Low-disturbance farming regenerates healthy critical zone towards sustainable agriculture

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Author Contributions

H.X. 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.

Abstract

Intensive conventional farming has degraded soil quality in farmlands and other ecosystems globally. Although low-disturbance practices have been widely adapted to restore soil health and save energy, the underlying mechanisms associated with farm sustainability 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 soil to retain water and nitrogen, regenerated whole-soil microbial diversity and function, and significantly improved corn yield at the drought year. This study implies that the NTSM practice could cut fertilizer-N input by 281.6 kg/ha to corn farmland in, at least, Northeast China and may potentially reduce China's total greenhouse gas emissions by 1.6% and save about 6.7% households energy while without reducing corn production.

Significance Statement

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

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 carbon in farmlands has not only changed global climate but also produced catastrophic cascade impacts on global food security, as soil carbon is the cornerstone for healthy and productive soil that will be needed to feed 10 billion people in 2050 (United Nations, World Population Prospects 2019). It is well known that intensive conventional farms with high energy inputs and disturbance (e.g. chemical fertilizers, tillage/compaction, burn/remove stover) have caused soil carbon loss with a series of environmental issues(2). Even worse, increasing the amount of chemical fertilizer is unlikely to continue the increase in quantity and quality of food products worldwide(3). Moreover, tillage particularly prevents root growth into deeper soil(4), thus affecting mineral weathering in deep soil(5) and reducing crop resilience to drought.

Since the 1970s, low-disturbance conservative practices (e.g. reduced tillage, no-tillage and stover mulching) have been gradually applied to restore soil health and reduce non-point source pollution(6). Growing evidence shows that no-tillage and stover mulching boosted top-soil organic carbon (SOC)(7-9), increased soil aggregate(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 and drive soil carbon transformation and stabilization(12, 13). However, most studies only focused on topsoil or soils within 1-m depth(14-16). Soil below 1 meter, which belongs to Earth's Critical zone, was often overlooked despite that some crops have roots over 1 meter deep and microbes in the deep soils (> 1 m) may substantially impact long-term carbon sequestration, mineral weathering and crop production(17-19). Furthermore, deep roots influence material cycles in surficial soil and microbes inhabiting in the deep soils, which plays important roles in bridging aboveground vegetation with parent soils and even acts as an essential buffer protecting underground water(20). To completely evaluate the impact of low-disturbance practices on agro-ecosystem, an urgent need is to test the changes and functions of microbial communities in deep soil(21). Since microbial activities in deep soil are generally limited by the availability of labile carbon, we hypothesized that low-disturbance practices are conducive to deeper root growth, which could provide labile carbon and nutrients(4) and accelerate mineral weathering(5), thus substantially influencing the microbial composition in the deep soils and in turn the sustainability of whole ecosystem.

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

Results

Soil properties and corn yield. Soil properties varied significantly among disturbance practices and at different soil depths (SI Appendix, 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 SI Appendix, Table S1). The no-tillage practices of 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 depth. 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), as an organic acid proxy, is positively associated with root density and can be an indicator of root depth in deep soil(5). The SEOC 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). Hence, 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)(4, 24). 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, p<0.05). The relative contributions of SEOC to SOC (SEOC/SOC) in the NTSM were also always higher than in the CT and the NTNS (Fig. 1c). Based on the estimated root depths, total soil inorganic nitrogen available for the coming growing season in the NTSM and the NTNS was 427.34 and 352.34 kg ha^{-1}, respectively, while only 179.63 kg ha^{-1} in conventional tillage.

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

Microbial diversity, composition, and structure. The microbial richness (Chao1), observed number of species (Observed-species) and diversity (Shannon-Index) first increased within 0-20 cm and decreased from 20 to 90 cm, then increased hereafter (Fig. 3). The low-disturbance practices significantly increased Chao1, Observed-species and Shannon-Index, particularly in 0-40 cm soil depths (Fig. 3). There were 54 microbial phyla across all soil samples. The dominant phyla (relative abundance > 1% across all soil samples) were Proteobacteria, Actinobacteria, Chloroflexi, Acidobacteria, Nitrospirae, Gemmatimonadetes, Planctomycetes, and these phyla accounted for 60-91% of the total microbial abundances in the whole soil profile (SI Appendix, Fig. S1a). Bacteroidetes, Verrucomicrobia, Latescibacteria, Parcubacteria, Firmicutes, Microgenomates and Saccharibacteria were less dominant (relative abundance > 0.1% across all soil samples) but were still found across all soil 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 treatment clustered 0-10 cm and 10-20 cm together, 120-150 cm, 150-200 cm, 200-250 cm and 250-300 cm separately, and the other depths show some separations as well (PERMANOVA-F=11.32, p=0.0001) (Fig. 5).

