RESEARCH ARTICLE

Soil bacterial depth distribution controlled by soil orders and soil forms

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HIGHLIGHTS

- Topsoil diversity was greater in phenoforms than genosols, but the trend was reversed in subsoils.
- Bacterial community in topsoils was influenced by both soil orders and soil forms, however, in subsoils it was more impacted by soil orders.
- Cropping increased the similarity of bacteria structures among different soil orders.

GRAPHICAL ABSTRACT

ABSTRACT

Human disturbances to soils can lead to dramatic changes in soil physical, chemical, and biological properties. The influence of agricultural activities on the bacterial community over different orders of soil and at depth is still not well understood. We used the concept of genoform and phenoform to investigate the vertical (down to 1 m depth) soil bacterial community structure in paired genosols (undisturbed forests) and phenosols (cultivated vineyards) in different soil orders. The study was conducted in the Hunter Valley area, New South Wales, Australia, where samples were collected from 3 different soil orders (Calcarosol, Chromosol, and Kurosol), and each soil order consists of a pair of genosol and phenosol. The bacterial community structure was analyzed using high-throughput sequencing of 16S rRNA. Results showed that bacterial-diversity decreased with depth in phenosols, however, the trend is less obvious in genosol profiles. Topsoil diversity was greater in phenosols than genosols, but the trend was reversed in subsoils. Thus, cropping not only affected topsoil bacteria community but also decreased its diversity in the subsoil. Bacterial community in topsoils was influenced by both soil orders and soil forms, however, in subsoils it was more impacted by soil orders. Constrained Analysis of Principal Coordinates revealed that cropping increased the similarity of bacterial structures of different soil orders. This study highlighted the strong influence of agricultural activities on soil microbial distribution with depth, which is controlled by soil order.

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1 Introduction

Soil microorganisms have crucial roles in the terrestrial ecosystem's multifunctionality (Rillig et al., 2019; Thakur et al., 2020), such as litter decomposition, nutrient cycling, primary production, and climate regulation (Van Der Heijden

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et al., 2008; Bardgett and Van Der Putten, 2014; Wagg et al., 2014; Delgado-Baquerizo et al., 2016). Climate, geographical distance, and soil properties are considered as critical drivers of soil spatial microbial variability at large scales (Nazaries et al., 2018; Plassart et al., 2019). Current studies widely use those environmental variables as a proxy to represent the variation of soil microbial activity in global biogeochemical models (Crowther et al., 2019).

At the landscape and local scale, soil properties are known to have a strong influence on the underground microbial communities, notably soil pH, nutrient availability like organic carbon and nitrogen (Trivedi et al., 2019), as well as soil texture (Xia et al., 2020). Pedologists recognize different soil types and classified them into soil orders that relate how the soils were formed and manifested in unique soil properties. Soil, a complex biomaterial, hosts a broad range of different microbial habitats (Young and Crawford, 2004). These habitats may be only micrometers, yet, they can differ considerably in the occurrence of particular microbial taxa as well as in the relative abundance, evenness, richness, and activity (Fierer, 2017). Pronounced microbial differences have been found between soil samples only a few centimeters apart (O'Brien et al., 2016). As soil is a three-dimensional body, parameters that affect microbial distribution can change significantly with depth, such as aeration, pH, texture, and soil organic matter (Hsiao et al., 2018). However, soil microbiomes studies have mostly focused on the active surface horizon (topsoil), probably because topsoil is more related to agricultural production. As an essential driver of the ecosystem, the microbiome information from deep soils is crucial, especially for carbon storage under climate change (Wang et al., 2010). Recent methodological advances in molecular techniques and bioinformatics (like High-Throughput Sequencing Technologies) have greatly expanded researchers' ability to explore a greater understanding of the microbial distribution and functions in vertical soil profiles (Fierer, 2017). There is an increasing number of studies on soil microbiology in subsoils or even deep soils (Li et al., 2014; Seuradje et al., 2016; Van Leeuwen et al., 2017; Hendgen et al., 2018; Schlatter et al., 2020). Table S1 listed recent studies on the microbial distribution along the vertical soil profiles. Generally, a decreasing trend of microbial abundance (Eilers et al., 2012; Scharroba et al., 2012; Stone et al., 2014) and diversity (Jiao et al., 2018; Brewer et al., 2019; Upton et al., 2020) with depth was found in most of the studies. Along the soil depth, the underground microbial structures are regulated by different soil properties. Past studies reported that soil properties like soil organic carbon and nitrogen drive the microbial abundance and diversity in the upper soil horizons (Wu et al., 2020). But for the deeper horizons, microbial distribution is controlled by carbon forms, soil inherent properties, underground hydrology and so on (Table S1). Thus, a comprehensive description of the microbial distribution and composition along the vertical profiles as modulated by soil orders and soil forms is essential.

Human intervention, such as cropping, changes soil

properties over time, and those changes regulate the underground microbial distributions accordingly. Thus, land used is an essential factor of the soil microbial assembly (Lagerlöf et al., 2014; Geisen et al., 2019; Sui et al., 2019). The concept of genoforms and phenoforms has been recognized as a means to identify soil changes. The idea of genoform and phenoform of soil was suggested two decades ago (Droogers and Bouma, 1997) and has been revitalized recently (Rossiter and Bouma, 2018). In the current definition, genoform soils (genosoils) refer to soils in its natural form and used as the basis for soil classification, while phenoform soils (phenosoils) were their persistent variants with some physical or chemical differences and those differences are sufficient enough to influence soil functions (Rossiter and Bouma, 2018). Thus, this begs the question of how soil microorganisms change between soil forms? Would the microbial community of a phenosoil still have a high affinity with its genosoil? What are the influences of those changes with depth? And vertically, how does agriculture affect the microbial assembly along with depth? Those questions are still unsolved. Thus, a better understanding of vertical parallels between microbial communities in different soil forms would provide a reference for understanding soil change.

