances > 0.005%) in the NTNS and the NTSM plots, respectively, while no indicator genera were found in the conventional tillage plots (Fig. 4 and SI Appendix, Table S2). The indicator genera in the NTNS plots belonged to Proteobacteria, Actinobacteria, Chloroflexi, Gemmatimonadetes and Planctomycetes, and most of them appeared in the surface soil (0-20 cm) with only 1 genus below 150 cm. Importantly, more extra indicator genera — including Bacteroidetes, Acidobacteria, Deferribacteres, Firmicutes, Verrucomicrobia, Chlorobi and Spirochaetae — existed in the NTSM plots, in which under 150 cm we observed 7 genera (Fig. 4 and SI Appendix, Table S2).

Microbial community structures were visualized by Non-metric multidimensional scaling (MDS) and tested by Permutational multivariate analysis of variance (PERMANOVA) based on Bray–Curtis. The microbial communities among treatments in the root zones were marginally different (PERMANOVA $p=0.08$); however, below the root zone they differed distinctively (PERMANOVA $p=0.02$). The disturbance practices influenced the vertical distribution dissimilarity in microbial community structure (Fig. 5). Three clusters — 0-10 cm and 10-20 cm, 20-150 cm and 150-300 cm — were observed in the CT plots (PERMANOVA- $F=9.57$, $p=0.0001$) (Fig. 5). In the NTNS plots, 0-10 cm formed an independent cluster, while other soil depths showed some separation (e.g. 20-120 cm were separated from 150-300 cm soil depths by axis 1); however, Bray-Curtis distances between adjacent depths were too close to be separated (PERMANOVA- $F=8.18$, $p=0.0001$) (Fig. 5). The NTSM

174 treatment clustered 0-10 cm and 10-20 cm together, 120-150 cm, 150-200 cm,
175 200-250 cm and 250-300 cm separately, and the other depths show some separations
176 as well (PERMANOVA-F=11.32, p=0.0001) (Fig. 5).

177 **Predicted Ecological functions of microbial communities.** According to the results
178 of microbial diversity, composition and structure, the metabolic capabilities of
179 microbial community in the whole 3-m soil profiles were predicted using Tax4Fun (SI
180 Appendix, Fig. S2). Results showed that low-disturbance practices significantly
181 increased the abundance of predicted functions related to carbohydrate metabolism,
182 nucleotide metabolism, glycan biosynthesis and metabolism, lipid metabolism and
183 metabolism related to cofactors and vitamins (SI Appendix, Fig. S2a). Moreover, the
184 relative abundances of genes encoding for assimilatory nitrate reduction in
185 low-disturbance practices were higher than that in conventional tillage practice (SI
186 Appendix, Fig. S3). The results suggested that in low disturbance practices, microbial
187 community prefer to convert the nitrate/nitrite to ammonia. We then further assessed
188 the impact of stover mulching on functional profiles (SI Appendix, Fig. S2b). The
189 extended error bar plot shows that the NTNS enriched the abundance of amino acid
190 metabolism and lipid metabolism, while the NTSM enriched the functions associated
191 to energy metabolism, carbohydrate metabolism, biosynthesis of secondary
192 metabolites, glycan biosynthesis and metabolism as well as metabolism of cofactors
193 and vitamins (SI Appendix, Fig. S2b).

194 **Relationships between microbial communities and soil properties.** Forward
195 selection in Redundancy analysis (RDA) revealed that soil depth (pseudo-F=48, p=
196 0.002), SOC (pseudo-F=11.5, p= 0.002), SM (pseudo-F=3.4, p= 0.012), soil pH
197 (pseudo-F=2.3, p=0.018) and soil NH_4^+ -N (pseudo-F=2.7, p= 0.026) significantly
198 affected the vertical distribution of microbial communities (SI Appendix, Fig. S4).

Furthermore, the soil properties that regulated the distribution of soil microbes were different under different disturbance practices. Under the CT treatment, soil microbial community was mainly affected by soil $\text{NH}_4^+\text{-N}$ (pseudo- $F=4$, $p=0.002$) and soil $\text{NO}_3^-\text{-N}$ (pseudo- $F=2.3$, $p=0.012$) that mainly came from applied fertilizer (Fig. 6). The microbial community positively correlated to soil $\text{NH}_4^+\text{-N}$ in the 0-20 cm soil, to soil $\text{NO}_3^-\text{-N}$ negatively within 20-150 cm, while to soil $\text{NO}_3^-\text{-N}$ positively after 150 cm (Fig. 6). Under the NTNS treatment, soil pH (pseudo- $F=3.7$, $p=0.004$) constrained the distribution of the microbial community, in which strong negative correlations occurred in 0-10 cm soil and a positive correlation in 90-150 cm (Fig. 6). Under the NTSM treatment, soil TN (pseudo- $F=11$, $p=0.002$), SM (pseudo- $F=2.6$, $p=0.004$) and C/N ratio (pseudo- $F=1.8$, $p=0.016$) significantly influenced the soil microbial community separation (Fig. 6). In general, the microbes positively correlated with the soil TN and C/N ratio in the surface soil layers (0-40 cm) and with SM in the middle layers (40-150 cm), while they were mainly influenced by depth in the deeper soil (150-300 cm) (Fig. 6).

