Soil priming effects and involved microbial community along salt gradients

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Abstract

Soil salinity mediates microorganisms and soil process, like soil organic carbon (SOC) cycling. Yet, how soil salinity affects SOC mineralization via shaping bacterial communities diversity and composition remains elusive. Therefore, soils were sampled along a salt gradient (salinity at 0.25%, 0.58%, 0.75%, 1.00% and 2.64%) and incubated for 90 days to investigate i) SOC mineralization (i.e., soil priming effects induced by cottonseed meal, as substrate) and ii) responsible bacteria community, by using high throughput sequencing and natural abundance ^{13}C isotopes (to partition cottonseed meal derived CO_2 and soil derived CO_2). We observed negative priming effect during first 28 days of incubation and turned to positive priming effect after day 56. Negative priming at the early stage might be due to the preferential utilization of cottonseed meal. The followed positive priming decreased with the increase of salinity, which might be caused by the decreased alpha diversity of microbial community in soil with high salinity. Specifically, soil pH and EC along salinity gradient were the dominant variables modulating the structure of microbial community and consequently SOC priming (estimated by distance-based multivariate analysis and path analysis). By adopting O2PLS, priming effects were linked with specific microbial taxa, e.g., *Proteobacteria* (*Luteimonas*, *Hoeflea* and *Stenotrophomonas*) were the core microbial genus that attributed to the substrate induced priming effects. Here, we highlight that the increase of salinity reduced the diversity of microbial community and shifted dominant microorganisms(*Actinobacteria* and *Proteobacteria* (*Luteimonas, Hoeflea* and *Stenotrophomonas*)) that determined SOC priming effects, which provides a theoretical basis for understanding of SOC dynamics and microbial drivers under salinity gradient.

Keywords: *Salt gradient, priming effects, bacterial community, core microorganisms*

1. Introduction

Soil salinization is an increasing environmental problem caused by natural and human activities in the arid and semi-arid area (Wichern et al., 2006). Salinization is often a major threat to crop productivity in agricultural land. Soil microorganisms suffer from osmotic stress. Soil salinity often cause microbial death or dormant. It was widely reported that the increased salinity decrease microbial biomass, enzymatic activity, and alpha diversity of microbial community (Laura, 1974; Pathak et al., 1998; Rietz et al., 2003). Soil salinity is reported to the major determinants of composition, activity of microbial community (Kamble et al., 2014). Although salinity is reported to be a vital factor in influencing microorganisms in the arid and semi-arid area, limited studies investigated C processes (e.g., priming effect) driven by microbial community in salinity soils (Sardinha et al., 2003).

Soil organic carbon (SOC) is the largest pool (1500 Pg C) in the terrestrial carbon (C) cycle, and contains twice as much C as the atmosphere (Filley et al., 2006; Wiesmeier et al., 2019). The input of substrate C can influence the output (i.e., CO_2 release) through a phenomenon called priming effect, which was firstly discovered by LÖhnis (1926). Substrate additions accelerate or decrease soil organic C mineralization, referred to positive or negative priming effects (Kuzyakov et al., 2000). The intensity of the priming effect affects the turnover of SOC and thus storage pool (Sullivan et al., 2013). Soil priming effects are affected by many biotic and abiotic factors (Lavelle et al.,1997; Martin et al.,2019), to investigate abiotic and biotic mechanisms underlying SOC priming enhance strong understanding of the SOC cycling.

Soil priming effects is affected by soil fauna animals (Scheu et al., 1994), activities, diversity and composition of microbial community (Fontaine et al., 2011). The microbial decomposers are the major player in the decomposition process of added C sources. The addition of substrate, such as composts (Xun et al., 2016), animal sludges (Hartmann et al., 2015), sewage sludges (Su et al., 2017; Wagner et al., 2011) and plant residues (Dai et al., 2017), generally increases soil microbial biomass C and stimulates the microbial activities thus enhanced the loss of SOC (positive priming effects) (Fontaine et al., 2003; Bird et al., 2011; Li et al., 2018; Ali et al., 2019).

Concerning abiotic factors, the priming effect can be controlled by climate variables (Hagemann, 2008), and soil properties, like pH, EC, TN(Blagodatskaya et al., 2008; Luo et al., 2017). To understand how environmental and edaphic factors affect the processes of SOC mineralization, is important to estimate terrestrial C pool (Lehmann et al., 2015). Although many studies have tested the effects of soil pH, SOC content, and other edaphic variables on soil priming effect, few study investigated soil priming effects in salinity soil (Asghar et al., 2012), especially linked with soil microbial community structure and their functions in C decomposition (Soina et al., 2018).

Thus, we sampled the soils along natural salinity gradients (0.25%, 0.58%, 0.75%, 1.00%, 2.64% apart from total water-soluble salt). Based on these soils, we conducted a 90 days of indoor incubation applying C3 substrate of cottonseed meal ($\delta^{13}C$=-23.47‰) to C4 soils with salt gradient ($\delta^{13}C$ between -14.21‰ and -16.01‰), to investigate: 1) mineralization rate of cottonseed meal and induced soil priming effects along salt gradients; 2) diversity of microbial community in the soils with increased salinity, and 3) identify the bacteria taxa associated Soil priming. We hypothesized that i) soil microbial community diversity and composition will be different with the different in soil variables particularly pH and EC along salinity gradients, and ii) Soil C processes like priming effects will be regulated mainly by microbial community and especially the core microbial species. To clarify the priming effects and involved microbial groups would help us better understanding C sequestration potential and underlying mechanisms in saline soils.