This research was designed to describe the vertical bacteria profile (down to 1 m in depth) in Australian soils and to study the changes of soil bacterial community between soil forms in different soil orders. The aim is to investigate the bacterial distribution with soil depth as a function of soil orders and to understand the interactive effects of soil orders, land use, and depth on soil bacterial distribution. It was hypothesized that soil forms might have more influence on the bacterial distribution in the topsoils, but soil orders may contribute more to the subsoils.

2 Materials and methods

2.1 Research area and soil sampling design

This study area is in the Private Irrigation District (PID) in the Hunter Valley, New South Wales (NSW), Australia (Fig. S1). Hunter Valley is located in the temperature region with a seasonal climate. Temperature varies from around 4°C to 30°C, while the average monthly rainfall varies from about 30 mm in winter to 95 mm in summer (Bureau of Meteorology, 2019). Topographically, this region is, for the most part, dominated by undulating hills that ascend to form low mountains to the south-west. Soil orders vary markedly and are dominated by *Chromosols* and *Kurosols*, interspersed with *Calcarosols*, *Hydrosols*, *Rudosols*, and *Tenosols* according to the Australian soil classification system (ASC) (Huang et al., 2018). Based on prevailing soil properties, Huang et al. (2018) classified these pre-European settlement soils into genosoils and phenosoils with the phenosoils further divided into three groups according to their degree of differentiation from their genosoil; with phenoform-1 the most closely related to the genoform and phenoform-3 with most changes.

Based on the soil map created by Huang et al. (2018), three sites were randomly selected to represent three major soil orders, *Chromosol*, *Kurosol*, and *Calcarosol* (ASC) or the WRB equivalent of *Luvisol*, *Acrisol*, and *Calcisol*. The north-east of the area is dominated by *Chromosol*, the north-west by *Kurosol*, and the southern part by *Calcarosol* (Fig. S1). *Chromosols* are soils with a strong texture contrast between the A and B horizon, with pH > 5.5 for the B horizon. *Kuro sols* are texture contrast soil with pH < 5.5 in the B horizon. *Calcarosols* are soils that are calcareous throughout.

At each soil order area, within a geographical distance of 1000 m, two soil forms were sampled, the genosol and phenosol (on phenoform-3 soils, the most differentiated form of the genosol) (Fig. S1). To minimise the influence of vegetation on the microbial community, the genosols were sampled from the undisturbed forest dominated by eucalyptus, and the paired phenosols were selected from cropping lands used as vineyards. The soil orders and forms were identified from the map created by Huang et al. (2018). At each sampling site, two 1 m long soil cores (approximately 10 cm in diameter) were sampled with a distance of 1 m from each other. In the forest, the soil cores were sampled at least 1 m from trees roots. In the vineyard, soil cores were taken from the middle of the vine rows and were approximately 1 m away from vine roots. Generally, there was some grass growing in between the rows of vines, that was regularly managed by the farmers via ploughing.

At each sampling site, soil down to 1 m depth or extended until bedrock was observed; soil samples were collected every 5 cm from 0 to 30 cm depth; depended on different texture change trend in the subsoils, it was sampled every 10 cm (for *Kurosol*) or 15 cm (for *Chromosol* and *Calcarosol*) from 40 cm to 100 cm depth (or until the core hits bedrock layer) (Fig. S1). For each sampled layer, approximately 20 g of soil was collected in the middle of the section and stored in a portable fridge at 4°C. The soil samples were then stored at -20°C before DNA extraction. Additional samples were also recovered from the same depths for soil physical and chemical analysis. In total, 138 soil samples were collected from the paired soil forms from three different soil orders.

2.2 Soil DNA extraction and sequencing

Soil DNA was extracted from 0.25 g soil using the DNeasy® Powersoil® Kit (QIAGEN, CA, USA) with the manufacturer's recommended protocol. The soil DNA quality was controlled using a spectrophotometer (NanoDrop™ 2000, Thermo Scientific, MA, USA) and agarose gel electrophoresis.

Total soil DNA was quantified using the Quantifluor dsDNA system (Promega, WI, USA) and expressed as ng DNA/soil (dry) after adjusting for soil moisture content. Bacterial community structure was determined from the V3-V4 variable region of the 16S rRNA gene using Pro341F and Pro805R as forward and reverse primers (Takahashi et al., 2014). The polymerase chain reaction (PCR) was performed using MyTaq™ Red DNA Polymerase system (Bioline, UK). All

PCRs were executed using a T100 thermal cycler (Bio-Rad, CA, USA). After an initial denaturation step at 95°C for 3 min, the DNA libraries were constructed using 25 PCR cycles of 95°C for 20 s(s), 60°C for 20 s, and 72°C for 45 s, followed by a final elongation step of 5 min at 72°C. The DNA libraries were then paired-end (PE300) sequenced using Illumina MiSeq instrument by the Australian Metagen Laboratory.

2.3 Soil physical and chemical properties

Soil moisture was determined gravimetrically from mass loss after drying at 105°C for 24 h. Soil pH and electric conductivity (EC) were measured using soil to water ratio 1:5 by SevenCompact™ pH/Ion S220 and Conductivity S230 instruments, respectively (both from Toledo Group, Switzerland). Other properties, such as soil organic carbon (SOC), cation exchange capacity (CEC) were assessed using Mid-infrared spectroscopy (MIR) according to the approach improved by Ng et al. (2017).

2.4 Data analysis

Fastq files were obtained from the sequencing company after the removal of adapters and low-quality sequences. The raw amplicon sequences were demultiplexed and processed to each sample by the demultiplex pipeline using python in QIIME2/2019.10 platform (Bolyen et al., 2019). Paired-end Illumina reads were then filtered, trimmed, denoised, merged, and removed from chimeras by Dada2 package in R 3.6 (RStudio, 2019) following Dada 2 pipeline tutorial from Callahan et al. (2019). Single sequences counted for less than 10 reads or found in less than two samples were cleaned from the analysis. The low sequence samples were also removed (see the sample rarefaction curves in Fig. S2). The remained unique amplicon sequence variants (ASVs) were assigned for taxonomy using Silva 132 as the reference library (Callahan, 2018). After the cleaning of Archaea taxa, the bacterial ASVs library was combined with taxonomy, phylogenetic tree, and sample metadata (soil order, soil forms, depth, moisture, pH, EC, SOC, and CEC) into a phyloseq object using the 'phyloseq' package in R (Callahan et al., 2016). Further analysis of the vertical bacterial distribution, α -diversity (i.e. Shannon and Simpson) were calculated in R using 'phyloseq' and 'vegan' packages (Dixon, 2003). The DNA sequences data set was rarefied for α -diversity calculation.