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

Relationships between microbial communities and soil properties. Forward selection in Redundancy analysis (RDA) revealed that soil depth (pseudo-F=48, p= 0.002), SOC (pseudo-F=11.5, p= 0.002), SM (pseudo-F=3.4, p= 0.012), soil pH (pseudo-F=2.3, p=0.018) and soil NH_4^+-N (pseudo-F=2.7, p= 0.026) significantly 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 NH_4^+-N (pseudo-F=4, p= 0.002) and soil NO_3^--N (pseudo-F=2.3, p= 0.012) that mainly came from applied fertilizer (Fig. 6). The microbial community positively correlated to soil NH_4^+-N in the 0-20 cm soil, to soil NO_3^--N negatively within 20-150 cm, while to soil NO_3^--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 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 (~200 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 in deep soil under NTSM can increase the resilience and resistance of maize to disturbances with higher grain production (Fig. 2). Zhang et al.(35) also observed litter-covered soil showed greater resistance to heating and copper addition due to the changes in soil properties and microbial community structure. Resistance to disturbance or stresses is the nature of a healthy soil and is essential for maintaining ecosystem functions, such as decomposing organic matter (35, 36). Under the NTSM treatment, the microorganisms associated with the degradation of relatively stable carbon compounds, such as Planctomycetes and Verrucomicrobia (SI Appendix, Table S3)(37, 38) as well as the indicator *Cellulomonas* and *Azospirillum* (Fig. 4 and SI Appendix, Table S2) with the function of cellulose decomposition(39, 40) were increased. The predicted functional profiles related to energy metabolism (Carbon fixation pathways in prokaryotes), carbohydrate metabolism (TCA cycle, amino sugar, nucleotide sugar, galactose, fructose), biosynthesis of secondary metabolites (Carotenoid and Betalain) and glycan biosynthesis were increased, suggesting a higher metabolic activity and a change in substrate quality (SI Appendix, Fig. S2). In addition, stover mulching also increased the ecological filter function of soil depth for selecting microbial communities as more indicator genera of each soil depths were identified under NTSM compared to NTNS and CT practices (Fig. 4 and SI Appendix, Table S2). And these indicators residing at different soil depths might enhance the anti-disturbance ability of NTSM. For example, denitrification bacteria *Caldithrix* and *Pseudomonas*(25, 26) were the indicator genera of 150-200 cm and 250-300 cm, respectively (Fig. 4 and SI Appendix, Table S2), which might explain the low nitrate in the deep soil in NTSM. *Ignavibacteria* and *Spirochaeta*, the indicator genera of deep soil, have the ability to grow under the conditions of strictly anaerobic(41) and severely limited nutrients(42), respectively. Surface indicator genera belonging to

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

Implications for climate change. It was observed that about 179.63, 352.34 and 427.34 kg ha^{-1} inorganic N were kept in the root-zone soil in the CT, NTNS and NTSM, respectively. Generally, corn roots reach their maximum depth at the silking stage(44), which is also the time when the heaviest rainfall occurs in northeastern China. We therefore expect that the available N kept in the root zone would be utilized by crops in the coming growing season, which means that fertilizer N could be cut to meet crop growth and also prevent reactive N losses. Since the nitrogen use efficiency (NUE) of maize system under the conventional management is 51% in northeast China (NUE is defined as the efficiency of fertilizer N transferring to harvested crop N)(45). Then, we conservatively calculate the required fertilizer N in the next year based on two assumptions: 1) the NUE of soil available N in root zone is equal to that NUE of applied fertilizer N, both of them are 50%; 2) the mineralized N during the coming growing season is neglected. Thus, N supply requirement = Fertilizer N×NUE + N in root zone ×NUE + Stover-N, where Stover-N for NTSM is 60 kg ha^{-1}. We estimated the N requirement for each disturbance practice by multiplying grain yield by grain N concentration (1.4%)(45) plus multiplying stover yield by stover N concentration (0.8%)(46). For CT, NTNS and NTSM, the mean annual corn yields were 10946.74, 12487.81 and 13416.81 kg ha^{-1}, and the stover yields were 966.67, 10083.33 and 10833.33 kg ha^{-1}, respectively. Thus, the N requirements were 230.6, 255.5 and 274.5 kg ha^{-1} for CT, NTNS and NTSM, respectively. Therefore, the theoretically conservative amounts of fertilizer N in the coming growing season are 281.6, 158.7 and 1.7 kg ha^{-1} for CT, NTNS and NTSM, respectively. No fertilizer-N is needed to apply without reducing corn yield in the NTSM plot. Compared to CT, the NTNS and NTSM could at least save respectively about 122.9 and 281.6 kg ha^{-1} N-fertilizer. For every kilogram of fertilizer-N produced and used on cropland, up to 87.9 MJ of energy is consumed(47) and 13.5 kg of CO_2-equivalent (eq) (CO_2-eq) is emitted(48). Hence, totally 24,752.6 MJ of energy consumption could be reduced and 3,801.6 kg CO_2-eq emission could be cut per hectare cornland in Northeast China at least by using NTSM tillage practice. If this could be applied to all maize farmland in China (42,000,000 ha, Source: China Statistics Yearbook 2018), 1.0 EJ of energy could be saved and 159.7 Mt of CO_2-eq could be reduced. Based on the average annual energy consumption for households of China in 2017 (15 EJ, China Statistics Yearbook 2018) and CO_2 emissions (9,839 Mt, Global Carbon Atlas), the NTSM practice in corn farming has the potential to save 6.7% of household energy and to reduce 1.6% of CO_2 emissions each year in China.