2. Materials and methods

2.1. Soil sampling and cottonseed meal production.

The soil type was gray desert soil, which was collected from farmlands (82.90° E, 44.96° N) in Xiao Yinpan town, Bole City, Bortala, northern Xinjiang Uygur Autonomous Region, northwest China. The farmlands soil is naturally formed original saline-salinity soil and with a continuous 30 years planting of maize (C4 crop) and maize straw returning to soil for 7-8 year. In September 2021, we determining the sampling area, and use the five-point sampling method to collecting non-rhizosphere soil. The soil samples were indoor air drying and hand-picked to remove visible other debris, animal and plant residues and then sieved at field moisture (<2mm) and subsequently adjusted to 40% of water holding capacity (WHC). Determination of five salinity gradients at 0.25%, 0.58%, 0.75%, 1.00% and 2.64% through soil salinity measurements.Texture was determined by the pipette method without carbonate in all soil samples. They were then incubated at 25 ºC for 7 days before starting the experiments, to allow any early sampling and sieving effects to subside.

Cottonseed meal is a kind of reddish or yellow granular material obtained by pressing, leaching and other cottonseed. The cottonseed meal was purchased from the market and dried at 105 °C for 24 h indoor, then further pulverized by a ball mill and passed through < 2 mm sieve.

2.2. Soil and substrate analyses

EC and pH of soil and cottonseed meal were measured at a soil: water ratio of 1:5 (weight/weight) (Bao, 2000). Air-dry soil (5 g, <2 mm) and 25 ml of deionised water were shaken together for 1 min and left to settle for 30 min, which was repeated once more before pH was determined with a pH electrode. Soil water-soluble salt was analyzed by weighted at a soil:water ratio of 1:5 (weight/weight). Air-dry soil (5 g, <1 mm) and 25 ml of deionised water were shaken together for 30 min, filtration to obtain clear filtrate, using thermostat water bath to evaporate and weigh(Bao, 2000). Soil total carbon (TC), total nitrogen (TN) are collect soil to be tested was dried and ground through a 0.15 mm screen, and a certain amount of treated soil sample was wrapped in tin foil and placed in an element analyzer for determinatio (air-dried, milled <150 μm) were determined by dry combustion (LECO CNS 2000, LECO Corporation, Michigan, USA). Soil microbial biomass C was determined by fumigation extraction (Vance et al., 1987; Wu et al., 1990). The K_2SO_4 extractable organic C was determined using an organic carbon autoanalyser (Shimadzu, Analytical Sciences, Kyoto, Japan). Soil microbial biomass C (Bc) was calculated from: Bc = 2.22 Ec, where Ec = [(organic C extracted from fumigated soil) minus (organic C extracted from non-fumigated soil)]. The natural $\delta^{13}C$ (‰) abundance of the soils (air-dried, milled <200 μm) was determined using an elemental analyser-isotope ratio mass spectrometer (Sercon Ltd, Crewe, UK). All measurements are given on an oven-dry weight basis (o.d., 105 °C, 24 h).

The $\delta^{13}C$ (‰) abundance of the cottonseed meal (air-dried, milled <200 μm) was determined using an elemental analyser-isotope ratio mass spectrometer (Sercon Ltd, Crewe, UK). The main elemental composition of the substrate was determined using elemental analysis (Vario EL Cube, Hanau, Germany), with the samples combusted at 1200 °C. Natural $\delta^{13}C$ (‰) abundance ,the total carbon, total nitrogen contents and C/N of the cottonseed meal was presented in Table 1.

2.3. Experimental design

After pre-incubation, five soils with salinity gradient(salinity at 0.25%, 0.58%, 0.75%, 1.00% and 2.64%) were thoroughly mixed with cottonseed meal at 20 mg C g^{-1} soil (d.w. basis), and incubated over 90 days following moisture adjustment to 40% of water-holding capacity (WHC) to investigate the substrate mineralization and priming effects. Each soil sample (40 g d.w. basis) was incubated in a 100 ml beaker inside a 1 L brown glass jar. Three jars with only water and NaOH were set as blank. All the jars were sealed with a rubber bung and incubated in a randomized block design at 25 °C for the 90 days of incubation. The NaOH vials were changed after 1, 3, 5, 7, 14, 28, 56 and 90 days for determination of evolved CO_2 and ^{13}C-CO_2 (‰). Meanwhile, soil biomass C, NH_4^+, NO_3^-, pH, EC, TC, TN and DNA extraction were measured at day 28.

2.4. Soil CO_2-C and its isotopic composition

Soil C evolved as CO_2-C in jars was measured by trapping CO_2 in 1 M NaOH (20 ml) during soil incubation. After the NaOH (20 ml) trapping CO_2 at different periods of soil incubation, 5 ml 1 M NaOH of each sample was mixed with 10 ml deionised water and titrated with 0.05 M standardised HCl by the TIM840 autotitrator (Radiometer Analytical, Villeurbanne Cedex, France). Meanwhile, the $\delta^{13}C$ (‰) of trapped CO_2-C was precipitated, with 8 ml of the 1 M NaOH (20 ml) mixed with 8 ml 1.5 M $BaCl_2$ in vials (Aoyama et al., 2000). The $BaCO_3$ precipitate was trapped on the glass fibre the filter, rinsed with deionised water several times, and dried overnight (80 °C), weighed (0.100-0.200 mg) into tin capsules, and analyzed for $\delta^{13}C$ on an elemental analyzer-isotope ratio mass spectrometer (Sercon Ltd, Crewe, UK).