The bacterial structure and soil factors relationships were explored using constrained Analysis of Principal Coordinates (CAP). It used principal coordinate analysis of relative bacterial abundances (by transferring bacterial abundance counts to fractional abundances), followed by a canonical discriminant analysis by soil orders and soil forms (Anderson and Willis, 2003). CAP was calculated using the cap scale function in the "vegan" package in R. Figures of the experimental results were created using "ggplot2" (Wickham, 2016) in R and JMP.

3 Results and discussion

3.1 Soil bacteria α -diversity

After DNA sequence processing and filtering, 130 samples remained with 13 091 unique ASVs in total. It is then rarefied to even depth and reduced to 12 772 ASVs, which were used for alpha diversity calculation. Vertically, the bacterial Shannon and Simpson index displayed similar trends (Figs. 1 and S3) which varied with depth. Generally, the bacterial alpha diversity decreased with depth, but in some cases, there was a rebound trend in some of the genoform profiles. The influence of land use on bacterial diversity was evident, as depicted in Fig. 1B. The Shannon index decline with depth was observed in the phenosoils, but this decreasing trend was less evident in the genosoils. For the genoform of *Kurosols* and *Chromosols*, after a slight decrease in the surface layers, the Shannon index increased again at 60 cm and 80 cm, respectively. This contradicts most findings that show microbial diversity decreases with depth, the same as microbial biomass (Eilers et al., 2012; Jiao et al., 2018). The alpha diversity was altered in all three soil orders by agricultural activities. For the topsoils (5–30 cm), cropping increased soil bacterial diversity in all soil orders. However, this trend was reversed at deeper layers (40 to 85 cm in *Calcarosols* and *Chromosols*, 80 to 100 cm in *Kurosols*), where genoform soils had higher diversity than phenoform soils. This finding reflects that agricultural activities can have an impact on soil microbial communities as far as the deep soil layers.

Comparing the contrasting land use, the higher bacteria diversity in the phenosoils at the surface layers (5 to 30 cm) was not expected. There has been a lot of evidence suggesting microbial diversity decline due to agriculture, especially due to practices such as chemical applications (Wenhui et al., 2007; Luo et al., 2015) and tillage (Lienhard et al., 2014; Wang et al., 2020). On the other hand, there are also findings that demonstrated cropping does not necessarily lead to a loss of microbial diversity, and with optimal agricultural management, higher soil microbial diversity might also be

found (Tardy et al., 2015). Since vegetation is also an essential factor for soil microbes (Millard and Singh, 2010), these results require further investigation in the future.

The decreased trend of bacterial diversity with depth was commonly reported in past studies (Will et al., 2010; Jiao et al., 2018). Eilers et al. (2012) reported a drop of 20% to 40% of microbial diversity from the top 10 cm to a depth of 180 cm. They explained that changes in environmental conditions with soil depth represent a strong ecological filter and that many surface-dwelling microorganisms are less likely to thrive in the environments of the deeper soil. In this study, SOC decreased with depth (Fig. 2), and there were also variations of CEC, pH, EC, and soil moisture along with depth. The combined effects of the nutrient availability (SOC and CEC), soil pH, and salinity (EC) filtered the vertical bacterial distribution. Notably, a parallel pattern of SOC and the extracted microbial DNA abundances was observed in our results (Fig. 2). Jones et al. (2018) reported that the microbial population was mainly limited by carbon in the subsoils, thus the significant decrease of SOC resulted in the low DNA concentrations in the subsoils in Fig. 2. This result is consistent with previous studies carried out in other regions (Eilers et al., 2012; Jones et al., 2018).

3.2 Soil bacteria depth distribution

At the genus level, there were 546 genera in all soil samples, and the top 85 genera accounted for 90% of the whole ASVs. The eight most abundant phyla dominated ~98% of the total ASVs and their vertical distributions are illustrated in Fig. 3A. The relative abundances of those bacterial communities varied with the depth, of each soil order and soil form. Will et al. (2010) reported that the bacterial communities were depth-specific despite some overarching dominant taxa. In this study, the bacterial phylum composition generally showed a similar trend between genosoils and phenosoils, although there were a few specific phyla were an exception. Chloroflexi, for example, were found to be more abundant in the deep phenosoils in *Calcarosol*. *Chromosol* presented a similar trend of Actinobacteria and Proteobacteria with depth, but less

Fig. 1 (A) Alpha diversity of bacteria along the vertical soil profiles (the lines are smoothing splines, smooth Lambda = 0.001); (B) The alpha diversity was compared between soil forms. Error bars are 1 standard error.

Fig. 2 Mean soil properties and the soil microbial DNA concentrations with depth of each profile from the paired soil forms of the three types (the lines are smoothing splines, smooth Lambda = 0.001).

Chloroflexi were found in the deep phenosols. Acidobacteria and Actinobacteria change significantly between soil forms in *Kurosol*.