Taken together, we provide new evidence that low-disturbance practice promotes deep-soil stability to cope with environmental stress through increasing water and nutrient holding capacity, microbial richness, microbial diversity and ecological functions. According to ecological theory(49, 50), microbial community assembly in the CT treatment was mainly based on deterministic processes and significantly influenced by environmental stress and fertilizer nitrogen. Stover mulching might alter these processes through deeper roots affecting the vertical heterogeneity in resource availability(4). When energy resources are richer in the soil, environmental stress tend to alleviate(51), and higher biodiversity was caused due to more stochastic processes introduced in community assembly(52). Moreover, low disturbance practice also showed the potential to increase the maize yield (Fig. 2), save energy, and decrease the risk of groundwater leaching and greenhouse gas emissions. In view of the importance of microbial community assembly in predicting ecosystem service functions(53, 54), our results demonstrated that the lowest disturbance-practice — no-tillage with stover mulching increases the sustainability of agro-ecosystems.

Materials and methods

Site description and soil sampling. The field experiment was established in 2007 at the Lishu Conservation Tillage Research and Development Station of the Chinese Academy of Sciences in Jilin province, Northeast China (43.19° N, 124.14° E). The region has a humid continental climate with a mean annual temperature of 6.9 °C and the mean annual precipitation of 614 mm. The soils are classified in the Mollisol order (Black Soil in Chinese Soil Classification) with a clay loam texture(55). The site has been continuously planted with maize since 2007. We set up an experiment by a randomized complete block design with four replicates and five treatments. Each plot area was 261m^2 (8.7×30m). The five treatments included conventional tillage (moldboard plowing to a depth around 30 cm), no-tillage (no soil disturbance and direct seeding), and no-tillage with three-level stover mulching (33%, 67% and 100% newly produced maize stover were evenly spread over the soil surface each fall). For each treatment, slow-release fertilizer was applied at one time when sowing, which was equal to 240 kg/ha N; 47 kg/ha P; 90 kg/ha K. The rainfall data were obtained from local meteorological admistration. The grain yield was estimated by manually harvesting 20 m^2 area, randomly taken from each plot.

In this experiment, in order to reduce the damage to the plots and reduce costs, 3 plots were randomly taken from each treatment including conventional tillage (CT), no-tillage without stover mulching (NTNS), no-tillage with 100% stover coverage (NTSM) as three comparative practices. In April 2017, triplicate soil cores (0-300 cm) were collected from each plot at the dormant season. After removing surface stover, we took soil cores by a stainless-steel hand auger and sliced each into ten layers: 0-10 cm, 10-20 cm, 20-40 cm, 40-60 cm, 60-90 cm, 90-120 cm, 120-150 cm, 150-200 cm, 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 (NH_4^+-N) and nitrate nitrogen (NO_3^--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 NH_4^+-N and NO_3^--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, 5μl 10× Pyrobest buffer and 0.3 U of Pyrobest polymerase (Takara Code: DR005A). The PCR cycle conditions were as follows: initial denaturation at 95°C for 5 min followed by 26 cycles of denaturation at 95°C for 45s, annealing at 50°C for the 30s, and extension at 72°C for 45s, with a final extension at 72°C for 10 min. Each sample was amplified for three replicates. The PCR products from the same sample were pooled, checked by 2% agarose gel electrophoresis and were then purified using AxyPrepDNA agarose purification kit (AXYGEN). Finally, purified PCR products were sequenced on an Illumina MiSeq platform PE300 sequencer (Illumina, USA).

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

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

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

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Data 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). All other relevant data are available from the corresponding author on request.

Supporting information

This article contains supporting information online at …..

Competing interests

The authors declare no competing interests.