2.5. DNA exaction and sequencing

The total soil DNA was extracted from 0.50 g of moist soil using a FastDNA Spin Kit (MP Biomedicals, Santa Ana, CA, USA) according to the manufacturer's protocol. The extracted DNA was dissolved in 50 μl of TE buffer, quantified using a spectrophotometer and stored at -20 °C until sequencing.

V3-V4 hypervariable regions of the bacterial 16S rRNA gene were amplified with primers 341F (5'-CCTAYGGRBGCASCAG-3') and 806R(5'-GGACTACHVGGGTWTCTAAT-3'). The PCR reactions were conducted with a thermocycler PCR system (GeneAmp 9700, ABI, USA) by using the following programs: 3 min of denaturation at 95 °C; followed by 27 cycles of 30 s at 95 °C, 30 s at 55 °C, and 45 s at 72 °C; and a final extension at 72 °C for 10 min with a thermocycler

PCR system (GeneAmp9700, ABI, USA). PCR amplicons pooled from the triplicate reactions were purified using a QIAquick PCR purification kit (Qiagen, Shenzhen, China), and quantified using a NanoDrop ND-1000 spectrophotometer (Thermo Scientific, Waltham, MA, USA). The PCR products were purified, mixed, and sent to Majorbio, Inc. (Shanghai, China) for sequencing based on the Illumina MiSeq platform.

2.6. Calculations

2.6.1. CO_2-$\delta^{13}C$ emission

The mineralisation of cottonseed meal was separated from SOC mineralisation according to the change of stable isotopic composition ($\delta^{13}C$) with time. The standard equation for determining $\delta^{13}C$ (‰) is derived from:

$$\delta^{13}C\ (‰) = [(R_{sample}/R_{VPDB}) - 1] \times 1000, \qquad \text{Eqn. 1}$$

where R_{sample} is the mass ratio of ^{13}C to ^{12}C of each sample and R_{VPDB} is the international PDB(Peedee Belemnite) limestone standard. The labeled ^{13}C (%) of cottonseed meal was then estimated from:

$$CO_2\text{-}^{13}C\ (\%) = (\delta_{treatment} - \delta C4) / (\delta C3 - \delta C4), \qquad \text{Eqn. 2}$$

where CO_2-^{13}C (%) is the proportion of evolved CO_2 from C3 (cottonseed meal) matter, $\delta_{treatment}$ is the $\delta^{13}C$ (‰) in treatments of soil with cottonseed meal, $\delta C4$ is the $\delta^{13}C$ (‰) in control soil and $\delta C3$ is the $\delta^{13}C$ (‰) from cottonseed meal. Thus, the CO_2-C produced from cottonseed meal during the incubation was calculated from:

$$CO_2\text{-}^{13}C\ (\mu g\ g^{-1}\ soil) = CO_2\text{-}^{13}C\ (\%) \times total\ CO_2\text{-}C\ (\mu g\ g^{-1}\ soil)/100, \qquad \text{Eqn. 3}$$

CO_2 from SOC was CO_2-^{13}C subtracted from total evolved CO_2-C. The absolute soil priming effect (or primed soil CO_2-C) with the addition of cottonseed meal was calculated from:

$$Primed\ soil\ CO_2\text{-}C\ (\mu g\ C\ g^{-1}\ soil) = CO_2\text{-}C_{treatment} - CO_2\text{-}C_{control} \qquad \text{Eqn. 4}$$

where CO_2-$C_{treatment}$ is the non-isotopically labeled CO_2-C evolved from cottonseed meal amended soil, CO_2-$C_{control}$ is non-isotopically labeled CO_2-C evolved from soil without cottonseed meal.

2.7. Statistics

The data of ^{16}S gene sequencing were processed using the Quantitative Insights Into Microbial Ecology (QIIME) 1.9.0-dev pipeline (Caporaso et al., 2010). In brief, Reads with less than length 200 bp and ambiguous bases were discarded. The sequences were then binned into operational taxonomic units (OTUs) by UCLUST (Edgar, 2010) based on 97% pairwise identity. Chimeric OTUs identified by USEARCH (Edgar et al., 2011) in QIIME were removed. The most abundant sequence from each OTU was selected to represent that OTU. Taxonomy was assigned to 16S OTUs against a subset of the Silva 104 database. The representative OTU sequences were aligned using PyNAST (Caporaso et al., 2010). We obtained between 64,425 and 89,989 clean_reads per sample for all experimental samples.

To avoid potential bias caused by sequencing depth, all sample datasets were rarefied for the bacteria α-diversity and β-diversity analyses. Faith's phylogenetic diversity was calculated to provide an integrated index of the phylogenetic breadth across taxonomic levels (Faith, 1992). To compare β-diversity between samples, principal coordinate analyses based on the unweighted and weighted UniFrac (Lozupone et al., 2007a) distances were calculated using the function 'pcoa' in the R package 'Ape'. Additionally, permutational multivariate analysis of variance (PERMANOVA) was carried out using the function 'adonis' in the R 'vegan' to measure effect size and significance on β-diversity. The variable influence projection (VIP) value was processed using the way of O2PLS analysis by the SIMCAP 14 (Version 14.1.0.2047) (Wang et al., 2016). The y-matrix was defined as the environmental factors datasets and the x-matrix was defined as the microbial community on genus level dataset.

