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Special Issue Article

The legacy of bacterial invasions on soil native communities

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Summary

Soil microbial communities are often not resistant to the impact caused by microbial invasions, both in terms of structure and functionality, but it remains unclear whether these changes persist over time. Here, we used three strains of *Escherichia coli* O157:H7 (*E. coli* O157:H7), a species used for modelling bacterial invasions, to evaluate the resilience of the bacterial communities from four Chinese soils to invasion. The impact of *E. coli* O157:H7 strains on soil native communities was tracked for 120 days by analysing bacterial community composition as well as their metabolic potential. We showed that soil native communities were not resistant to invasion, as demonstrated by a decline in bacterial diversity and shifts in bacterial composition in all treatments. The resilience of native bacterial communities (diversity and composition) was inversely correlated with invader's persistence in soils (R^2 = 0.487, p < 0.001). Microbial invasions also impacted the functionality of the soil communities (niche breadth and community niche), the degree of resilience being dependent on soil or native community diversity. Collectively, our results indicate that bacteria invasions can potentially leave a footprint in the structure and functionality of soil communities, indicating the need of assessing the legacy of introducing exotic species in soil environments.

Introduction

Soil health and functionality are strongly associated with soil biodiversity, which also determines the ability of soil native communities to withstand disturbances (Griffiths and Philippot, 2013; Wagg *et al*., 2014). Biological diversity plays a substantial role in soil resilience, which is necessary to sustain desirable ecosystem states in unstable environments (Peterson *et al*., 1998; Elmqvist *et al*., 2003). The importance of soil diversity for resilience and multifunctionality applies to both macro and microbial ecology (Girvan *et al*., 2005; Kowalchuk *et al*., 2002), although the latter might be blurred by its extreme high functional redundancy (Balser and Firestone, 2005; Jurburg and Salles, 2015). As known, macro-ecology seeks to find the relationships between organisms and environment at large spatial scales to characterize and explain statistical pattern of abundance, distribution and diversity (Brown and Maurer, 1989). With ever-growing research on microbial ecology, ecologists proved that microbial communities can expand species numbers and macro-ecological individuals by different orders of magnitude (Curtis *et al*., 2006). Microbial systems can also easily test the macro-ecological hypotheses, while large organisms would be very difficult to test (Jessup *et al*., 2004). Overall, bacterial, archaeal and eukaryotic organisms share similar ecological patterns which are accordance with that species' functional and ecological similarities are shaped by common ancestry patterns despite of their dependent entities (Felsenstein, 1985; Soininen, 2012). Therefore, we unified the macro- and micro-ecological descriptions towards invasion mechanisms.

Disturbances can occur in response to the arrival of alien species – those that are transported outside their native area – due to the rise of globalization, anthropogenic activities and habitat degradation, potentially becoming invasive once introduced into a new range (Hulme, 2009; Blackburn *et al*., 2011). When introduced into new environment,

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invasive species may displace resident taxa and alter community functioning, potentially leading to 'perturbation' or 'stress' in the system (Rykiel, 1985; Franz *et al*., 2014; Mallon *et al*., 2015a). In contrast, resident communities can also resist alien species invasion. The invasion resistance is related to the diversity of native communities, as more diverse communities have greater ability to exploit and compete for available resources than simpler ones, thus leaving less resources available for the invasive species (Tilman, 1977; Rykiel, 1985; Mallon *et al*., 2015b). In plant communities, high diversity increases resistance to invasion by exploring larger patches, which decreases the availability of light and nutrients for alien plant species (Naeem *et al*., 2000). Similar rules also apply for soil microbiome, where microbial communities with increased species diversity are more resistant or resilient to changes induced by exotic microbial species due to their ability to exploit space and resource in a complete and efficient fashion (van Elsas *et al*., 2012; Mallon *et al*., 2015b; Lourenço *et al*., 2018).

Once overcoming the biotic resistance of resident community, invasive species successfully expand their population, permanently displacing the non-resilient resident taxa and potentially generating a new, alternative, stable state (Levine *et al*., 2004). The concept of invasion is often studied from a success perspective, that is, the impact of invasion is determined for communities that are successfully colonized by alien species. However, previous studies showed that invasive species can also leave footprints in a new environment even if their elimination is imminent (Yao *et al*., 2014; Mallon *et al*., 2018). Hence, unsuccessful invasions, such as the release of *Escherichia coli* O157:H7 (*E. coli* O157:H7) in the environment during animal defecation or manure amendment, can have legacy effects on the resident communities, modifying its diversity and functioning (Mallon *et al*., 2018).