Discussion

No-tillage practices promote soil health and corn yield. No-tillage promotes root growth into deep soil, up to 150 cm in the NTSM. The root exudates with various organic acid and dead roots likely contributed to the lower soil pH and higher SEOC in the NTNS and the NTSM, which in turn increased mineral weathering(5) and diversified the microbial communities with multi-ecological functions. The increased fine roots in deeper soil retained more nutrients including nutrients in dead roots and converting nitrate to more stable ammonium (SI Appendix, Fig. S3) and also provided labile carbon (Fig. 1b) to remove leaked nitrate through denitrification in deeper soil (below 1.5 m), as higher relative abundance of the denitrification bacteria

(*Pseudomonas* and *Caldithrix*)(25, 26) (Fig. 4 and Table. S2) and denitrification genes (SI Appendix, Fig. S3) were detected in low disturbance practices — particularly in no-tillage with stover mulching. However, shallower roots in the CT treatment can't provide enough labile carbon to remove extra soil NO_3^- -N in deep soil, thus causing nitrite accumulation and leaching into deeper soil layers. The amount of inorganic nitrogen accumulated in the root zones under NTSM ($427.34 \text{ kg ha}^{-1}$) likely could provide plenty of nitrogen for corn growth in the coming growing season (Fig. 2), based on the removed nitrogen in the grain ($\sim 200 \text{ kg ha}^{-1}$). Additionally, in line with many studies that show stover mulching reduces water evaporation and surface runoff and increase soil moisture in top soils(11, 27), we found that the soil moisture was significantly higher in the NTSM than in the CT plots. Therefore, no-tillage with stover mulching not only restores soil health by increasing the holding capacities for nutrients and water, thus reducing energy input to farm, but also tended to reduce the risk of nitrate leaching to groundwater. And more importantly, the healthy soil in turn raises corn production and promote the crop resistance to drought (Fig. 2). All these are critical to the development of sustainable agriculture and the associated ecosystems.

No-tillage with stover mulching promotes microbial diversity, richness, and ecological function contributing to sustainable farming. Under the CT treatment, tillage heavily disturbed the topsoil and liberated occluded organic materials. Microbes tended to rapidly use available nutrients in the plowed layer (e.g. NH_4^+ -N)(28), thereby causing the reduction of microbial metabolic diversity (SI Appendix, Fig. S2a). Then, the resistance of the soil to stress or disturbance may also decrease(29). In deeper soil layers, due to shallower roots, NO_3^- -N could quickly move downward and accumulate in deeper soil (Fig. 1i), which not only contaminated

the underground water but also limited the activity of non-dominant microbes with important ecological functions, as no indicator genera were identified for each soil depth in CT treatment (Fig. 4 and SI Appendix, Table S2). Because the microbial communities were closely associated with inorganic nitrogen, the microbes under CT were mainly influenced by added chemical fertilizer. Although the dominant microbial communities in CT were similar to those in the NTNS and NTSM, the loss of function resulted from the difference of non-dominant microbes, indicating that the soil under CT had degraded.

Under the NTNS treatment, soil pH was the major edaphic factor affecting the microbial community and the indicator genera (Fig. 6 and SI Appendix, Table S2). The lower soil pH possibly was caused by deeper roots as shown by higher SEOC that is generally positively related to root density(5). Soil pH is often observed as a major factor determining the microbial composition and structure in natural ecosystems(30, 31), as microbes often show a narrow tolerance to soil pH. In addition, soil pH regulates the availability of nutrient and mitigate ion toxicity(30-32). Under NTNS, soil pH and depth only explained 35% distribution of the microbial community (Fig. 6). We speculated that other edaphic factors (e.g. salinity and iron) directly or indirectly related to soil pH and SEOC also influenced the changes in the microbial community.

Under NTSM treatment, TN and C/N significantly correlated with soil microbial community due to the high C/N ratio of stover and roots (Fig. 6). Prior studies have reported that, following maize stover mulching, more organic N, amino acid N, and amino sugar N were observed in soil(33, 34), which increased the retention time of nitrogen, hence meeting the nutrient requirement of corn growth and reducing nitrate loss to underground water. The increased available nitrogen, labile carbon and water

274 in deep soil under NTSM can increase the resilience and resistance of maize to
 275 disturbances with higher grain production (Fig. 2). Zhang et al.(35) also observed
 276 litter-covered soil showed greater resistance to heating and copper addition due to the
 277 changes in soil properties and microbial community structure. Resistance to
 278 disturbance or stresses is the nature of a healthy soil and is essential for maintaining
 279 ecosystem functions, such as decomposing organic matter (35, 36). Under the NTSM
 280 treatment, the microorganisms associated with the degradation of relatively stable
 281 carbon compounds, such as Planctomycetes and Verrucomicrobia (SI Appendix, Table
 282 S3)(37, 38) as well as the indicator *Cellulomonas* and *Azospirillum* (Fig. 4 and SI
 283 Appendix, Table S2) with the function of cellulose decomposition(39, 40) were
 284 increased. The predicted functional profiles related to energy metabolism (Carbon
 285 fixation pathways in prokaryotes), carbohydrate metabolism (TCA cycle, amino sugar,
 286 nucleotide sugar, galactose, fructose), biosynthesis of secondary metabolites
 287 (Carotenoid and Betalain) and glycan biosynthesis were increased, suggesting a
 288 higher metabolic activity and a change in substrate quality (SI Appendix, Fig. S2). In
 289 addition, stover mulching also increased the ecological filter function of soil depth for
 290 selecting microbial communities as more indicator genera of each soil depths were
 291 identified under NTSM compared to NTNS and CT practices (Fig. 4 and SI Appendix,
 292 Table S2). And these indicators residing at different soil depths might enhance the
 293 anti-disturbance ability of NTSM. For example, denitrification bacteria *Caldithrix* and
 294 *Pseudomonas*(25, 26) were the indicator genera of 150-200 cm and 250-300 cm,
 295 respectively (Fig. 4 and SI Appendix, Table S2), which might explain the low nitrate
 296 in the deep soil in NTSM. *Ignavibacteria* and *Spirochaeta*, the indicator genera of
 297 deep soil, have the ability to grow under the conditions of strictly anaerobic(41) and
 298 severely limited nutrients(42), respectively. Surface indicator genera belonging to