Among the dominant phyla, the top five (i.e., Actinobacteria, Proteobacteria, Chloroflexi, Acidobacteria, and Verrucomicrobia) accounted for 95% of the total sequences. Comparing the total phylum proportion between the paired genosol and phenosol profiles (Fig. 3B), Actinobacteria had a higher proportion in the genosols, with an average of 47.4% versus 29.9% in the phenosols. Proteobacteria was the subdominant phylum with a similar range between genosols and phenosols close to 20%. The mean abundance of Chloroflexi,

Acidobacteria, Verrucomicrobia seemed to be increased by cropping. The significant variation in different soil forms was mainly found in Actinobacteria, Acidobacteria, and Verrucomicrobia (Fig. 3B). This observation suggests that agricultural activities had decreased the proportion of Actinobacteria. Actinobacteria, which share the characteristics of both bacteria and fungi, are widely distributed in soil. They play an essential role in recycling refractory biomaterials when decomposing complex organic materials (Anandan et al., 2016). Actinobacteria are also associated with the ability to produce soil organic matter (Suela Silva et al., 2013), which was compromised in the cropping system. Thus, this result

Fig. 3 (A) Bacterial distribution with depth in the phylum level of the paired genoform and phenoform profiles in three soil orders. (B) Box plot of the compositions of the most abundant 5 phyla in the paired genoform and phenoform soils. The dots represent the phyla proportion of each sample colored by soil order. The phylum with significant differences by *t*-test was marked with stars: $0.01 \leq p \leq 0.05$ were marked with *, $0.001 \leq p \leq 0.01$ were marked with **, $p \leq 0.001$ were marked with ***.

suggests that phenosols would be more susceptible to a decrease in soil organic carbon. The Acidobacteria phylum typically prefers soil with oligotrophic properties (Barns et al., 1999; Conradie and Jacobs, 2020). The higher abundance of Acidobacteria in the phenosols, indicating a nutrient decrease of soil profiles after cropping. It is reported that Verrucomicrobia can establish beneficial plant-microbe interactions in the rhizosphere (Aguirre-von-Wobeser et al., 2018). They are found to be more abundant in phenoforms suggesting that cropping may enhance their habitat.

3.3 Factors influencing soil microbial distribution

To further discover drivers of bacteria distribution in this study area, a multivariate analysis called Constrained Analysis of Principal Coordinates (CAP) was performed including soil properties (SOC, CEC, pH, EC, moisture, and depth) as explanatory variables. The first two principal components accounted for 16.4% and 5.4% of the bacterial variation (Fig. 4). The sample distance was calculated based on the UniFrac indicator (Lozupone et al., 2011).

Significant differences were observed in the microbial communities' similarities between the genosols and phenosols (Fig. 4). In the less disturbed genosols, samples displayed greater dissimilarity between soil orders, indicating a greater variance of bacteria structure in different soil orders. However, these differences were altered in cropping soils. Indeed, the phenosols from different soil orders showed to host more similar communities and even to overlap with each other at the surface layers, in particular (Fig. 4A). These results suggest that agricultural activities tend to homogenize soil bacterial community composition. Similar findings were reported (Xue et al., 2018) where the authors attributed the dissimilarity to the altered soil properties after cropping or the increased similarities of substrate derived from the decomposition of the same plant materials.

The beta diversity response to cropping, however, was not the same for all soil orders (Fig. 4B). All soil orders have shown a decrease in the similarity between soil layers in cropping soils, especially for *Kurosols* and *Calcarosols*. It indicates that agricultural activities not only decreased differences of soil bacterial composition between soil orders but also reduced the dissimilarity between soil horizons, i.e. a decrease in beta diversity. Soil management and disturbances during cultivation, such as tillage (Jiang et al., 2011) might be a cause for soil homogenization and microbial similarities. The dissimilarity between soil forms within same soil orders revealed the significant importance of land use, which is consistent with the report that within similar soil clusters, land use would be a major driver of the bacterial communities (Plassart et al., 2019). Additionally, among the analyzed soil properties, soil pH and SOC showed significant effects in the first principal component (Fig. 4), suggesting strong influences of soil pH and nutrients on bacterial community structures. These results are consistent with previous reports (Tripathi et al., 2013; Wu et al., 2020). Both

soil moisture and CEC presented positive effects in the second principal component on the bacterial communities. Additionally, soil EC influenced by depth presented a significant control on the bacterial community composition, indicating a critical effect of salt concentration on the soil microorganisms in the deep soil layers.

Comparing the bacterial community structure of the topsoil (0–5 cm) with subsequent depth layers, the UniFrac distance increased with depth (Fig. 5A). The changes in deeper soil environments might lead to changes in microbial inhabitants. This is particularly true in the *Calcarosols* that soil pH changed to quite alkaline in the deeper layers resulted in an increasing dissimilarity there. However, for *Kurosols* and *Chromosols*, there is a decreasing UniFrac distance in the deeper zones (70 cm to 100 cm), which indicates that the bacterial communities of those deeper horizons tended to similar to the ones at the surface, to some extent. The similarity between the surface soils and the deep soils was also reported by Eilers et al. (2012) and it might drive by the same type of carbon between the deep and surface soils.

To visualize the dissimilarity of bacteria structure with depths between soil forms, UniFrac distances were calculated by each successive depth, as shown in Fig. 5C. Different soil orders presented different variance patterns between the genoforms and phenoforms with depth. Overall, the dissimilarities between the paired soil forms decreased with depth in the upper zones (~5 to 40 cm) – except for *Calcarosol* (the alkaline soil modulated bacterial distribution, Fig. 2). The highest dissimilarity between soil forms showed in the surface soils (5–10 cm) for *Chromosol* and *Kurosols*, it then decreased with depth (15–40 cm for *Chromosol* and 10–60 cm for *Kurosols*) (Fig. 5C). This result suggests a significant influence of agriculture on the bacterial distribution in the topsoils and it decreased with depth. It might because agriculture activities altered soil properties in the topsoils (Söderström et al., 2014). However, in the deeper zones (~60 to 100 cm), there is an increasing trend of dissimilarities between soil forms with depth found in all soil orders. This is likely due to changes in soil properties other than SOC (e.g., pH, EC, texture) or hydrologic conditions (moisture) in the deeper layers (Fig. 2) (Eilers et al., 2012). In summary, the bacterial vertical distribution is a combined result of land use and soil properties, with decreasing influence of land use in the upper layers. In the deeper zones, the soil bacteria is mostly controlled by underground soil conditions (i.e. inherent soil properties and hydrologic conditions).