Data were logarithmically transformed and analyzed by ANOVA. All analyses were performed using SPSS software (13th edition). Pearson's correlation analyses were performed to assess the linear correlation among soil physio-chemical properties and microbial community. MULTIVARIATE analysis were operated to investigate interaction of salinity treatments on bacteria community parameters.

3. Results

3.1. Soil physicochemical properties along salt gradients

The major soil physicochemical properties along salt gradients were presented (Table 1) and all of soil physicochemical properties has significant difference ($p < 0.05$). The total soluble salinity content in the soils ranged from 0.25% to 2.64% of salinity soils, soil salt gradients increasing gradually from salinity 1 samples to salinity 5 samples. The pH and EC in soils ranged from 8.45 to 8.85 and from 1.06 ms cm^{-1} to 7.75 ms cm^{-1}. Soil total C and N were increased with salinity, ranging from 3.16% to 3.57%, and from 0.18% to 0.26%. The $\delta^{13}C$ value for soils are between -14.21‰ and -16.01‰, which were relatively enriched compared to cottonseed meal (-23.47‰). This allowed separation of soil derived CO_2 from total evolved CO_2, according to the classic mixed modeling.

3.2. Total CO_2 evolution

During the whole 90 days of incubation, the cumulative CO_2 evolved had similar trends, which the amount of CO_2 increased with the incubation times (Fig. S1). The cumulative CO_2 evolved increased more rapidly with the addition of cottonseed meal before 14 days, compared to non-amended soils. At 90 days of incubation. The cumulative CO_2 evolved in the soil with the lowest salinity (Salinity 1) gave the lowest CO_2 emission (597 μg C g^{-1}) in the non-amended soils (Fig. S1, $p < 0.001$).

3.3. Cottonseed derived $^{13}CO_2$ and soil priming effects

The total cumulative CO_2-C was divided three parts based the $\delta^{13}C$ value, including basal soil-derived CO_2, cottonseed meal-derived CO_2 and primed soil CO_2 (Fig.1). The cottonseed meal-derived CO_2 had a significant contribution to the total CO_2

evolved during the early incubation period. The cottonseed meal-derived CO_2 was significantly higher in Salinity 1, Salinity 2 and Salinity 3 than in Salinity 4 and Salinity 5 before 28 days incubation. Meanwhile, the soil priming effects was negative in all amended soil treatments before 28 days incubation and the direction of priming effect in most of soil samples turned into positive after 28 days. During the whole 90 days incubation, there was a negative correlation between cottonseed meal-derived CO_2 and primed soil CO_2 (Fig. 2).

3.4. Bacterial diversity and community structure

The number of sequences ranged from 64,425 to 91,261 for per sample (average valve of 80,602). About 27,990 OTUs in total were obtained under different five treatments. Bacterial community diversity was measured by a series of OTU-based analyses of alpha diversity including chao1 estimator, and observed_species in the QIIME pipeline (Fig. 3). Chao1 diversity estimator and observed_species was significantly different in treatments, being the highest in Salinity 1, followed by Salinity 3, Salinity 2, Salinity 4 and Salinity 5 ($p < 0.01$). In general, bacterial community diversity decreased with increasing salinity (Fig. 3).

The most abundant phylum in the soils and their correlation with salinity were shown in Fig. 4. Among them, *Actinobacteria* was the dominant taxa in all soils, with the abundance ranging from 50.07 % (Salinity 3) to 68.99 % (Salinity 4). The relative abundance of *Bacteroidetes*, *Firmicutes* and *Deinococcus-Thermus* increased with the salinity, while *Acidobacteria* decreased with salinity degree.

Based on OTUs of five gradient salt treatments, the PCA analysis showed that treatments from Salinity 2 and Salinity 4 clustered together. Meanwhile, soil samples of Salinity 1, Salinity 3 and Salinity 5 distributed in the first, fourth and three quadrant, which indicated that these treatments had large environmental heterogeneity (Fig. S4).

In order to visualize the relationship between environmental factors and microbial community, *Canonical Correspondence Analysis* (CCA) was conducted, showing that

NO_3^--N, EC and TC had a more obvious impact than other factors for microbial community (Fig. 3). Soil EC were positively correlated with pH, NH_4^+-N, and negatively correlated with TN, TC and MBC. Mantel test and Distance-based multivariate analysis showed the contribution rate of different environmental factors account for 78% of the variability of microbial communities (Table 2). The value of pH (31%) and EC (12%) had a strong influence on microbial community.

3.5. Relation between soil microbial community and C dynamics

Based on the O2PLS analysis, the variable influence projection (VIP) values of bacterial genus more than 1.00% were showed their contributions to C decomposition of cottonseed meal-derived C, basal soil-derived C, and primed soil C (Table 3). There were many microbial taxa positively correlating to soil primed CO_2, for insatnce, genera of *Actinomarinales*, *Luteimonas*, *Nocardioides*, *Hoeflea*, *Intrasporangium*, *Nitrolancea*, *Pseudarthrobacter* and *Stenotrophomonas* had a positive correlation with primed CO_2. In order to further to evaluate the relationship between soil properties, soil bacterial communities and C decomposition, we used the structural equation modeling (SEM) to suggest the direct and indirect impacts of salinity and microbial community on soil C decomposition (Fig. 4). The result showed that soil pH and EC had negative contribution to bacterial diversity, while bacterial diversity had a strong positive influence on the primed soil C (Fig. S4). For instance, salinity properties of EC had a directly negative influence on the bacterial diversity but positive influence on the primed soil C. Meanwhile, pH were negatively correlated with bacterial diversity and positively correlated with substrate derived C.