299 Bacteroidetes might have the ability to degrade organic matter that is difficult to
300 decompose(43).

301 **Implications for climate change.** It was observed that about 179.63, 352.34 and
302 427.34 kg ha⁻¹ inorganic N were kept in the root-zone soil in the CT, NTNS and
303 NTSM, respectively. Generally, corn roots reach their maximum depth at the silking
304 stage(44), which is also the time when the heaviest rainfall occurs in northeastern
305 China. We therefore expect that the available N kept in the root zone would be
306 utilized by crops in the coming growing season, which means that fertilizer N could
307 be cut to meet crop growth and also prevent reactive N losses. Since the nitrogen use
308 efficiency (NUE) of maize system under the conventional management is 51% in
309 northeast China (NUE is defined as the efficiency of fertilizer N transferring to
310 harvested crop N)(45). Then, we conservatively calculate the required fertilizer N in
311 the next year based on two assumptions: 1) the NUE of soil available N in root zone is
312 equal to that NUE of applied fertilizer N, both of them are 50%; 2) the mineralized N
313 during the coming growing season is neglected. Thus, N supply requirement =
314 Fertilizer N×NUE + N in root zone ×NUE + Stover-N, where Stover-N for NTSM is
315 60 kg ha⁻¹. We estimated the N requirement for each disturbance practice by
316 multiplying grain yield by grain N concentration (1.4%)(45) plus multiplying stover
317 yield by stover N concentration (0.8%)(46). For CT, NTNS and NTSM, the mean
318 annual corn yields were 10946.74, 12487.81 and 13416.81 kg ha⁻¹, and the stover
319 yields were 966.67, 10083.33 and 10833.33 kg ha⁻¹, respectively. Thus, the N
320 requirements were 230.6, 255.5 and 274.5 kg ha⁻¹ for CT, NTNS and NTSM,
321 respectively. Therefore, the theoretically conservative amounts of fertilizer N in the
322 coming growing season are 281.6, 158.7 and 1.7 kg ha⁻¹ for CT, NTNS and NTSM,

323 respectively. No fertilizer-N is needed to apply without reducing corn yield in the
 324 NTSM plot. Compared to CT, the NTNS and NTSM could at least save respectively
 325 about 122.9 and 281.6 kg ha⁻¹ N-fertilizer. For every kilogram of fertilizer-N produced
 326 and used on cropland, up to 87.9 MJ of energy is consumed(47) and 13.5 kg of
 327 CO₂-equivalent (eq) (CO₂-eq) is emitted(48). Hence, totally 24,752.6 MJ of energy
 328 consumption could be reduced and 3,801.6 kg CO₂-eq emission could be cut per
 329 hectare cornland in Northeast China at least by using NTSM tillage practice. If this
 330 could be applied to all maize farmland in China (42,000,000 ha, Source: China
 331 Statistics Yearbook 2018), 1.0 EJ of energy could be saved and 159.7 Mt of CO₂-eq
 332 could be reduced. Based on the average annual energy consumption for households of
 333 China in 2017 (15 EJ, China Statistics Yearbook 2018) and CO₂ emissions (9,839 Mt,
 334 Global Carbon Atlas), the NTSM practice in corn farming has the potential to save 6.7%
 335 of household energy and to reduce 1.6% of CO₂ emissions each year in China.

336 Taken together, we provide new evidence that low-disturbance practice promotes
 337 deep-soil stability to cope with environmental stress through increasing water and
 338 nutrient holding capacity, microbial richness, microbial diversity and ecological
 339 functions. According to ecological theory(49, 50), microbial community assembly in
 340 the CT treatment was mainly based on deterministic processes and significantly
 341 influenced by environmental stress and fertilizer nitrogen. Stover mulching might
 342 alter these processes through deeper roots affecting the vertical heterogeneity in
 343 resource availability(4). When energy resources are richer in the soil, environmental
 344 stress tend to alleviate(51), and higher biodiversity was caused due to more stochastic
 345 processes introduced in community assembly(52). Moreover, low disturbance practice
 346 also showed the potential to increase the maize yield (Fig. 2), save energy, and
 347 decrease the risk of groundwater leaching and greenhouse gas emissions. In view of

348 the importance of microbial community assembly in predicting ecosystem service
349 functions(53, 54), our results demonstrated that the lowest disturbance-practice —
350 no-tillage with stover mulching increases the sustainability of agro-ecosystems.

351 **Materials and methods**

352 **Site description and soil sampling.** The field experiment was established in 2007 at
 353 the Lishu Conservation Tillage Research and Development Station of the Chinese
 354 Academy of Sciences in Jilin province, Northeast China (43.19° N, 124.14° E). The
 355 region has a humid continental climate with a mean annual temperature of 6.9 °C and
 356 the mean annual precipitation of 614 mm. The soils are classified in the Mollisol
 357 order (Black Soil in Chinese Soil Classification) with a clay loam texture(55). The site
 358 has been continuously planted with maize since 2007. We set up an experiment by a
 359 randomized complete block design with four replicates and five treatments. Each plot
 360 area was 261m² (8.7×30m). The five treatments included conventional tillage
 361 (moldboard plowing to a depth around 30 cm), no-tillage (no soil disturbance and
 362 direct seeding), and no-tillage with three-level stover mulching (33%, 67% and 100%
 363 newly produced maize stover were evenly spread over the soil surface each fall). For
 364 each treatment, slow-release fertilizer was applied at one time when sowing, which
 365 was equal to 240 kg/ha N; 47 kg/ha P; 90 kg/ha K. The rainfall data were obtained
 366 from local meteorological administration. The grain yield was estimated by manually
 367 harvesting 20 m² area, randomly taken from each plot.