Escherichia coli is commonly used as a model species for the study of molecular biology as well as ecological invasion due to being the pathogenic agent of intestinal and urinary tract diseases and public health indicator, whose secondary habitats can be water, sediment and soil (Brown *et al*., 2006; He *et al*., 2010; Mallon *et al*., 2018). From human/animal intestinal system to open environment, *E. coli* can experience biphasic lifestyles including host-independent and host-associated lifestyles which changed the strain core genomes to adapt to new habitats (van Elsas *et al*., 2011). The ways to obtain abundant nutrients and promote the growth efficiency in new habitats under fluctuating environmental conditions depend on the evolution of the metabolic functional genomes (Tao *et al*., 1999; Ihssen and Egli, 2005). Once in its secondary environment, the interaction between *E. coli* and the native soil microbiome, especially its ability to compete for resources, will determine the success of the invasion (Savageau, 1983; Mallon *et al*., 2018; Xing *et al*., 2019). Importantly, when outside the animal gut, *E. coli* can reach the roots and leaves of vegetables through irrigation or manure compost application, potentially leading to (deadly) outbreaks (Berger *et al*., 2010). For instance, Shiga toxin-producing *E. coli* (STEC) strains known for causing bacterial severe gastrointestinal illness in children (Siegler and Oakes, 2005) and elderly have caused an important outbreak (United States for example) when found colonizing in fresh lettuce and spinach (Brandl, 2008; Grant *et al*., 2008; Neil *et al*., 2012).

Here, we were interested in determining to what extent the invasion by *E. coli* can induce changes in native soil bacterial communities – that is, soil resistance and resilience to invasion – and whether this legacy is dependent on the genetic background of the invasive strain, initial soil diversity and soil biotic and abiotic components. We have previously shown that soil indigenous biotic and abiotic community properties affect the survival of *E. coli* O157:H7, confirmed the importance of biodiversity upon invasion and revealed that virulence genes negatively influenced *E. coli* survival (Xing *et al*., 2019). However, the consequence of these invasions for the native soil bacterial communities remained unclear. In this study, we hypothesized that invasion will generate a soil legacy on both bacterial community composition and functioning and that the extent of this legacy is driven by the duration of the invasion (survival time). To test this hypothesis, we followed shifts in bacterial community structure, niche breadth (carbon sources utilization) and community niche in response to the inoculation of three *E. coli* carrying different Shiga toxic genes into four soils for 120 days after invasion.

Results

Four natural soils included laterite from Lingao (LG), yellow soil from Wenzhou (WZ), yellow cinnamon from Nanjing (NJ) and cinnamon from Penglai (PL), which showed different physical and chemical characteristics (Supporting Information Table S2). According to our recent research results, the most important soil characteristic that affects the *E. coli* survival time in soil is pH value, 4.30 for LG, 4.51 for WZ, 6.53 for NJ and 7.23 for PL. Also, the difference of soil types observed with different biotic indexes including α-diversity, microbial composition and functional indicators also left significant effects on *E. coli* invasion time (Xing *et al*., 2019).

Shifts in bacterial α-diversity as a function of E. coli invasion

In order to address the potential impact of invasion, we quantified bacterial diversity measurements before and at 4, 40 and 120 days after the invasion (Fig. 1 and Supporting Information Fig. S1). Overall, invasion led to a decrease in phylogenetic diversity (Fig. 1) and species richness (Supporting Information Fig. S1), regardless of soil types, *E. coli* strains or dilution treatments.