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Figure Legends

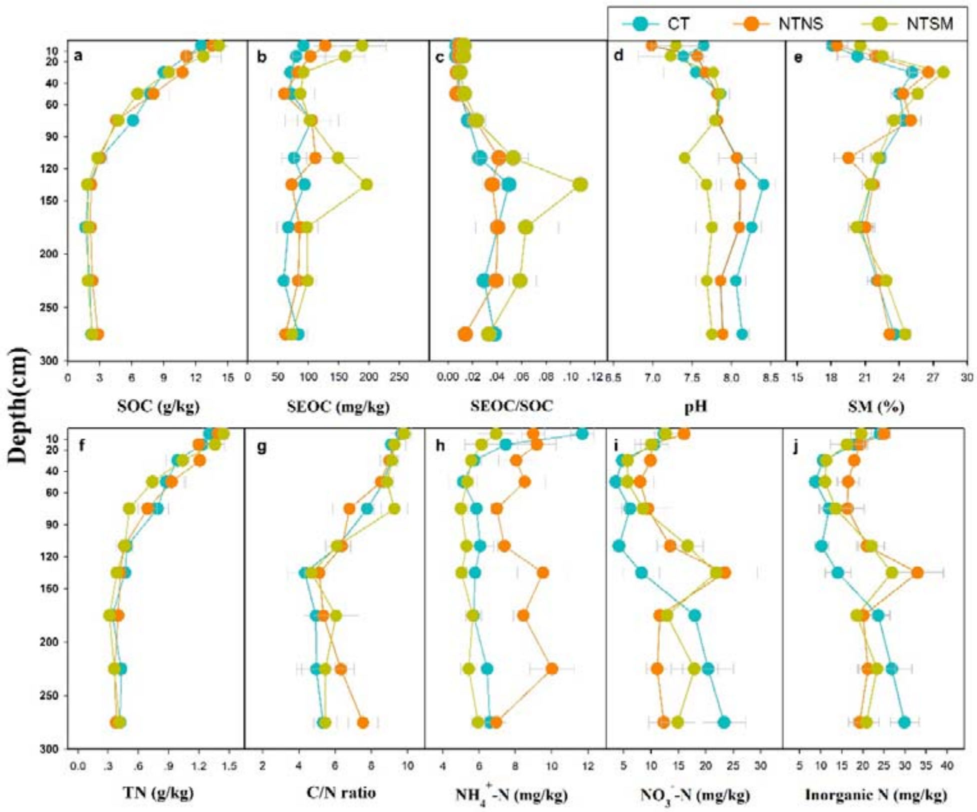

Figure 1. Soil properties (mean±SE, n = 3) along soil depth under different practices. SOC = soil organic carbon, SEOC = salt-extractable organic carbon, SEOC/SOC = ratio of SEOC to SOC, SM = soil moisture, TN = total nitrogen content, C/N = ratio of SOC to TN, NH_4^+-N = ammonium nitrogen, NO_3^--N = nitrate nitrogen, Inorganic N = NH_4^+-N + NO_3^--N.

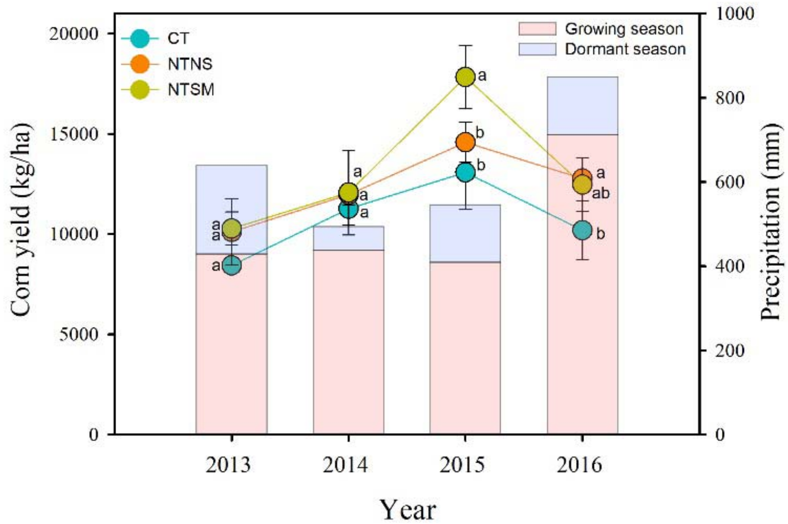

Figure 2. Corn yield (line+symbol) and annual rainfall during growing and dormant seasons (bar) under different disturbance practices during 2013-2016. Error bars indicate standard errors (n = 3 or 4), different letters indicate significant differences at $P < 0.05$.