368 In this experiment, in order to reduce the damage to the plots and reduce costs, 3
 369 plots were randomly taken from each treatment including conventional tillage (CT),
 370 no-tillage without stover mulching (NTNS), no-tillage with 100% stover coverage
 371 (NTSM) as three comparative practices. In April 2017, triplicate soil cores (0-300 cm)
 372 were collected from each plot at the dormant season. After removing surface stover,
 373 we took soil cores by a stainless-steel hand auger and sliced each into ten layers: 0-10
 374 cm, 10-20 cm, 20-40 cm, 40-60 cm, 60-90 cm, 90-120 cm, 120-150 cm, 150-200 cm,
 375 200-250 cm, 250-300 cm. In total, 90 soil samples were collected and transported to

the laboratory within 3 hours, then passed through a 2-mm sieve. All visible roots, crop residues and stones were removed. Each soil sample was divided into three subsamples: one subsample for DNA extraction and soil salt-extractable organic carbon (SEOC) measurement that was immediately placed into a polyethylene plastic bag and stored at -80 °C, one for chemical measurements including ammonium nitrogen ($\text{NH}_4^+\text{-N}$) and nitrate nitrogen ($\text{NO}_3^-\text{-N}$) (within one day), and the remaining one was air dried for other soil physicochemical properties.

Soil properties. Soil total nitrogen (TN) content was measured by an Element analyzer Vario EL III (Elementar Analysensysteme GmbH, Hanau, Germany). Soil organic carbon (SOC) was converted from soil organic matter (SOM) that was measured by potassium dichromate oxidation(56). Soil pH was measured in deionized free- CO_2 water (1:2.5 w/v). Gravimetric soil moisture was determined by oven-drying fresh soil to a constant weight at 105 °C. Soil $\text{NH}_4^+\text{-N}$ and $\text{NO}_3^-\text{-N}$ were extracted from fresh soil by 2 M KCl and measured by a continuous flow analytical system (AA3, SEAI, Germany). To reflect soil soluble, exchangeable, mineral-bound OC, soil salt-extractable organic carbon (SEOC) was extracted from the frozen soil samples with 0.5 M K_2SO_4 (1:5 w/v)(4, 57, 58).

DNA extraction, PCR amplification and pyrosequencing. Soil DNA was extracted from the frozen soil samples (0.5 g wet weight) by using MoBio PowerSoil DNA isolation kit (MoBio Laboratories, Carlsbad, CA, USA) following the instructions of the manufacturer. The quality of DNA was determined by 1% agarose gel electrophoresis. The V3–V4 region of the bacterial 16S rRNA gene was amplified by PCR using the primers 338F and 806R with barcode for Illumina MiSeq sequencing. PCR was performed in a total volume of 50 μl containing 30 ng DNA as a template, 20 mol of each primer, 10mM dNTPs, 5 μl 10 \times Pyrobest buffer and 0.3 U of Pyrobest

401 polymerase (Takara Code: DR005A). The PCR cycle conditions were as follows:
 402 initial denaturation at 95°C for 5 min followed by 26 cycles of denaturation at 95°C
 403 for 45s, annealing at 50°C for the 30s, and extension at 72°C for 45s, with a final
 404 extension at 72°C for 10 min. Each sample was amplified for three replicates. The
 405 PCR products from the same sample were pooled, checked by 2% agarose gel
 406 electrophoresis and were then purified using AxyPrepDNA agarose purification kit
 407 (AXYGEN). Finally, purified PCR products were sequenced on an Illumina MiSeq
 408 platform PE300 sequencer (Illumina, USA).

409 The raw sequence data were further analyzed by the following protocol.
 410 Low-quality sequences with an average quality score of less than 20 were filtered by
 411 employing Trimmomatic(59). The FLASH software was used to merge overlapping
 412 ends and treat them as single-end reads(60). The non-amplified region sequences,
 413 chimeras and shorter tags were also removed using Usearch and Mothur(61, 62). The
 414 resulting high-quality sequences were clustered into Operational Taxonomic Units
 415 (OTUs) at 97% sequence similarity using Usearch (Version 8.1.1861
 416 <http://www.drive5.com/usearch/>) (Edgar, 2013). OTUs were then classified against
 417 the Silva (Release119 <http://www.arb-silva.de>) database and the taxonomic
 418 information of each OTU representative sequence was annotated using the RDP
 419 Classifier(63-65). A total of 3,255,693 high-quality reads were obtained from all soil
 420 samples, which were clustered into 9,573 unique OTUs at a 97% sequence similarity.
 421 The Good's coverage of all the samples ranged from 0.93 to 0.98, which indicates an
 422 adequate level of sequencing to identify the majority of diversity in the samples.

423 **Statistical analyses.** Soil properties were analyzed and plotted using Sigmaplot 12.5
 424 software. Alpha diversity indices were calculated in Qiime (version v.1.8) and used to
 425 reflect the diversity and richness of the microbial community in different samples.