4. Discussion

4.1. Soil priming effects along salty gradients

Understanding soil C dynamics along salinity gradients is crucial to predict C sequestration in salty soils. In the early stage of the incubation, we observed that the cumulative substrate derived CO_2 in the soils with lower salinity was significantly higher than soils with higher salinity (Fig. 1), which can be possibly explained by that high salinity inhibited microbial activity. Many studies have reported the influence of soil salinity on organic matter decomposition, mostly, the decomposition of organic matter are decreased by salinity (Wichern et al., 2006; Ghollarata et al., 2007; Tripathi et al., 2007; Setia et al., 2012). Yet, the response of microbial community to the increasing levels of salinity and consequent effects on soil priming effects remains largely unknown.

Here, we found soil priming effects was gradually changed from negative to positive priming effect (Fig. 1). The early pattern of the dynamics of the priming effect in this study was similar to other studies showing preferential utilization of labile C substance. The first phase of negative priming effects was likely to be caused by microbial assimilation of substrate. The soil microbes turned to use the new added substrate and thus used less of the original SOC. This was attributed to "preferential substrate utilization" (Perelo et al., 2005).

Soil microbial biomass-related growth predominating in the first phase were most likely to utilize SOC, leading to a positive priming effects after substrate was largely vanished. The magnitude of priming effects depends on soil microbial biomass size (Schneckenberger et al., 2008). It was found that the amount of added easily available organic C is beyond 50% of microbial biomass C (Blagodatskaya et al., 2008). Namely, the second phase of positive PEs probably was due to increased biomass size and enhanced demand on SOC. Secondly, C that was assimilated into microbial biomass in the first stage may also be mineralized in the second stage due to the turnover of microbial biomass (Shahbaz et al., 2017; Perelo et al., 2005).

4.2. Microbial community along salt gradients

Previous studies concerning the impact of salinity on soil microbial community used different soils with a range of salt levels. In the present study we investigated the influence of soil salinity on microbial communities in soils from the closed area covering a range of salt content. Similarly, Rousk et al. (2011) also used agricultural soils from the same area representing a range of soil salinity. Here, we found microbial diversity (alpha diversity) decreased with increasing salinity (Fig. 3). The negative impact on microbial diversity can be explained by that the accumulation of large amounts of salt in the soil raised the extracellular osmotic concentration (Rath et al., 2015; Oren, 2011). The high osmotic pressures made it difficult for many microorganisms to adapt to and thus reduce their biological activity. The changes of soil microbial community structure were also explained by salinity (Herlemann et al., 2011; Campbell et al., 2013). We found that *Bacteroidetes*, *Firmicutes*, *Acidobacteria* and *Deinococcus-Thermus* were dominant in these soils (Fig. 4). These results are supported by previous findings that *Firmicutes* possess the high salinity resistance. Other studies also found that *Bacteroidetes* is dominant taxa in alkaline saline soil because of its resistant to salt (Valenzuela-Encinas et al., 2009; Keshri et al., 2013). Other study shows that the dominant phyla are *Bacteroidetes* and followed by *Proteobacteria* in the haloalkaline soil (Keshri et al., 2013). These results are consistent with the esuarine or marine environments, despite some studies suggest that soil salinity is not found to be a decisive factor for bacterial community and their growth (Rousk et al., 2011).

The difference of microbial community structure is affected by many soil variables, and pH and EC were the most important ones (Fig. 3; Table 2). Our results showed that the value of soil pH and EC would significantly affect the microbial community structure and the combined contribution rate of these two variables to microbial community was 43% (Table 2). At high levels of salt and alkaline arid condition, soil pH has been also shown to have a very powerful influence on the soil bacterial community structures (Bååth et al., 2003; Fierer et al., 2006; Rousk et al., 2010). Meanwhile, it is consequently unlikely that soil pH differences between the studied soils obscured the influence of salt (Rousk et al., 2011). Salinity has been identified as one of the most potent environmental factors that determine assembly of microbiome. Salinity has been regarded to play the vital role in shaping microbial community in different ecosystem. This, despite the clear evidence from aquatic microbial ecology (Lozupone et al., 2007b), show a potential for salt to affect soil microbial communities apart from that of pH (Rath et al., 2015).

4.3. The core microbial taxa regulating C decomposition along salinity gradient

The correlation of microbial taxa and SOC decomposition (priming) were found according to the results of O2PLS and SEM (Table 3; Fig. S4). Here we showed that *Streptomyces* (*Actinobacteria*), *Glycomyces* (*branch of Actinobacteria*), *Agromyces* (*branch of Actinobacteria*), and *Sphingomonas* (*branch of Proteobacteria*) at the genus level were significantly correlated with the C process particularly primed soil-drived C. Most of these functional taxa belonged to *Actinobacteria* and *Proteobacteria*. In a recent study, Ren et al. (2018) found that *Actinobacteria* had negative impact on SOC mineralization across land-use change (Fierer et al., 2007; Goldfarb et al., 2011) and *Proteobacteria* drove the positive soil respiration (He et al., 2012; Stevenson et al., 2004), indicating the balance of soil C dynamics were largely regulated by these two phyla. We found similar result that *Streptomyces* (*branch of Actinobacteria*) had a negative correlation with primed soil CO_2. *Actinobacteria* are able to grow preferentially on the C-rich refractory materials and relatively easily decompose the cellulose, lignocellulose (Khodadad et al., 2011), indicating these microorganisms preferentially use the C source that is used partially by others.