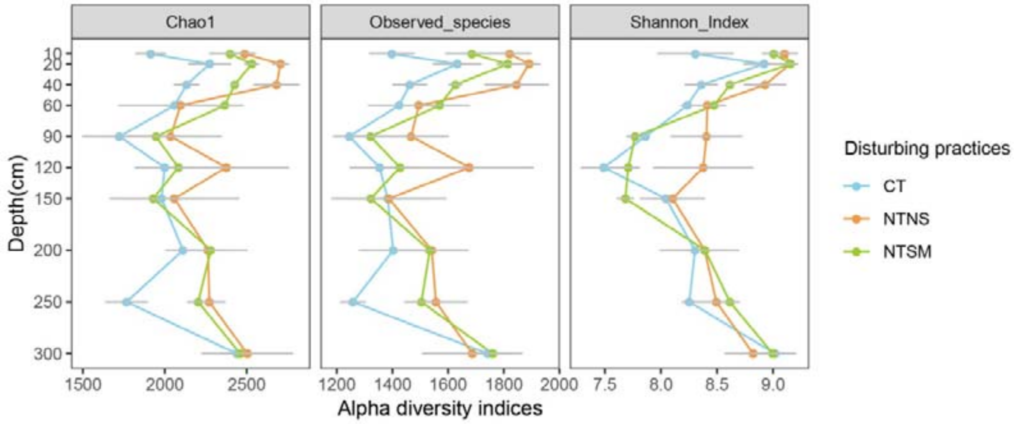

Figure 3. Microbial richness (Chao1), observed number of species (Observed_species) and diversity (Shannon_Index) in the CT (conventional tillage), NTNS (no-tillage without stover mulching) and NTSM (no-tillage with stover mulching) plots. Error bars indicate standard deviation (n = 3).

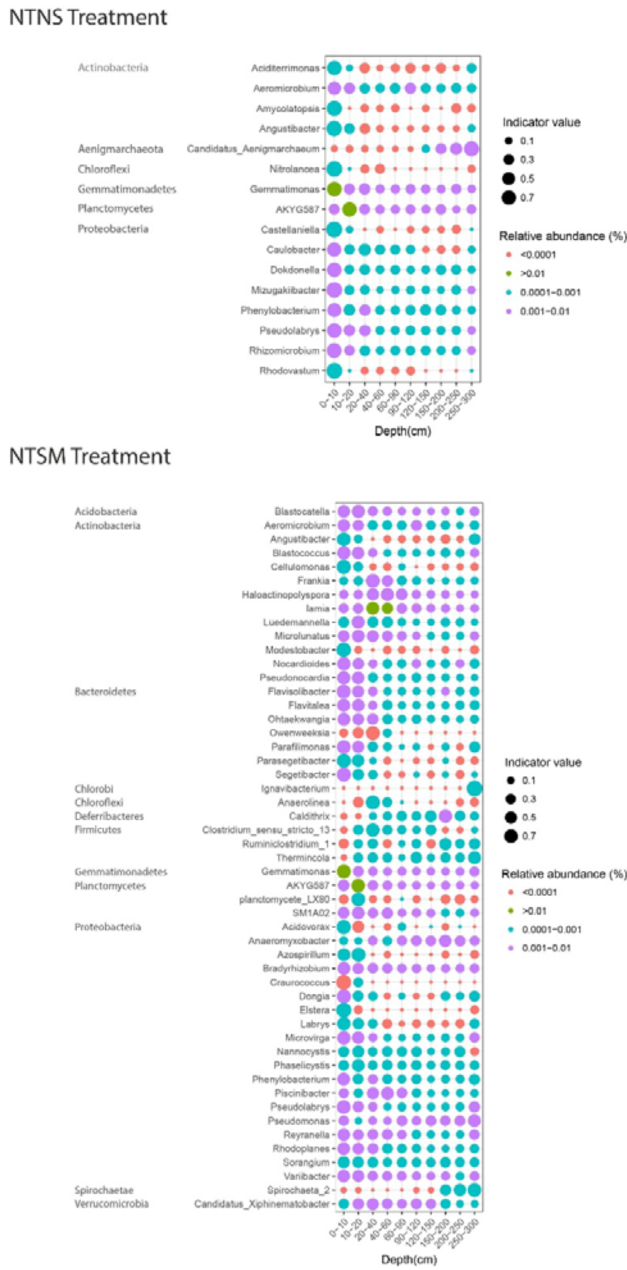

Figure 4. Indicator genera significantly (q < 0.1) associated with tillage practices. The size of each circle represents the indicator value of a specific genus in the different soil depths. The color indicates the relative abundance of each indicator genus. Taxonomic information, indicator values, P-values,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