The variance of the bacterial assembly was compared between different soil orders in Fig. 5D. Bacteria community across soil orders tend to be similar in the surface soils (0–10 cm), however, the sample dissimilarity then increased in the subsoils. The relatively smaller variance of soil bacterial community in the topsoils between different soil orders has not been reported previously. As discussed in Fig. 4, it suggests a significant soil homogenization effect of agriculture in the topsoils. Additionally, these results might also be influenced by the above vegetation (Brandt et al., 2013), especially the

Fig. 4 Constrained Analysis of Principal Coordinates (CAP) of bacterial structure and soil properties base on the UniFrac distance (4A), and compared between different soil orders (4B). The samples were linked with depth downward of each file by the connected lines. All plots used the same axes scales.

same type of vegetation of the soil orders collected in this study. With increasing depth, the effect of soil orders became more evident in the subsoils (Fig. 5D).

The relative influence of soil forms and soil orders on soil bacterial community was compared in Fig. 5B. The results show variation with depth. It is interesting to find that the dissimilarities between soil orders and soil forms were relatively close at 5–10 cm. Not as hypothesized, these results suggested a relatively similar influence of soil order and soil

form (land use) on the topsoils. Then a larger dissimilarity was found between soil orders at ~15 cm to 80 cm, indicating a larger influence of soil order than soil form in the subsoils. This region is where soil properties vary significantly and thus affected soil bacteria distribution and override the effect of land use. At the deepest layers (80–100 cm), the sample dissimilarity between soil forms and soil orders fluctuated but approached a close range again. This fluctuation indicated unstable drivers in the deep soils, it might attribute to the

Fig. 5 (A) UniFrac Distances between top layer (0–5 cm) and below layers of each vertical soil profile in the paired soil forms; (B) Box plot of the sample dissimilarity compared between soil forms (within same soil order) and soil orders, and the line smoothers are the mean values varied with depth (smoothness Lambda = 0.001). (C) UniFrac Distances of each soil horizons between the paired soil forms within each soil order. (D) UniFrac Distances of each soil horizons are compared between different soil orders. Error bars are standard errors. The broken dash lines are the measured values, and the lines are smoothed spline (smoothness Lambda = 0.001).

stronger effect of soil properties or underground hydrology (Eilers et al., 2012).

4 Conclusions

This study compared soil bacterial structure between paired soil forms in three different soil orders. Vertically, the bacterial distribution was influenced by both soil orders and soil forms (land use). The bacterial α -diversity decreased with depth was observed in most of the soil profiles, but there was a rebounding trend in the deep layers in some genosols. More microbial diversity was observed in the topsoils of phenosols, while at deeper layers, genosols presented greater diversity. The influence of cropping on microbial distribution might be deeper than assumed. Moreover, agricultural activities have homogenized soil bacteria populations from different soil orders via altered soil properties and vegetation, especially in the topsoils. The degree of these

changes was not the same for different soil orders. Beyond soil pH and SOC, an understanding of soil orders and forms is more crucial in determining soil bacterial composition. The influence of soil forms (land use) decreased with depth in the upper layers, while soil order presented more significant impacts in the subsoils (15–80 cm). All in all, the distribution of soil bacterial in depth provides key information for soil ecological studies and agricultural management, and the next step should be a deeper exploration into the functions of those microbial communities and their role in agriculture production.