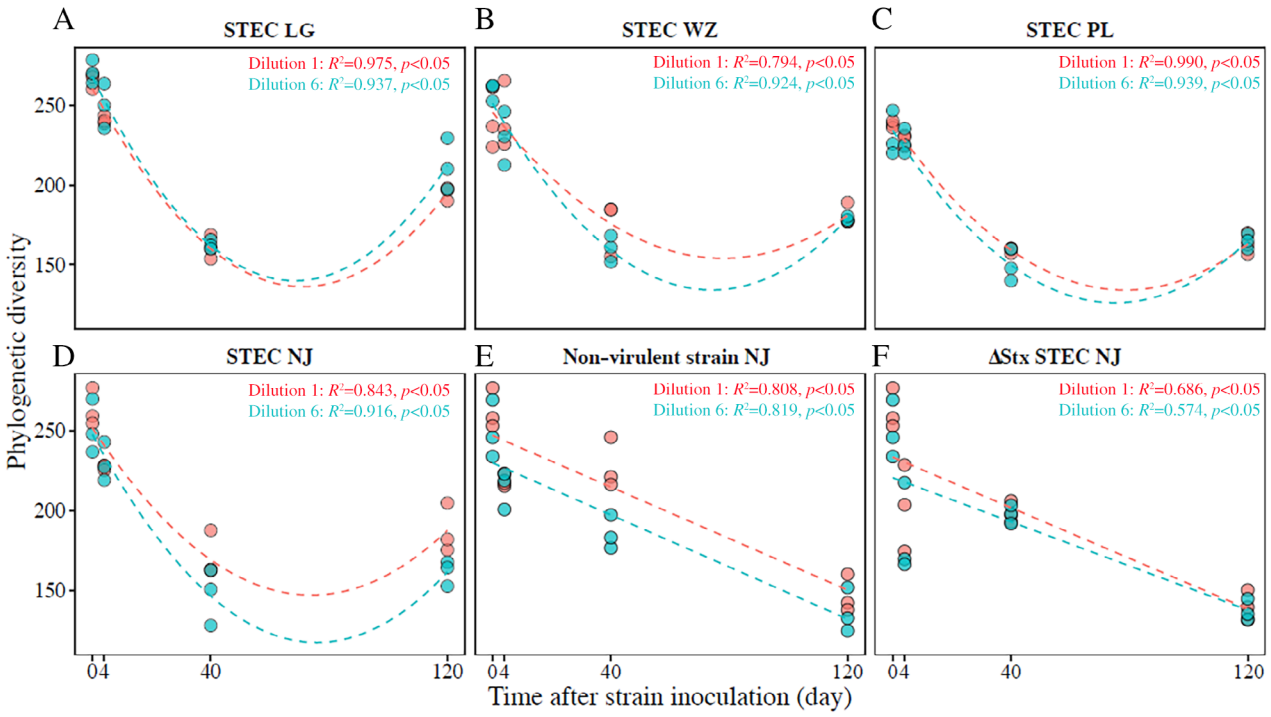

Fig 1. Decline in the phylogenetic diversity of native soil bacterial communities from four Chinese soils upon invasion with three *E. coli* strains. Strain STEC was inoculated in all four soils (LG, WZ, PL and NJ; A–D, respectively) whereas the non-virulent and ΔStx STEC strains were inoculated in soil NJ only (D–F, respectively). Strains were inoculated in soils harbouring a gradient of diversity (10^{-1} and 10^{-6} dilution treatments) and sampled before and at 4, 40 and 120 days after inoculation. Regression lines, R^2 values and significance are indicated for each dilution, in each graph. Data points indicate the average values of three replicates and samples followed by the same letter were not significantly different (Tukey test, $p > 0.05$). Red symbols and lines, treatment 10^{-1} dilution; blue symbols and lines, treatment 10^{-6} dilution. [Color figure can be viewed at wileyonlinelibrary.com]

The effect of soil type on the invasion was verified by selecting STEC that invaded into four types of soil. Comparisons across soils inoculated with STEC revealed that the decrease of diversity followed a binomial regression. Although diversity statistically increased over time in some cases (LG, both dilutions and WZ), dilution 10^{-6}, Fig. 1A and B), indicating tendency to recover in the later period, soils inoculated with STEC strain were not resilient to the decrease in phylogenetic diversity triggered by invasion. The effect of the strain on the invasion was verified by the selection of three *E. coli* strains (including STEC, non-virulent and ΔStx STEC strains) to invade into NJ soil (Fig. 1E and F), although the changes in diversity followed a linear decline, being stronger at 120 days after inoculation took place. Interestingly, these results suggest that the impact caused by invasion is dependent on the *E. coli* strain and on its capacity to survive, because the longer the invader survives in soils (Xing *et al*., 2019), the bigger legacy effect on bacterial α-diversity.

Shifts in bacterial β-diversity as a function of E. coli invasion

To explore temporal variability in the overall bacterial community composition upon *E. coli* invasion over the sampling period, we did principal coordinates analysis (PCoA) based on Bray-Curtis distance (Fig. 2). Similar to what was observed for α-diversity, our data revealed dynamic changes in community composition. Overall, invasion led to rapid shifts in beta diversity in all soils, for all strains and both dilution treatments. Except for strain STEC in soils LG and WZ, which tended to recover to the initial community composition 120 days after invasion (Fig. 2A and B; analysis of molecular variance [AMOVA], $p > 0.05$), communities in the remaining treatments did not return to the original composition within the timeframe of the experiment (Fig. 2 and Supporting Information Fig. S2). Comparisons between the bacterial communities before and 120 days after invasion revealed that the largest turnovers were observed for soils inoculated with non-virulent and ΔStx STEC strains, and the lowest for STEC strain in soils LG and WZ, for both 10^{-1} and 10^{-6} dilution treatments (Supporting Information Fig. S3A), suggesting that the resilience of bacterial community composition to *E. coli* invasion was dependent on strain and soil, as previously observed for *E. coli* survival (Xing *et al*., 2019). Indeed, the correlation between survival time of each strain in the soil and the turnover in bacterial community composition (Bray-Curtis distance) between day 0 and day 120 revealed that 48.7% ($p < 0.001$) of the variation in turnover