Although some studies suggest soil salinity may not be a vital factor for C decomposers (Rousk et al., 2011), the composition of microbial community are considered to play a decisive role in determining C dynamic processes in response to salt stress (Ramsey et al., 2005; Schimel et al., 2007; Nottingham et al., 2009). Here, SEM analysis showed that soil pH and EC in salted soils reduced microbial diversity and thus limited the utilization of SOC by microbial community, It was reported that high pH and salinity are the major determinants of soil microbial activity and community structure (Kamble et al., 2014).

5. Conclusion

Cotton meal is a kind of organic material with high nitrogen content, adding cotton meal in salinised soil can stimulate and promote the release of soil nutrients. The microorganisms mainly use the organic matter in the cotton meal in the pre-culture period, so the soil carbon excitation is negative excitation, Soil priming effect turned from negative to positive at the later stage of incubation (day 28), because microorganisms turned to decompose SOC from the labile substrate. With the increase of salinity, the diversity of microbial community decreased. Soil microbial community was mainly controled by soil pH and EC. By O2PLS, we found *Actinobacteria* and *Proteobacteria* (*Luteimonas, Hoeflea* and *Stenotrophomonas*) dominant in these soils were the core microbial taxa that affecting the process of organic C mineralization, particularly soil primed CO_2.

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Data availability

The datasets used and analysed during the current study available from the corresponding author on reasonable request.

Author contributions

K.W. conceptualized and conducted the experiment. H.Z. and D.C. conducted the data analysis and wrote the manuscript, conducted the indoor experiment. C.M. and

Z.Z. assisted in conducting the experiment. All authors reviewed the manuscript.All authors contributed to the manuscript and approved the submitted version.

Competing interests

The authors declare no competing interests.

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Table 1. Soil samples and Cottonseed meal properties

	Salinity 1	**Salinity 2**	**Salinity 3**	**Salinity 4**	**Salinity 5**	**Cottonseed meal**
Total C (%)	3.38b	3.18c	3.16c	3.57a	3.35b	42.98
Total N (%)	0.18d	0.19d	0.20c	0.22b	0.26a	5.84
C/N ratio	18.32a	16.56b	15.71c	16.54b	12.94d	7.38
δ^{13}C value (‰)	-14.21a	-14.79c	-14.60b	-14.55b	-16.01d	-23.47
pH (H_2O)	8.85a	8.45c	8.58b	8.59b	8.55b	7.63
EC (dS m^{-1})	1.06e	1.96c	1.28d	2.64b	7.75a	2.56
Salinity (%)	0.25e	0.58d	0.75c	1.00b	2.64a	ND

Table 2. Mantel test and Distance-based multivariate analysis relevance and contribution rate between soil properties and bacterial community compositions.

	pH	EC	NO_3^-–N	NH_4^+–N	MBC	TN	TC
Correlation	0.74**	0.56**	0.36**	0.68**	0.31**	0.11	0.27
Contribution	0.31**	0.12**	0.05	0.04	0.16	0.03	0.07**

Note:* $p < 0.05$, ** $p < 0.01$

Table 3. The variable influence projection (VIP) value and Spearman's correlation between the relative abundances of genera and C dynamic.

Phylum-Genus	VIP	Cottonseed meal CO_2-C(μg g^{-1})	Primed soil CO_2-C(μg g^{-1})	Basal soil CO_2-C(μg g^{-1})
Actinobacteria-Actinomarinales	1.36		0.63**	
Proteobacteria-Luteimonas	1.31		0.80**	
Actinobacteria-Nocardioides	1.30		0.54*	
Proteobacteria-Hoeflea	1.29		0.73**	
Actinobacteria-Streptomyces	1.27		-0.84**	
Actinobacteria-Glycomyces	1.26	0.63**		
Actinobacteria-Marmoricola	1.26	-0.52		
Proteobacteria-Nitrosospira	1.23		0.59	
Actinobacteria-Intrasporangium	1.22		0.60*	
Actinobacteria-Agromyces	1.19			0.58*
Proteobacteria-Sphingomonas	1.18			0.65**
Actinobacteria-Myceligenerans	1.16			
Chloroflexi-Nitrolancea	1.15		0.65**	
Actinobacteria-Pseudarthrobacter	1.06		0.62**	
Proteobacteria-Stenotrophomonas	1.00	-0.50	0.72**	