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References

- Aguirre-von-Wobeser, E., Rocha-Estrada, J., Shapiro, L.R., de la Torre, M., 2018. Enrichment of Verrucomicrobia, Actinobacteria and Burkholderiales drives selection of bacterial community from soil by maize roots in a traditional milpa agroecosystem. *PLoS One* 13, e0208852.
- Anandan, R., Dharumadurai, D., Manogaran, G.P., 2016. An Introduction to Actinobacteria. In: Dhanasekaran, D., Jiang, Y., eds. *Actinobacteria-Basics and Biotechnological Applications*. Intechopen.
- Anderson, M.J., Willis, T.J., 2003. Canonical analysis of principal coordinates: a useful method of constrained ordination for ecology. *Ecology* 84, 511–525.
- Bardgett, R.D., Van Der Putten, W.H., 2014. Belowground biodiversity and ecosystem functioning. *Nature* 515, 505–511.
- Barns, S.M., Takala, S.L., Kuske, C.R., 1999. Wide distribution and diversity of members of the bacterial kingdom Acidobacterium in the environment. *Applied and Environmental Microbiology* 65, 1731–1737.
- Bolyen, E., Rideout, J.R., Dillon, M.R., Bokulich, N.A., Abnet, C.C., Al-Ghalith, G.A., Alexander, H., Alm, E.J., Arumugam, M., Asnicar, F., Bai, Y., Bisanz, J.E., Bittinger, K., Brejnrod, A., Brislawn, C.J., Brown, C.T., Callahan, B.J., Caraballo-Rodríguez, A.M., Chase, J., Cope, E.K., Da Silva, R., Diener, C., Dorrestein, P.C., Douglas, G. M., Durall, D.M., Duvallet, C., Edwardson, C.F., Ernst, M., Estaki, M., Fouquier, J., Gauglitz, J.M., Gibbons, S.M., Gibson, D.L., Gonzalez, A., Gorlick, K., Guo, J., Hillmann, B., Holmes, S., Holste, H., Huttenhower, C., Huttley, G.A., Janssen, S., Jarmusch, A.K., Jiang, L., Kaehler, B.D., Kang, K.B., Keefe, C.R., Keim, P., Kelley, S.T., Knights, D., Koester, I., Kosciulek, T., Kreps, J., Langille, M.G. I., Lee, J., Ley, R., Liu, Y.X., Lofffield, E., Lozupone, C., Maher, M., Marotz, C., Martin, B.D., McDonald, D., McIver, L.J., Melnik, A.V., Metcalf, J.L., Morgan, S.C., Morton, J.T., Naimey, A.T., Navas-Molina, J.A., Nothias, L.F., Orchanian, S.B., Pearson, T., Peoples, S.L., Petras, D., Preuss, M.L., Priesse, E., Rasmussen, L.B., Rivers, A., Robeson, M.S. II, Rosenthal, P., Segata, N., Shaffer, M., Shiffer, A., Sinha, R., Song, S.J., Spear, J.R., Swafford, A.D., Thompson, L.R., Torres, P.J., Trinh, P., Tripathi, A., Turnbaugh, P. J., Ul-Hasan, S., van Der Hooft, J.J.J., Vargas, F., Vázquez-Baeza, Y., Vogtmann, E., Von Hippel, M., Walters, W., Wan, Y., Wang, M., Warren, J., Weber, K.C., Williamson, C.H.D., Willis, A.D., Xu, Z.Z., Zaneveld, J.R., Zhang, Y., Zhu, Q., Knight, R., Caporaso, J.G., 2019. Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. *Nature Biotechnology* 37, 852–857.
- Brandt, A.J., de Kroon, H., Reynolds, H.L., Burns, J.H., 2013. Soil heterogeneity generated by plant–soil feedbacks has implications for species recruitment and coexistence. *Journal of Ecology* 101, 277–286.
- Brewer, T.E., Aronson, E.L., Arogyaswamy, K., Billings, S.A., Botthoff, J.K., Campbell, A.N., Dove, N.C., Fairbanks, D., Gallery, R.E., Hart, S.C., Kaye, J., King, G., Logan, G., Lohse, K.A., Maltz, M.R., Mayorga, E., O'Neill, C., Owens, S.M., Packman, A., Pett-Ridge, J., Plante, A.F., Richter, D.D., Silver, W.L., Yang, W.H., Fierer, N., 2019. Ecological and genomic attributes of novel bacterial taxa that thrive in subsurface soil horizons. *mBio* 10, e01318–e01319.
- Bureau of Meteorology, 2019. Climate statistics for Australian locations. Australian Government.
- Callahan, B., 2018. Silva taxonomic training data formatted for DADA2 (Silva version 132).
- Callahan, B., Sankaran, K., Fukuyama, J.A., McMurdie, P.J., Holmes, S.P., 2016. Bioconductor workflow for microbiome data analysis: from raw reads to community analyses. *F1000 Research* 5:1492.
- Callahan, B., Sankaran, K., Fukuyama, J.A., McMurdie, P.J., Holmes, S.P., 2019. DADA2 Pipeline Tutorial (1.12).
- Conradie, T., Jacobs, K., 2020. Seasonal and agricultural response of Acidobacteria present in two fynbos rhizosphere soils. *Diversity (Basel)* 12, 277.
- Crowther, T.W., Van den Hoogen, J., Wan, J., Mayes, M.A., Keiser, A., Mo, L., Averill, C., Maynard, D.S., 2019. The global soil community and its influence on biogeochemistry. *Science* 365, eaav0550.
- Delgado-Baquerizo, M., Maestre, F.T., Reich, P.B., Jeffries, T.C., Gaitan, J.J., Encinar, D., Berdugo, M., Campbell, C.D., Singh, B.K., 2016. Microbial diversity drives multifunctionality in terrestrial ecosystems. *Nature Communications* 7, 10541.
- Dixon, P., 2003. VEGAN, a package of R functions for community ecology. *Journal of Vegetation Science* 14, 927–930.
- Droogers, P., Bouma, J., 1997. Soil survey input in exploratory modeling of sustainable soil management practices. *Soil Science Society of America Journal* 61, 1704–1710.
- Eilers, K.G., Debenport, S., Anderson, S., Fierer, N., 2012. Digging deeper to find unique microbial communities: The strong effect of depth on the structure of bacterial and archaeal communities in soil. *Soil Biology & Biochemistry* 50, 58–65.
- Fierer, N., 2017. Embracing the unknown: disentangling the complexities of the soil microbiome. *Nature Reviews. Microbiology* 15, 579–590.
- Fierer, N., Schimel, J.P., Holden, P.A., 2003. Variations in microbial community composition through two soil depth profiles. *Soil Biology & Biochemistry* 35, 167–176.
- Garcia-Pichel, F., Johnson, S.L., Youngkin, D., Belnap, J., 2003. Small-scale vertical distribution of bacterial biomass and diversity in biological soil crusts from arid lands in the Colorado Plateau. *Microbial Ecology* 46, 312–321.
- Geisen, S., Wall, D.H., van der Putten, W.H., 2019. Challenges and opportunities for soil biodiversity in the Anthropocene. *Current Biology* 29, R1036–R1044.
- Hartmann, M., Lee, S., Hallam, S.J., Mohn, W.W., 2009. Bacterial, archaeal and eukaryal community structures throughout soil horizons of harvested and naturally disturbed forest stands. *Environmental Microbiology* 11, 3045–3062.
- Hendgen, M., Hoppe, B., Döring, J., Friedel, M., Kauer, R., Frisch, M., Dahl, A., Kellner, H., 2018. Effects of different management regimes on microbial biodiversity in vineyard soils. *Scientific Reports* 8, 9393.
- Hsiao, C.J., Sassenrath, G.F., Zeglin, L.H., Hettiarachchi, G.M., Rice, C.W., 2018. Vertical changes of soil microbial properties in claypan soils. *Soil Biology & Biochemistry* 121, 154–164.
- Hu, Y., Xiang, D., Veresoglou, S.D., Chen, F., Chen, Y., Hao, Z.,