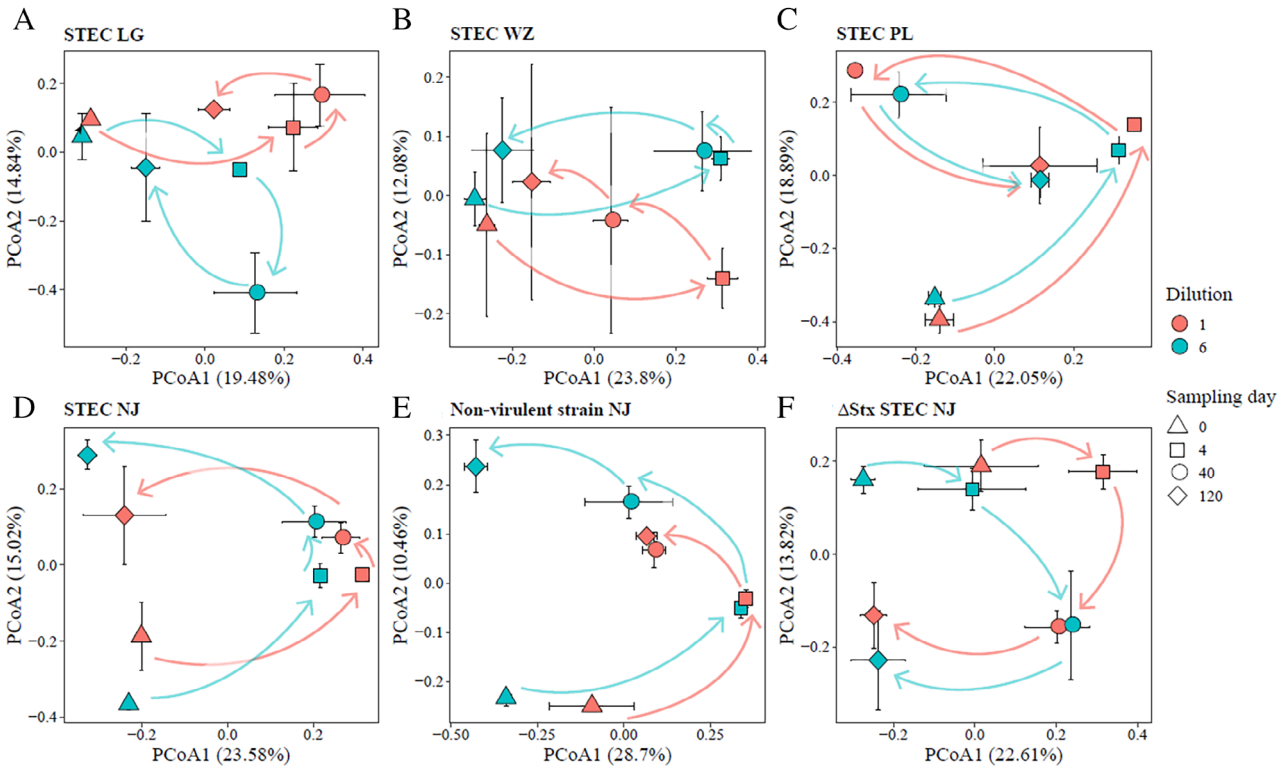

Fig 2. Beta diversity of native soil bacterial communities in response to inoculation with *E. coli* strains. Principal component analyses (PCoA) was based on Bray-Curtis dissimilarity of data generated from bacterial community composition (16S rRNA sequencing). Strain STEC was inoculated in all four soils (LG, WZ, PL and NJ; A–D, respectively) whereas the non-virulent and ΔStx STEC strains were inoculated in soil NJ only (D–F, respectively). Strains were inoculated in soils harbouring a gradient of diversity and sampled before and at 4, 40 and 120 days after inoculation. Each symbol represents the average of three replicates and contains the standard error. Arrows indicate the trajectory of communities from before the invasion (triangle symbols) up to 120 days (triangle, before invasion; square, 4 days; circle, 40 days; diamond, 120 days). Red symbols and lines, treatment 10^{-1} dilution; blue symbols and lines, treatment 10^{-6} dilution. [Color figure can be viewed at wileyonlinelibrary.com]

time could be explained by *E. coli* persistence in soils, with the legacy of invasion (larger turnover in composition) being positively correlated with invader's survival (Fig. 3).