426 The relative abundances of individual phyla in different samples were computed by R
 427 packages. The indicator analysis based on genera-specific to each soil depth was
 428 conducted using indicpecies package of R with 9999 permutations, and the P-values
 429 were corrected for multiple testing using qvalue package of R(14, 66). Functional
 430 profiles of the microbial community were predicted by Tax4fun (an open-source
 431 package in R)(67) and further statistical analysis was conducted by STAMP using
 432 Welch's t-test(68). Non-metric multidimensional scaling (MDS) was performed by
 433 "vegan" package of R to describe differences in microbial community structure
 434 among samples. Permutational multivariate analysis of variance (PERMANOVA) was
 435 employed on Bray-Curtis distances to test the differences in soil microbial
 436 communities among various sample groups. The Redundancy analysis (RDA, Canoco
 437 5 software) were conducted to identify the correlations between microbial community
 438 composition and environmental variables. One-way and two-way ANOVA tests were
 439 conducted by SPSS Version 22. Percentage data were transformed using arcsine
 440 square root function before ANOVA test. All statistical tests were significant at $p \leq$
 441 0.05.

442 **Acknowledgments**

443 We would like to thank Dr. William H. Schlesinger at the Cary Institute of Ecosystem
 444 Studies for his comments and Dr. Randy Neighbarger at Duke University for language
 445 editing. This work was supported by the "National Key R&D Program" (No.
 446 2016YFD0800103, 2016YFD0200307) and the National Natural Science Foundation
 447 of China (grant number, 41671297). We would like to thank Pengshuai Shao,
 448 Xuesong Ma and many individuals for assistance with sample collection, processing
 449 and analysis.

450 **Data availability.** All sequencing data that support the findings of this study have
451 been deposited in the National Center for Biotechnology Information
452 (<https://www.ncbi.nlm.nih.gov/>), in the Sequence Read Archive (SRA) database
453 (BioProject number: PRJNA488172). All other relevant data are available from the
454 corresponding author on request.

455 **Supporting information**

456 This article contains supporting information online at

457 **Competing interests**

458 The authors declare no competing interests.

459

460

461

462 **References**

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Figure Legends

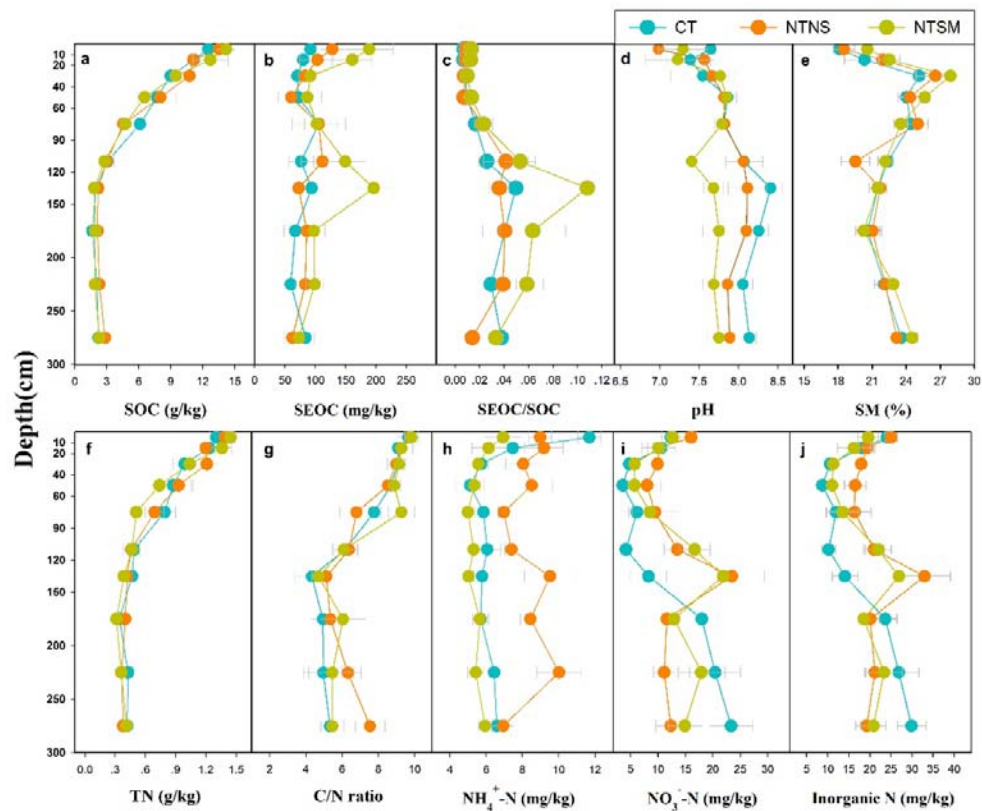
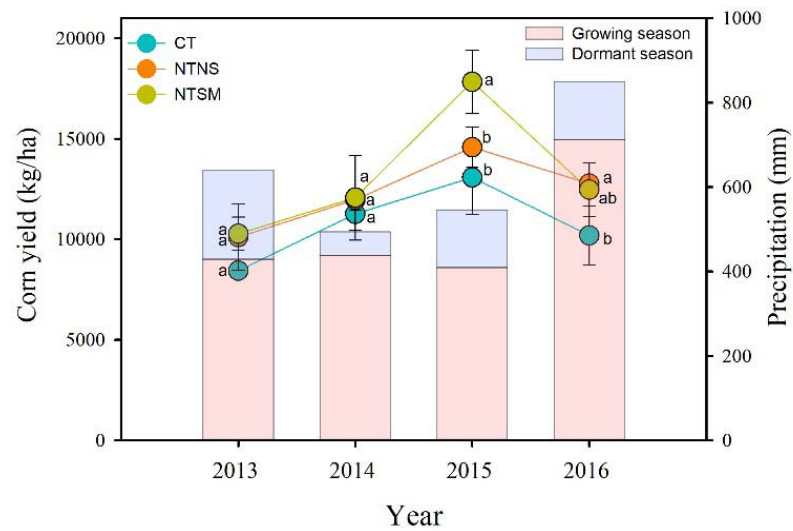


Figure 1. Soil properties (mean±SE, n = 3) along soil depth under different practices.