- Zhang, X., Chen, B., 2014. Soil organic carbon and soil structure are driving microbial abundance and community composition across the arid and semi-arid grasslands in northern China. *Soil Biology & Biochemistry* 77, 51–57.
- Huang, J., McBratney, A.B., Malone, B.P., Field, D.J., 2018. Mapping the transition from pre-European settlement to contemporary soil conditions in the Lower Hunter Valley, Australia. *Geoderma* 329, 27–42.
- Jiang, X., Wright, A.L., Wang, J., Li, Z., 2011. Long-term tillage effects on the distribution patterns of microbial biomass and activities within soil aggregates. *Catena* 87, 276–280.
- Jiao, S., Chen, W., Wang, J., Du, N., Li, Q., Wei, G., 2018. Soil microbiomes with distinct assemblies through vertical soil profiles drive the cycling of multiple nutrients in reforested ecosystems. *Microbiome* 6, 1–13.
- Johan, H., Corrie, S., 2015. Effects of conservation agriculture and fertilization on soil microbial diversity and activity. *Environments* 2, 358–384.
- Jones, D.L., Magthab, E.A., Gleeson, D.B., Hill, P.W., Sánchez-Rodríguez, A.R., Roberts, P., Ge, T., Murphy, D.V., 2018. Microbial competition for nitrogen and carbon is as intense in the subsoil as in the topsoil. *Soil Biology & Biochemistry* 117, 72–82.
- Lagerlöf, J., Adolfsson, L., Börjesson, G., Ehlers, K., Vinyoles, G.P., Sundh, I., 2014. Land-use intensification and agroforestry in the Kenyan highland: Impacts on soil microbial community composition and functional capacity. *Applied Soil Ecology* 82, 93–99.
- Li, C., Yan, K., Tang, L., Jia, Z., Li, Y., 2014. Change in deep soil microbial communities due to long-term fertilization. *Soil Biology & Biochemistry* 75, 264–272.
- Lienhard, P., Terrat, S., Prévost-Bouré, N.C., Nowak, V., Régnier, T., Sayphoummie, S., Panyasiri, K., Tivet, F., Mathieu, O., Levêque, J., Maron, P.A., Ranjard, L., 2014. Pyrosequencing evidences the impact of cropping on soil bacterial and fungal diversity in Laos tropical grassland. *Agronomy for Sustainable Development* 34, 525–533.
- Lozupone, C., Lladser, M.E., Knights, D., Stombaugh, J., Knight, R., 2011. UniFrac: an effective distance metric for microbial community comparison. *ISME Journal* 5, 169–172.
- Luo, P., Han, X., Wang, Y., Han, M., Shi, H., Liu, N., Bai, H., 2015. Influence of long-term fertilization on soil microbial biomass, dehydrogenase activity, and bacterial and fungal community structure in a brown soil of northeast China. *Annals of Microbiology* 65, 533–542.
- Millard, P., Singh, B., 2010. Does grassland vegetation drive soil microbial diversity? *Nutrient Cycling in Agroecosystems* 88, 147–158.
- Nazaries, L., Karunaratne, S.B., Delgado-Baquerizo, M., Campbell, C. D., Singh, B.K., 2018. Environmental drivers of the geographical distribution of methanotrophs: Insights from a national survey. *Soil Biology & Biochemistry* 127, 264–279.
- Ng, W., Malone, B.P., Minasny, B., 2017. Rapid assessment of petroleum-contaminated soils with infrared spectroscopy. *Geoderma* 289, 150–160.
- Nogueira, M., Ribeiro Vasconcelos, A., Hungria, M., 2016. Shifts in taxonomic and functional microbial diversity with agriculture: How fragile is the Brazilian Cerrado? *BMC Microbiology* 16, 42.
- O'Brien, S.L., Gibbons, S.M., Owens, S.M., Hampton-Marcell, J., Johnston, E.R., Jastrow, J.D., Gilbert, J.A., Meyer, F., Antonopoulos, D.A., 2016. Spatial scale drives patterns in soil bacterial diversity. *Environmental Microbiology* 18, 2039–2051.
- Plassart, P., Prévost-Bouré, N.C., Uroz, S., Dequiedt, S., Stone, D., Creamer, R., Griffiths, R.I., Bailey, M.J., Ranjard, L., Lemanceau, P., 2019. Soil parameters, land use, and geographical distance drive soil bacterial communities along a European transect. *Scientific Reports* 9, 605.
- Rillig, M.C., Ryo, M., Lehmann, A., Aguilar-Trigueros, C.A., Buchert, S., Wulf, A., Iwasaki, A., Roy, J., Yang, G., 2019. The role of multiple global change factors in driving soil functions and microbial biodiversity. *Science* 366, 886–890.
- Rossiter, D.G., Bouma, J., 2018. A new look at soil phenofoms—Definition, identification, mapping. *Geoderma* 314, 113–121.
- Scharroba, A., Dibbern, D., Hünninghaus, M., Kramer, S., Moll, J., Butenschön, O., Bonkowski, M., Buscot, F., Kandeler, E., Koller, R., Krüger, D., Lueders, T., Scheu, S., Ruess, L., 2012. Effects of resource availability and quality on the structure of the micro-food web of an arable soil across depth. *Soil Biology & Biochemistry* 50, 1–11.
- Schlatter, D.C., Kahl, K., Carlson, B., Huggins, D.R., Paulitz, T., 2020. Soil acidification modifies soil depth-microbiome relationships in a no-till wheat cropping system. *Soil Biology & Biochemistry* 149, 107939.
- Seuradze, B.J., Oelbermann, M., Neufeld, J.D., 2016. Depth-dependent influence of different land-use systems on bacterial biogeography. *FEMS Microbiology Ecology* 93, fiw239.
- Söderström, B., Hedlund, K., Jackson, L.E., Kätterer, T., Lugato, E., Thomsen, I.K., Bracht Jørgensen, H., 2014. What are the effects of agricultural management on soil organic carbon (SOC) stocks? *Environmental Evidence* 3, 2.
- Stone, M., DeForest, J., Plante, A., 2014. Changes in extracellular enzyme activity and microbial community structure with soil depth at the Luquillo Critical Zone Observatory. *Soil Biology & Biochemistry* 75, 237–247.
- Suela Silva, M., Naves Sales, A., Teixeira Magalhães-Guedes, K., Ribeiro Dias, D., Schwan, R.F., 2013. Brazilian Cerrado soil Actinobacteria ecology. *BioMed Research International* 2013, 503805–503805.
- Sui, X., Zhang, R., Frey, B., Yang, L., Li, M.H., Ni, H., 2019. Land use change effects on diversity of soil bacterial, Acidobacterial and fungal communities in wetlands of the Sanjiang Plain, northeastern China. *Scientific Reports* 9, 18535.
- Takahashi, S., Tomita, J., Nishioka, K., Hisada, T., Nishijima, M., 2014. Development of a prokaryotic universal primer for simultaneous analysis of bacteria and archaea using next-generation sequencing. *PLoS One* 9, e105592.
- Tardy, V., Spor, A., Mathieu, O., Lévêque, J., Terrat, S., Plassart, P., Regnier, T., Bardgett, R.D., van der Putten, W.H., Roggero, P.P., Seddaiu, G., Bagella, S., Lemanceau, P., Ranjard, L., Maron, P.A., 2015. Shifts in microbial diversity through land use intensity as drivers of carbon mineralization in soil. *Soil Biology & Biochemistry* 90, 204–213.
- Thakur, M.P., Phillips, H.R.P., Brose, U., De Vries, F.T., Lavelle, P., Loreau, M., Mathieu, J., Mulder, C., Van der Putten, W.H., Rillig, M.