Community functioning changes in response to invasion

The impact of invasion on bacterial community functioning was verified by comparing potential shifts in the metabolic potential of the communities before and 120 days after the invasion, using BIOLOG plates and by performing univariate (community niche) and multivariate analyses (PCoA).

The assessment of the community niches showed that the invasion leads to a significant increase (ranging from 111.88% to 275.35%) in metabolic potential of the bacterial communities after STEC invasion regardless of soil. The increment of community niche is higher at 10^{-1} dilution than 10^{-6}, except LG soil invaded by STEC and NJ soil invaded by ΔStx STEC strains (Fig. 4). Comparisons across *E. coli* strains in the same soil showed variable patterns indicating strain-specific responses. Specifically, the non-virulent and ΔStx STEC strains contributed less to shifts in community niche, with values varying from 87.79% to 172.61% of the control (Fig. 4). In summary, this univariate measure of metabolic potential revealed a lack of resilience of the community upon invasion, where soil types, strains and diversity determined the degree of observed changes.

Principal coordinate analysis of the metabolic potential of different soils when invaded by a STEC strain revealed, in general, large differences among soils, dilution treatments and invasion (before and 120 days after invasion), the latter being often discriminated along the first PCoA (42.3% of the variation; Fig. 5A). Except for soil NJ, dilution 10^{-1} and, to a lesser extent, soil LG, dilution 10^{-6}, invasion lead to strong changes in the metabolic potential, indicating lack of functional resilience. This effect that was observed for both dilutions indicating that initial soil diversity played a smaller role when communities are invaded by STEC strain. In contrast, comparison across strains in the same soil (NJ soil) discriminated the dilution treatments along the first PCoA (63.4% of the variation) whereas shifts in metabolic potential in response to invasion (before and 120 days after the invasion) were related to the second

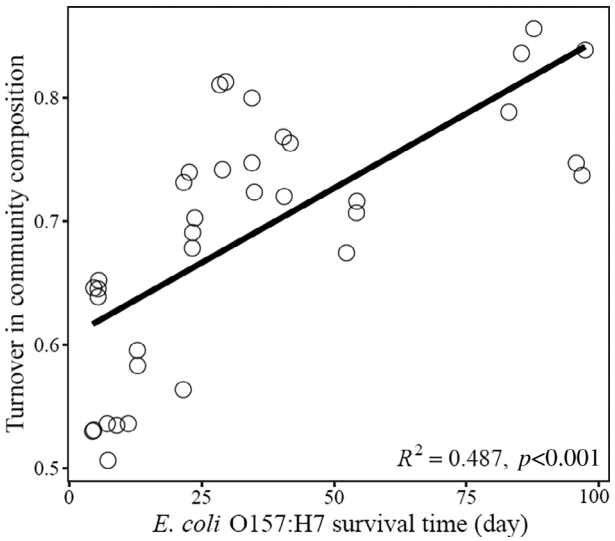

Fig 3. Relationship between turnover in the composition of native soil communities before and after invasion and survival time. Turnover in community composition (16S rRNA gene sequencing) was calculated based on Bray-Curtis distance between day 0 and 120 after inoculation. Survival time of all three *E. coli* O157:H7 strains in four soils is indicated in days and refer to data published in Xing *et al*. (2019). Each dot represents the invader survival time and its corresponding turnover value. Line indicates linear regression and R^2 and p values are indicated in the graph.

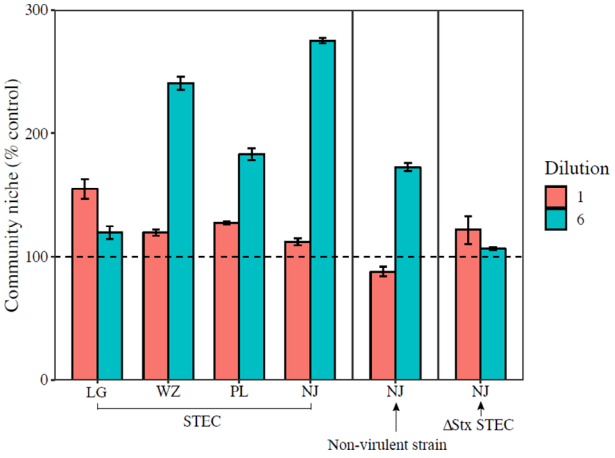

Fig 4. Community niche of soil native bacterial communities at day 120 after invasion, in percentage. Community niche was calculated according to Salles *et al*. (2009) and Mallon *et al*. (2018) and reflects univariate metric of metabolic potential (BIOLOG ECO plates) of each soil bacterial community. Line at 100% represents the community niche values at day 0 (before inoculation). Soils LG, WZ, PL and NJ were inoculated with STEC strain, whereas non-virulent and ΔStx STEC strains were inoculated only in soil NJ. Red bars, treatment 10^{-1} dilution; blue bars, treatment 10^{-6} dilution. [Color figure can be viewed at wileyonlinelibrary.com]

axis (18.5%; Fig. 5B). Thus, the effect of initial soil microbiome diversity seems to be dependent on strain.