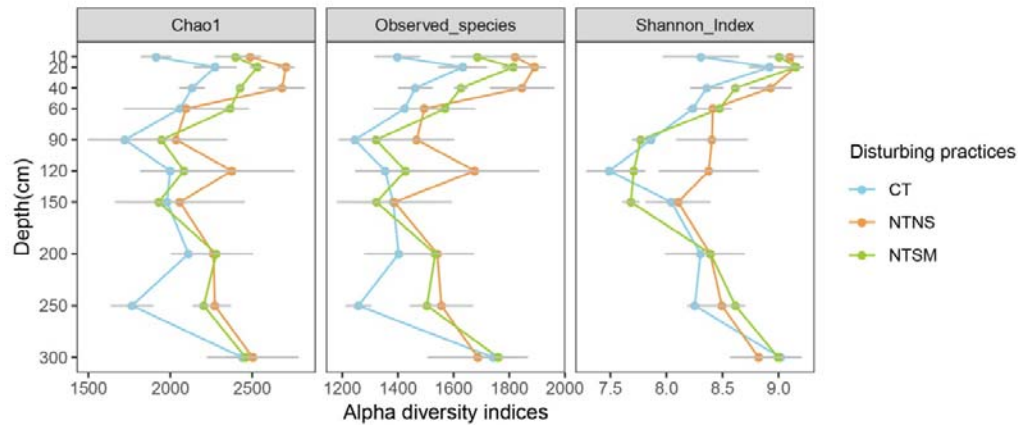
SOC = soil organic carbon, SIOC = salt-extractable organic carbon, SIOC/SOC = ratio of SIOC to SOC, SM = soil moisture, TN = total nitrogen content, C/N = ratio of SOC to TN, $\text{NH}_4^+\text{-N}$ = ammonium nitrogen, $\text{NO}_3^-\text{-N}$ = nitrate nitrogen, Inorganic N = $\text{NH}_4^+\text{-N} + \text{NO}_3^-\text{-N}$.



641

642 **Figure 2.** Corn yield (line+symbol) and annual rainfall during growing and dormant
643 seasons (bar) under different disturbance practices during 2013-2016. Error bars
644 indicate standard errors (n = 3 or 4), different letters indicate significant differences at
645 $P < 0.05$.

646



647

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

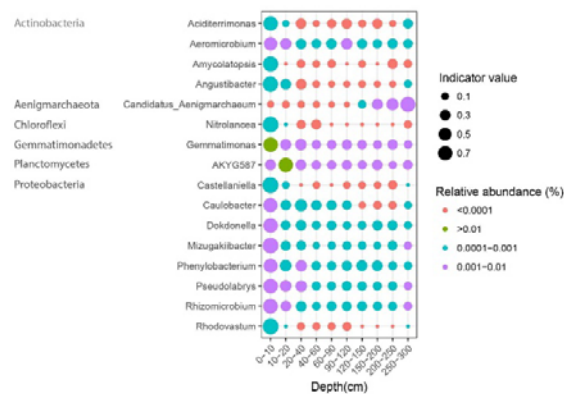
649 and diversity (Shannon_Index) in the CT (conventional tillage), NTNS (no-tillage

650 without stover mulching) and NTSM (no-tillage with stover mulching) plots. Error

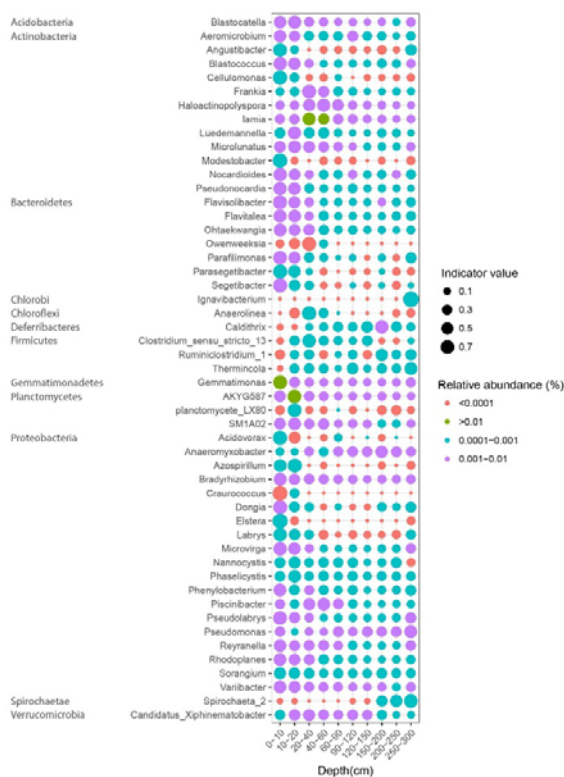
651 bars indicate standard deviation (n = 3).