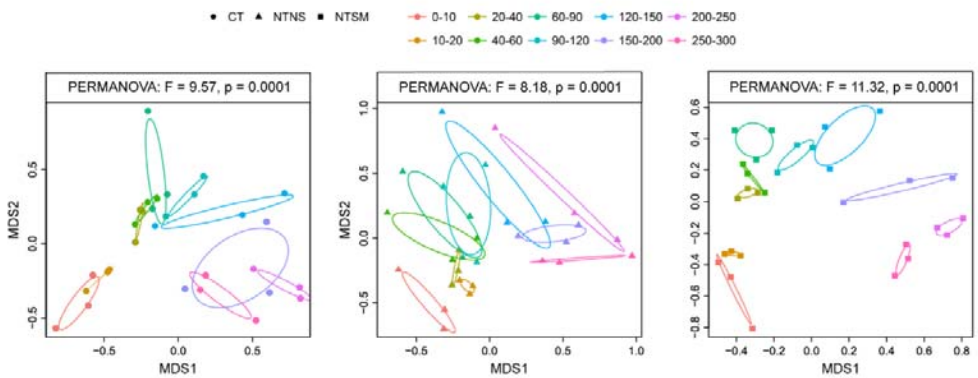

Figure 5. Non-metric multidimensional scaling (MDS) ordination of soil microbial community structures based on Bray-Curtis distances among soil depths at different agricultural disturbance practices. Permutational multivariate analysis of variance (PERMANOVA) revealed that the overall microbial community structures among soil depth were significantly different at each disturbance practice. Circles, triangles and squares represent CT (conventional tillage), NTNS (no-tillage without stover mulching) and NTSM (no-tillage with stover mulching), respectively.

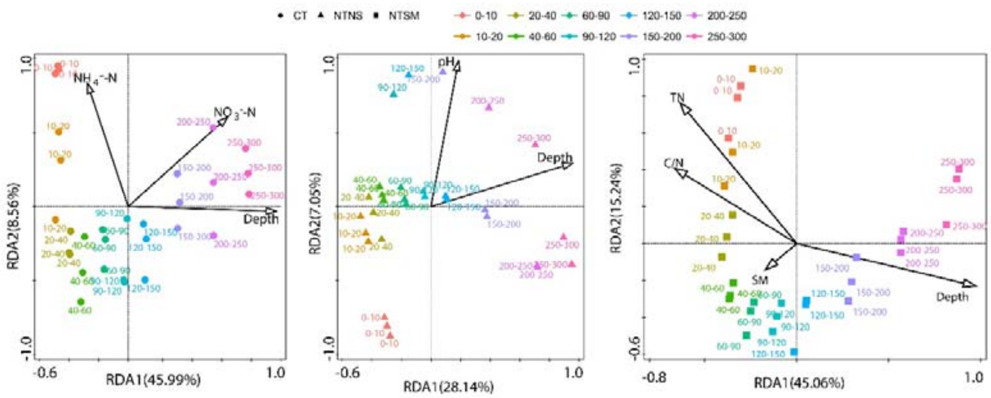

Figure 6. Redundancy analysis (RDA) of the soil microbial community originating from microbial phyla constrained by soil properties under different agricultural practices. Only soil variables that significantly explained variability in microbial community structure in the forward selection procedure were selected to the ordination (arrows). TN, total nitrogen content; C/N, a ratio of carbon to nitrogen content; NH_4^+-N, ammonium nitrogen; NO_3^--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.

Low-disturbance farming regenerates healthy critical zone towards sustainable agriculture

Supplementary Information

Supplementary Figures

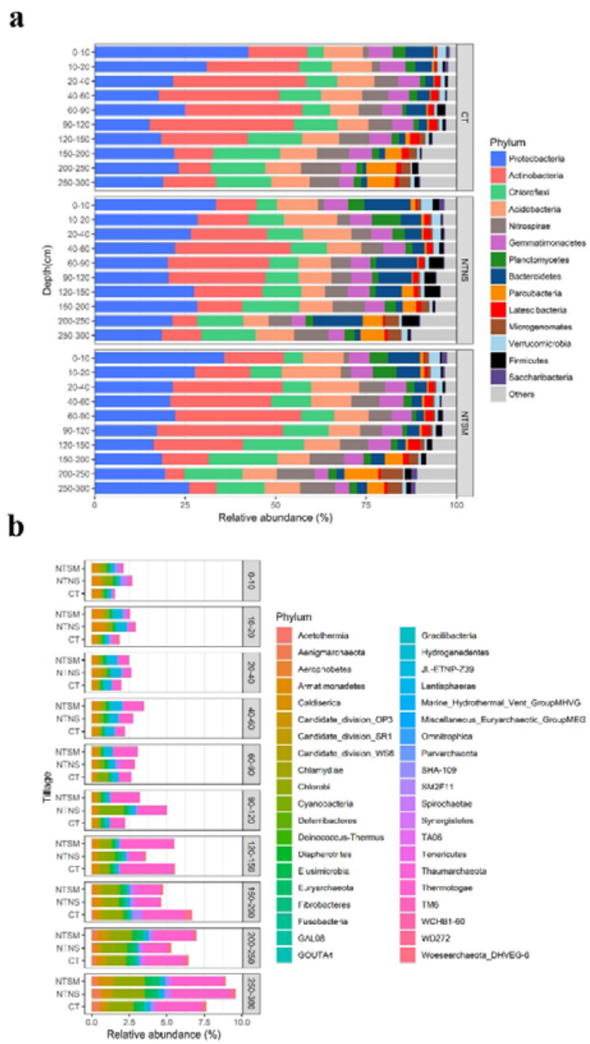

Figure S1. The relative abundance of bacterial community composition at the phylum level. **a** Only the bacterial phyla with the relative abundance > 0.1% across all soil samples were shown. **b** “Others” in the (**a**) panel represents the sum of bacterial phyla that individual relative abundance < 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).