Note:* $p < 0.05$, ** $p < 0.01$

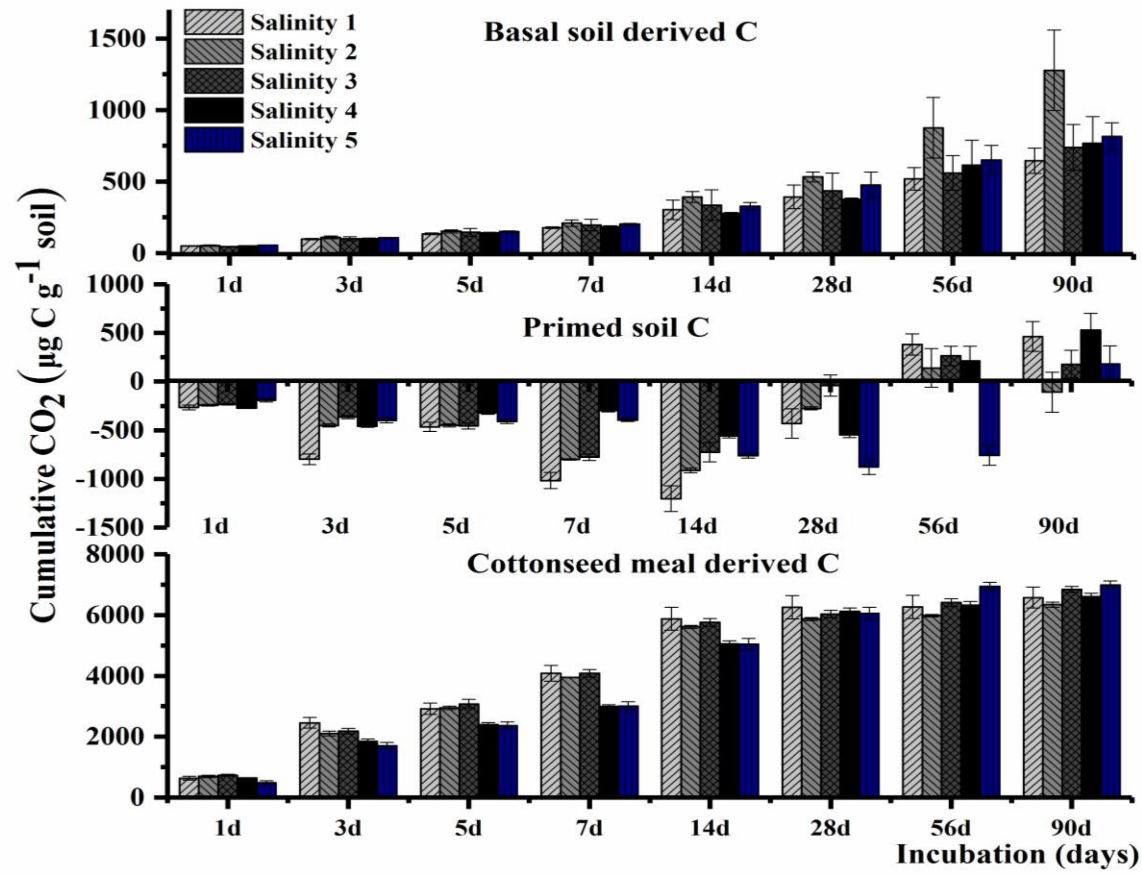

Fig. 1. Partitioning of CO_2 evolution after addition of cottonseed meal in different five salinity soils. Cumulative CO_2 evolved from salinity soil of 0.25 % (a) , 0.58 % (b) , 0.75 % (c) ,1.00% (d) and 2.64%(e) . Error bars represent standard errors of the means (n = 3).

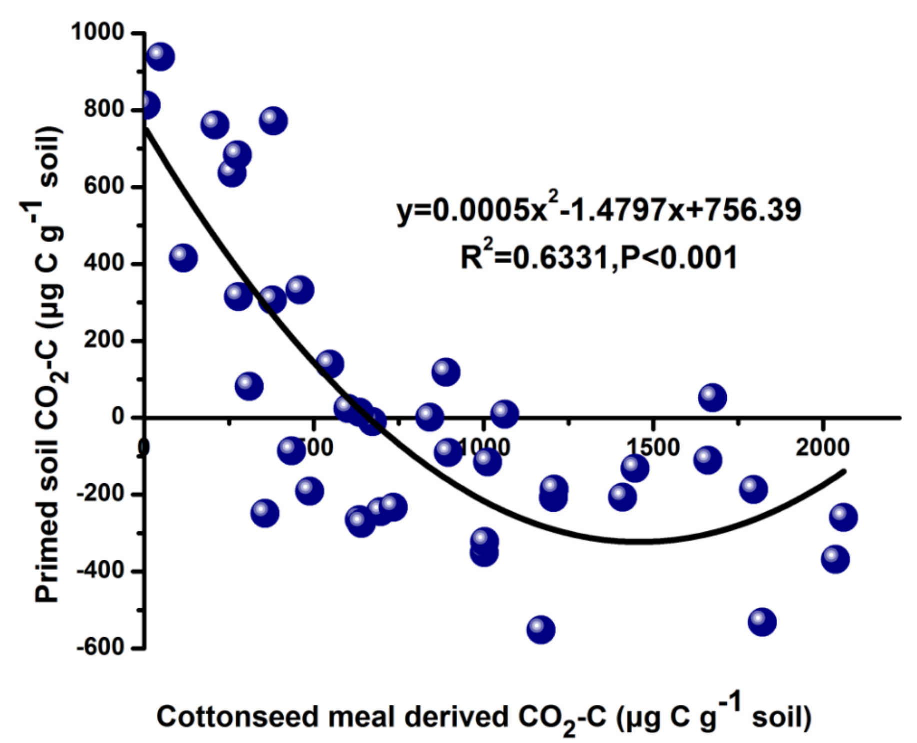

Fig. 2. Correlation between primed soil mineralisation and cottonseed meal mineralisation following different five salinity soils during 90 days incubation