652

NTNS Treatment



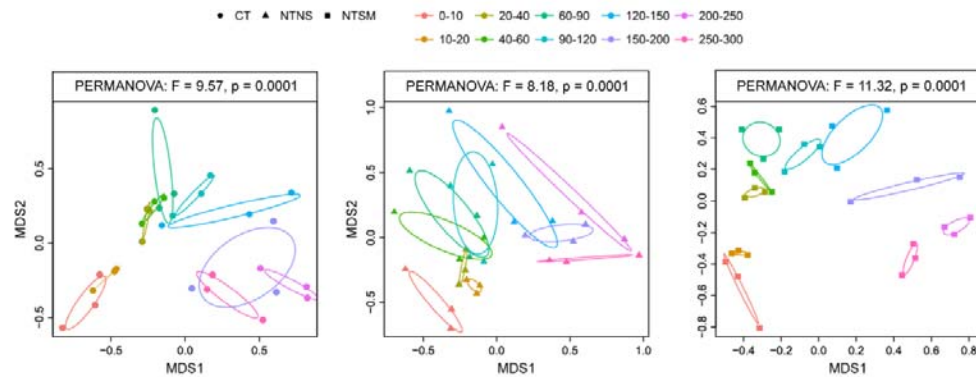
NTSM Treatment



653

654 **Figure 4.** Indicator genera significantly ($q < 0.1$) associated with tillage practices. The
655 size of each circle represents the indicator value of a specific genus in the different
656 soil depths. The color indicates the relative abundance of each indicator genus.
657 Taxonomic information, indicator values, P-values, and q-values of all indicator
658 genera are given in SI Appendix, Table S2. Zero indicator genera were identified in
659 CT treatment.

660



661

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

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

664 agricultural disturbance practices. Permutational multivariate analysis of variance

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

666 depth were significantly different at each disturbance practice. Circles, triangles and

667 squares represent CT (conventional tillage), NTNS (no-tillage without stover

668 mulching) and NTSM (no-tillage with stover mulching), respectively.

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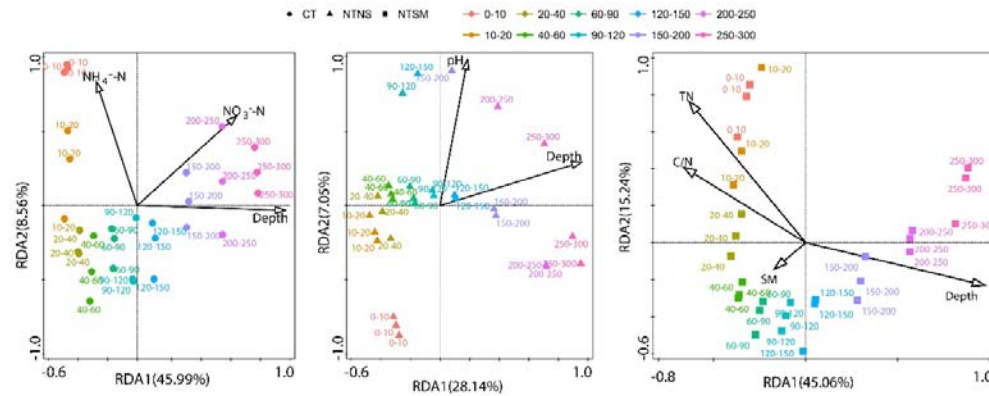


Figure 6. Redundancy analysis (RDA) of the soil microbial community originating from microbial phyla constrained by soil properties under different agricultural practices. Only soil variables that significantly explained variability in microbial community structure in the forward selection procedure were selected to the ordination (arrows). TN, total nitrogen content; C/N, a ratio of carbon to nitrogen content; $\text{NH}_4^+\text{-N}$, ammonium nitrogen; $\text{NO}_3^-\text{-N}$, nitrate nitrogen ; SM, soil moisture. Circles, triangles and squares represent CT (conventional tillage), NTNS (no-tillage without stover mulching) and NTSM (no-tillage with stover mulching), respectively.