In addition, we also quantified the turnover difference based on Bray-Curtis distance during the invasion period (day 0 and day 120) and observed that the turnover in metabolic potential differed between strains and dilutions (Supporting Information Fig. S3B). Highest turnover was observed for the STEC strain in 10^{-6} treatment, across all soils ($p < 0.05$), except for LG, where the 10^{-6} treatment also showed high turnover, similarly to the 10^{-1} treatment. The non-virulent and ΔStx STEC strains invaded into 10^{-1} treatment showed lower turnover in comparison to STEC strain, albeit the values were relatively high in low diversity 10^{-6} treatment. Generally speaking, *E. coli* invasion changed the functioning (carbon utilization patterns) of the native bacterial communities regardless of soil and strain, an effect that seemed larger in the soils from the 10^{-6} treatment, where *E. coli* persistence was the longest (Supporting Information Fig. S3B).

Specific changes in metabolic potential

Differential carbon sources profiles revealed differences between natural soil community (control) and *E. coli* invaded soil community (Supporting Information Fig. S4). In high diversity communities (10^{-1} dilution treatment), STEC invasion increased the utilization of all six carbon classes (polymers, carboxylic acids, carbohydrates, amino acids, amines and others) in all four soils although the relative contribution of each class was soil-dependent, indicating a soil effect on carbon source utilization. The non-virulent and ΔStx STEC strains aroused different shifts in carbon utilization when compared to STEC strain and among themselves in the same soil and dilution (NJ, 10^{-1} treatment), showing increase in the use of carboxylic acid, amines and other sources (Supporting Information Fig. S4) upon invasion, whereas invasion by the ΔStx STEC strain led to increase mostly in sources belonging to the carboxylic acid class. Details concerning carbon sources utilization patterns were listed in Supporting Information Table S1. The patterns observed by invasion by each of the three strains in four soils with 10^{-1} treatment (Supporting Information Fig. S4A–F) differed greatly from those observed for the lowest diversity treatment (10^{-6} treatment, Supporting Information Fig. S4G–L).

Discussion

Soil ecosystems harbour highly diverse communities which has been estimated to contain over 10^9 bacteria g^{-1} soil and a wide range of fungal hyphae, nematodes, earthworms, to mention a few (Roesch *et al*., 2007; Wagg *et al*., 2014). Microbial communities are at the heart of all soil ecosystems, where these microorganisms play essential roles in nutrient cycling and primary productivity (Jenkinson, 1988; Jangid *et al*., 2011; Shade *et al*., 2012; Makarov *et al*., 2016). As such, exotic microorganisms are often introduced into soils for agricultural or bioremediation purposes, following patterns associated with ecological invasions