- C., Wardle, D.A., Bach, E.M., Bartz, M.L.C., Bennett, J.M., Briones, M.J.I., Brown, G., Decaëns, T., Eisenhauer, N., Ferlian, O., Guerra, C.A., König-Ries, B., Orgiazzi, A., Ramirez, K.S., Russell, D.J., Rutgers, M., Wall, D.H., Cameron, E.K., 2020. Towards an integrative understanding of soil biodiversity. *Biological Reviews of the Cambridge Philosophical Society* 95, 350–364.
- Tripathi, B.M., Kim, M., Lai-Hoe, A., Shukor, N.A., Rahim, R.A., Go, R., Adams, J.M., 2013. pH dominates variation in tropical soil archaeal diversity and community structure. *FEMS Microbiology Ecology* 86, 303–311.
- Trivedi, C., Reich, P.B., Maestre, F.T., Hu, H.W., Singh, B.K., Delgado-Baquerizo, M., 2019. Plant-driven niche differentiation of ammonia-oxidizing bacteria and archaea in global drylands. *ISME Journal* 13, 2727–2736.
- Upton, R.N., Chęcinska Sielaff, A., Hofmockel, K.S., Xu, X., Polley, H. W., Wilsey, B.J., 2020. Soil depth and grassland origin cooperatively shape microbial community co-occurrence and function. *Ecosphere* 11, e02973.
- Uri, Y.L., Tracy, K.T., Robertson, G.P., Thomas, M.S., 2011. Agriculture's impact on microbial diversity and associated fluxes of carbon dioxide and methane. *ISME Journal* 5, 1683–1691.
- Van Der Heijden, M.G., Bardgett, R.D., Van Straalen, N.M., 2008. The unseen majority: soil microbes as drivers of plant diversity and productivity in terrestrial ecosystems. *Ecology Letters* 11, 296–310.
- Van Leeuwen, J., Djukic, I., Bloem, J., Lehtinen, T., Hemerik, L., de Ruiter, P., Lair, G., 2017. Effects of land use on soil microbial biomass, activity and community structure at different soil depths in the Danube floodplain. *European Journal of Soil Biology* 79, 14–20.
- Wagg, C., Bender, S.F., Widmer, F., van der Heijden, M.G., 2014. Soil biodiversity and soil community composition determine ecosystem multifunctionality. *Proceedings of the National Academy of Sciences of the United States of America* 111, 5266–5270.
- Wang, Y., Li, Y., Ye, X., Chu, Y., Wang, X., 2010. Profile storage of organic/inorganic carbon in soil: From forest to desert. *Science of the Total Environment* 408, 1925–1931.
- Wang, Z., Li, T., Li, Y., Zhao, D., Han, J., Liu, Y., Liao, Y., 2020. Relationship between the microbial community and catabolic diversity in response to conservation tillage. *Soil & Tillage Research* 196, 104431.
- Wenhui, Z., Zucong, C., Lichu, Y., He, Z., 2007. Effects of the long-term application of inorganic fertilizers on microbial community diversity in rice-planting red soil as studied by using PCR-DGGE. *Acta Ecologica Sinica* 27, 4011–4018.
- Wickham, H., 2016. *ggplot2: elegant graphics for data analysis*. Springer, Berlin.
- Will, C., Thurmer, A., Wollherr, A., Nacke, H., Herold, N., Schrupf, M., Gutknecht, J., Wubet, T., Buscot, F., Daniel, R., 2010. Horizon-specific bacterial community composition of german grassland soils, as revealed by pyrosequencing-based analysis of 16S rRNA genes. *Applied and Environmental Microbiology* 76, 6751.
- Wu, H., Adams, J.M., Shi, Y., Li, Y., Song, X., Zhao, X., Chu, H., Zhang, G.L., 2020. Depth-dependent patterns of bacterial communities and assembly processes in a typical red soil critical zone. *Geomicrobiology Journal* 37, 201–212.
- Xia, Q., Rufty, T., Shi, W., 2020. Soil microbial diversity and composition: Links to soil texture and associated properties. *Soil Biology & Biochemistry* 149, 107953.
- Xue, P., Carrillo, Y., Pino, V., Minasny, B., McBratney, A.B., 2018. Soil properties drive microbial community structure in a large scale transect in south eastern Australia. *Scientific Reports* 8, 11725.
- Young, I.M., Crawford, J.W., 2004. Interactions and self-organization in the soil-microbe complex. *Science* 304, 1634–1637.
- Zhalnina, K., Dias, R., Quadros, P., Davis-Richardson, A., Camargo, F., Clark, I., McGrath, S., Hirsch, P., Triplett, E., 2015. Soil pH determines microbial diversity and composition in the park grass experiment. *Microbial Ecology* 69, 395–406.