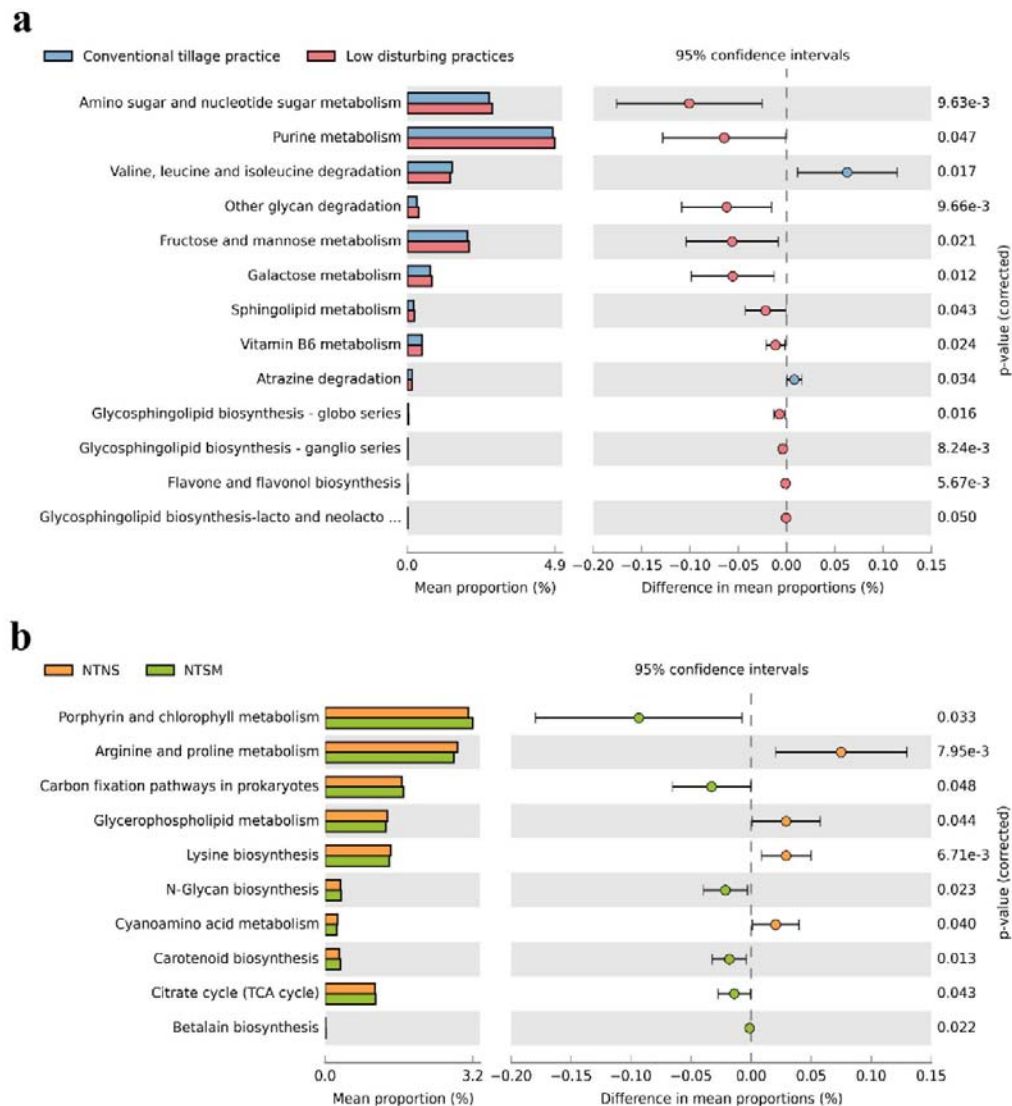
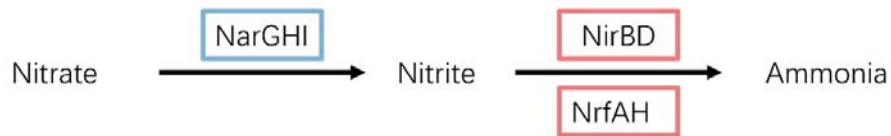


Figure S2. Extended error bar plots showing significant differences of 16S rRNA gene-predicted functional profiles obtained with Tax4Fun. **a** difference between mean proportions of conventional practice and low disturbance practices; **b** differences between mean proportions of NTNS (no-tillage without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

Denitrification

Dissimilatory nitrate reduction



Assimilatory nitrate reduction



Nitrification



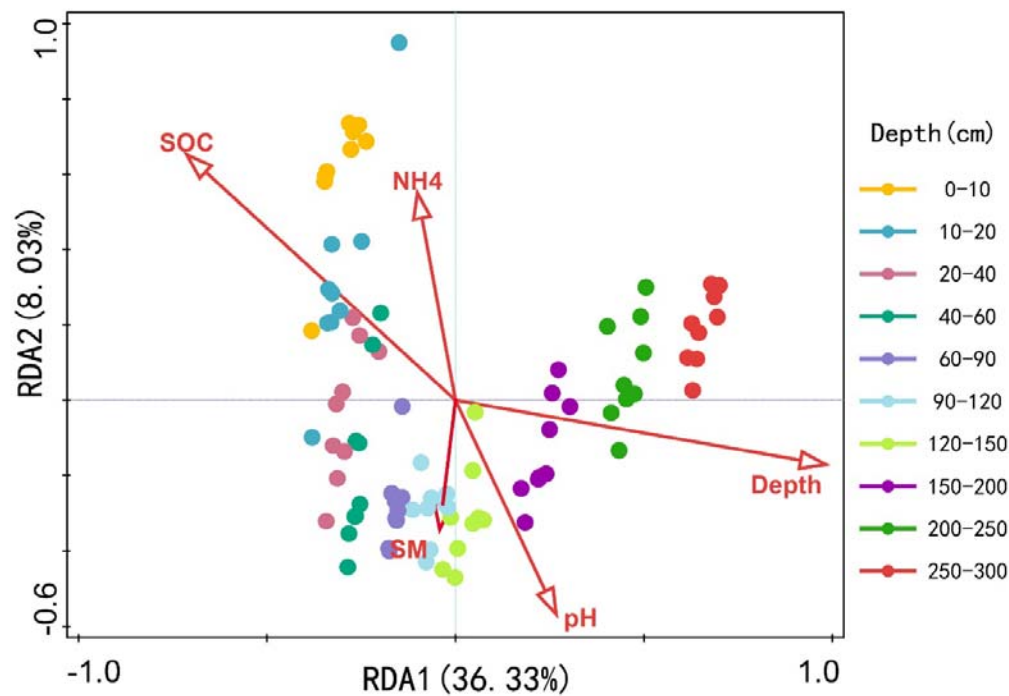
■ Conventional tillage practice
 ■ Low disturbing practices

697

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

702

703



704

705

706 **Figure S4.** Redundancy analysis (RDA) of soil microbial community originating
707 from microbial phyla constrained by soil properties among soil depths. Only soil
708 variables that significantly explained variability in microbial community structure in
709 the forward selection procedure were selected to the ordination (arrows).
710 Abbreviations: SOC, soil organic carbon; NH4, ammonium nitrogen; SM, soil
711 moisture.

712