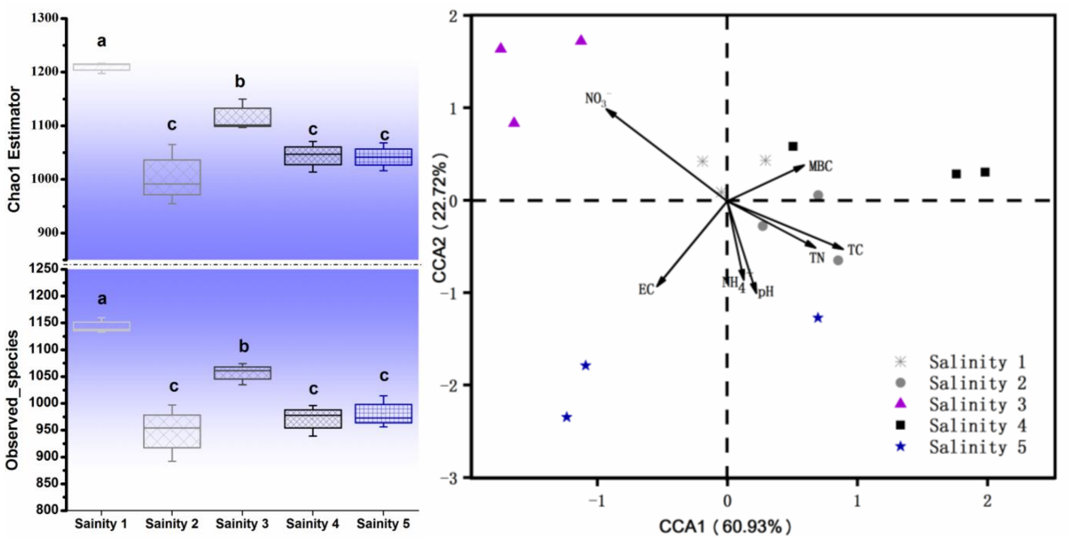

Fig. 3. Microbial community alpha diversity (Chao1) observed_species and beta diversity. Within each panel, boxplot data refer to maximum date (top line), 99%(the second line),mean (the third line), 1% (the fourth line) and minimum date (bottom line) of the different treatments, with statistical significance ($p < 0.05$).

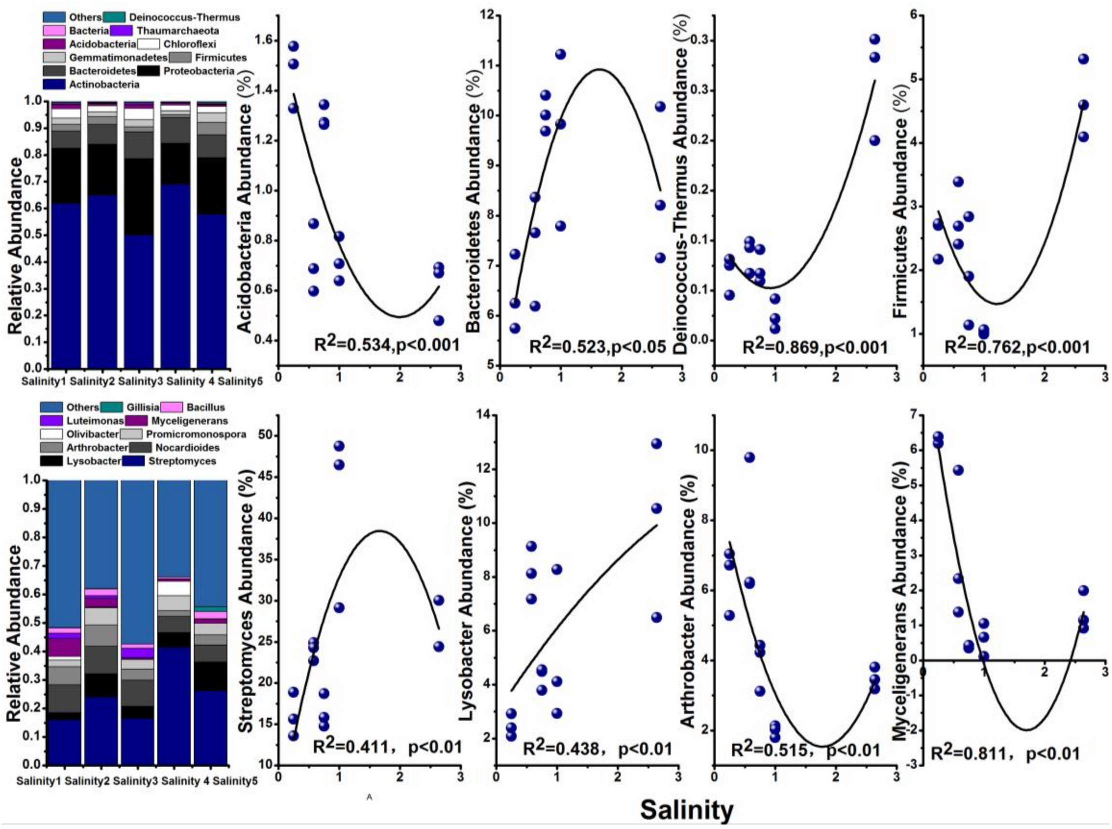

Fig. 4. The top 10 of phylums and genes in bacterial community in soils with a gradient of salinity