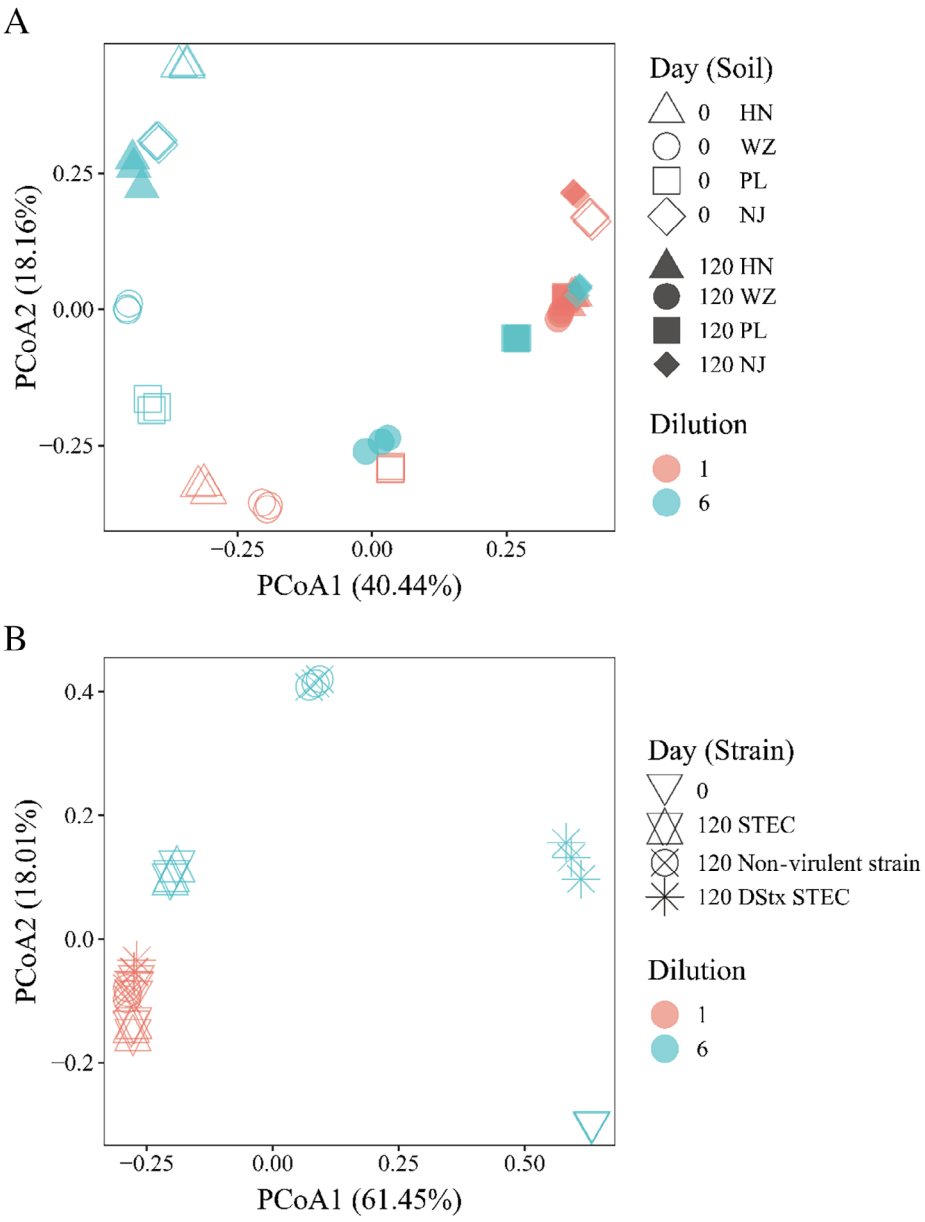

Fig 5. Shifts in the niche structure of the native soil bacterial communities in response to invasion. PCoA analysis was constructed using Bray-Curtis distance generated from the carbon sources utilization profile (BIOLOG ECO plate) of each microcosm, before and 120 days after inoculation.
A. Niche structure of bacterial communities from soils LG, WZ, PL and NJ (10^{-1} and 10^{-6} dilution treatments) after invasion with STEC strain.
B, Niche structure of bacterial communities from soil NJ (10^{-1} and 10^{-6} dilution treatments) when invaded by STEC, non-virulent and ΔStx STEC strains. Red symbols, treatment 10^{-1} dilution; blue symbols, treatment 10^{-6} dilution. Empty symbols, niche structure at day 0 (before invasion); Full symbols, niche structure at day 120 after invasion. [Color figure can be viewed at wileyonlinelibrary.com]

(Litchman, 2010; Amalfitano *et al*., 2015; Mallon *et al*., 2015a). However, the impact of these invasions on the native communities remains questionable, specially giving the low survival times of microbial invasive species (Mallon *et al*., 2015a).

Recent studies showed that *E. coli* invasion does lead to an impact on soil native communities (lack of resistance), despite its low persistence in soils (Mallon *et al*., 2018), opening two important questions. First, that the functionality and composition of soil native communities are resilient to the disturbances caused by microbial invasion remains unclear. Resilience happens when ecological interactions dampen disruptions and can be quantified by the time it takes to go back to the initial condition following disturbance (Peterson *et al*., 1998). Alternatively, in the absence of resilience, the community may shift to a different equilibrium from which it becomes difficult to return to the previous composition or function (Botton *et al*., 2006; Shade *et al*., 2012). Second, as community resilience is associated with environmental factors and ecosystems attributes (Griffiths and Philippot, 2013; Chambers *et al*., 2014), it is important to verify what extent the soil resilience to invasion is dependent on soil biotic and abiotic factors or invasive species.