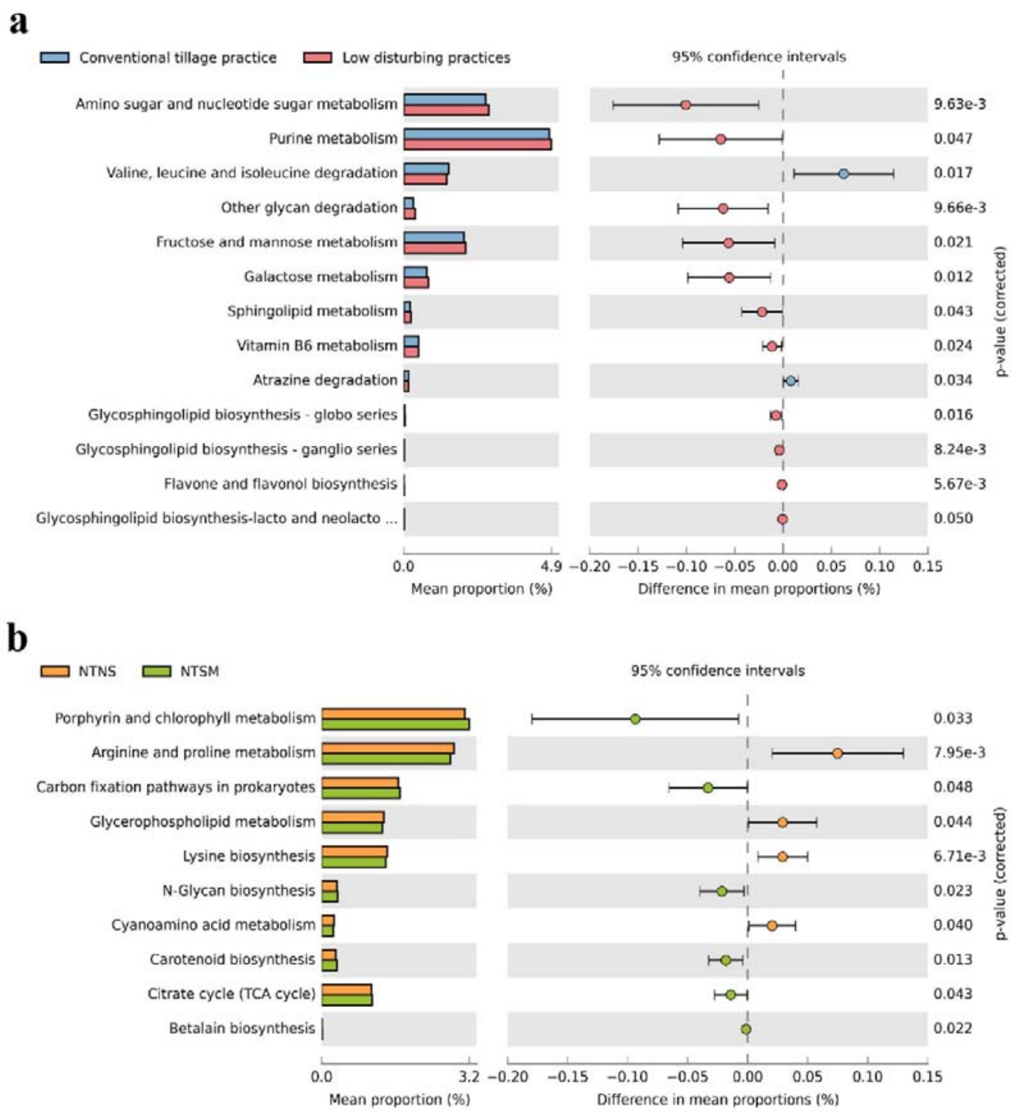

Figure S2. Extended error bar plots showing significant differences of 16S rRNA gene-predicted functional profiles obtained with Tax4Fun. **a** difference between mean proportions of conventional practice and low disturbance practices; **b** differences between mean proportions of NTNS (no-tillage without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

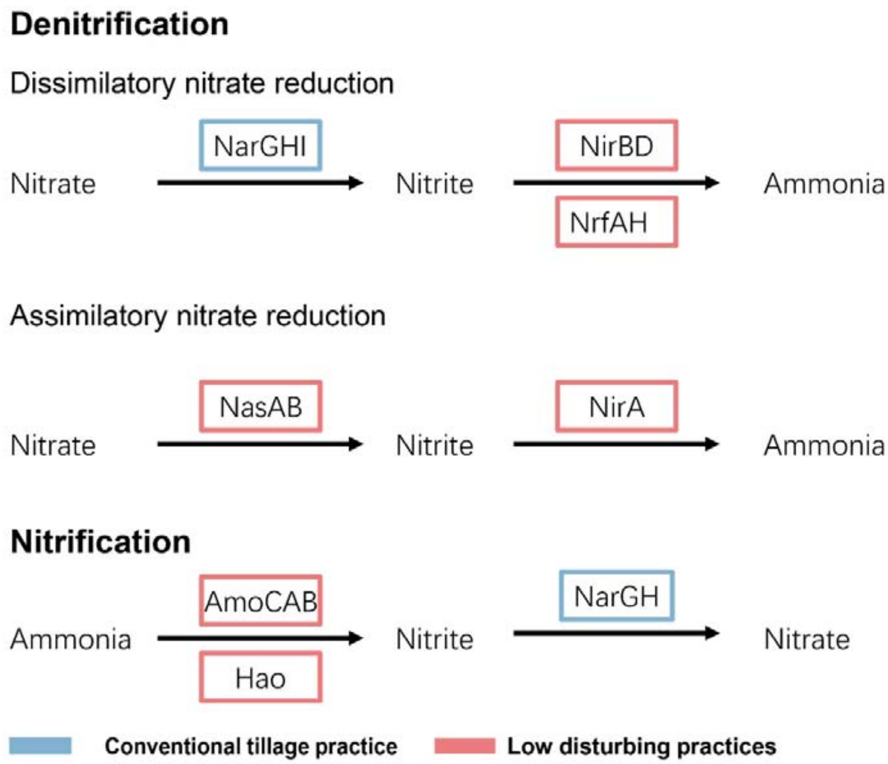

Figure S3. The denitrification and nitrification genes that influenced by different tillage practices. Genes in red rectangles means higher abundance in low disturbing practices; Genes in blue rectangles means higher abundance in conventional tillage practice.

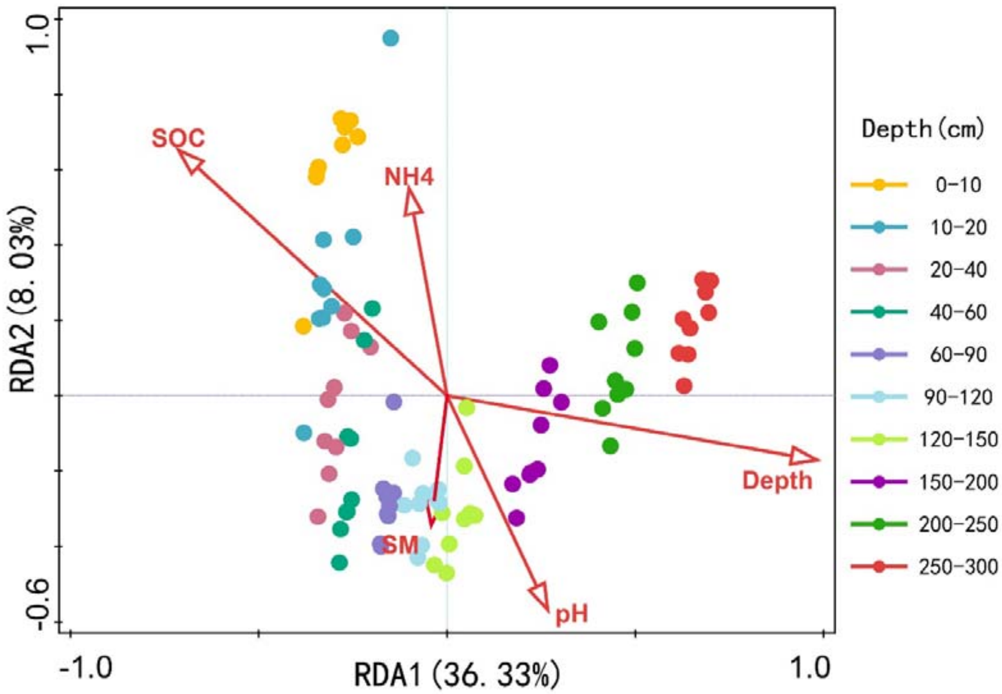

Figure S4. Redundancy analysis (RDA) of soil microbial community originating from microbial phyla constrained by soil properties among soil depths. Only soil variables that significantly explained variability in microbial community structure in the forward selection procedure were selected to the ordination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