Drivers of resilience in community diversity and structure

Our results clearly demonstrated that the taxonomic component of the soil microbiome was not resistant and, to a

larger extent, not resilient to invasions, a fact that was consistent across four forest soils from China eastern area, irrespective of soil, initial diversity or invasive strain. Community response to environmental disturbance is influenced by individual, population and community-level biological attributes. As such, the quality and healthy status of soils and associated bacterial diversity have been previously attributed to the resilience to disturbances such as heavy metal, pH and organic amendments (van Bruggen and Semenov, 2000; Girvan *et al*., 2005; Botton *et al*., 2006; Semenov *et al*., 2008). In the case of invasions, community diversity also plays an important role in hampering the establishment of exotic species and both species richness and evenness are positively associated with resistance to invasion (Case, 1990; Elton, 2000; van Elsas *et al*., 2012; De Roy *et al*., 2013; Mallon *et al*., 2015b; May, 2019; Xing *et al*., 2019). The effect of diversity is often linked to mechanisms associated with resource competition networks, including connectance and nestedness, as well as niche overlap (Wei *et al*., 2015). To verify the effect of soil type on the invasion, only STEC strain was considered in this part. In the context of our experiment, *E. coli* invasion caused rapid changes in the α- and β-diversities of soil resident bacterial communities. Although these soils followed different recovery paths, albeit not returning to the original state, some seem to engage faster in a recovery pattern, indicating higher resilience. Especially, LG and WZ soils tended to revert to the initial community state before invasion both in diversity and composition, whereas the other soils showed a continued decline in diversity as well as lack of resilience, at least in the time frame considered here. Finally, although native soil diversity is often considered as a major driver of resilience, we showed that in most cases, the degree of changes followed similar paths for both dilution treatments.

Interestingly, the intricate relationship between biotic (soil microbiome) and abiotic (soil characteristics) components in controlling soil resilience to invasions was directly linked to the time spam the invasive species remain in soils, given that *E. coli* survival time explained almost 50 % of the observed variation in the turnover of bacterial community composition. This result suggests that the persistence of invasive species can potentially have large, long-term effects on native community, by influencing the physiological, behavioural or morphological plasticity of native species (Griffiths and Philippot, 2013). Our finding is also supported by a plant invasion study which pointed out the system becomes less resilient with the extend of invasion period, mainly due to an incremental loss of native species (Marchante *et al*., 2011). This effect also impacted the belowground communities, as both soil microbial biomass C and activity were influenced by the time since *Acacia longifolia* invasion (Marchante *et al*., 2008). Similarly, South African "fynbos" invaded by *Acacia* species revealed decreased community resilience in response to invasion duration (Holmes and Cowling, 1997). In the context of microbial invasions, we have previously shown that soil pH and microbial diversity determined the invader's survival time in the soils used here (Xing *et al*., 2019). The fact that invasion legacy is correlated with survival time provides indirect evidence for the role played by both soil biotic and abiotic characteristics in constraining the resilience of soil bacterial communities when exposed to invasion disturbance, which integrates both resource availability and soil microbiome. Thus, both soil biotic and abiotic properties, as well as the genetic background of the invasive species, lead to patterns associated with the resistance and resilience of soil native microbiome to microbial invasions.

Drivers of resilience in community functioning

The lack of resilience observed in diversity and composition of native communities were translated into changes in their functionality, prompted by differences in the metabolic potential of the soil microbiome. Specifically, our data revealed large functional shifts in the niche breadth, niche structure and community niche of the native communities over the period of 120 days after invasion, confirming the lack of resilience also at the functional level. Examples borrowed from general invasion ecology show that exotic species can alter soil physical characteristics that may control soil community composition and function (Wolfe and Klironomos, 2005). For instance, invasion processes can decrease the growth of native tree species, change soil enzyme activities and respiration profiles as well as nitrogen mineralization rates (Belnap and Phillips, 2001; Roberts, 2001; Kourtev *et al*., 2002). However, contrary to the results observed for bacterial community structure and diversity, the functional resilience of native communities was mainly related to soil origin and dilution treatment, and to a lesser extent to the *E. coli* strain. To verify the effect of *E. coli* strains on the invasion, only NJ soil was considered in this part of study. All the 10^{-1} dilutions treatments from NJ soil were either resistant or resilient to changes in metabolic diversity in response to invasion by the three *E. coli* strains, an effect that was absent in the less diverse treatment (dilution 10^{-6}), where greater functional changes were detected. Since the metabolic potential was evaluated only at two time points, before invasion and 120 days after, we cannot conclude whether the less diluted microbiome of NJ soil was functionally resistant or resilient to invasion. Thus, it remains to be tested whether the large community diversity and composition shifts observed for NJ soil lead to small (functionally resistant communities) or large but highly resilient functional changes (Bissett *et al*., 2013). In any case, the lack of relationship between community

structure and functionality for NJ soil emphasizes three important aspects: First, the relevance of functional redundancy in the soil microbiome and community dynamics – driven here by shifts in rare and abundant bacterial species – are essential for maintaining stable processes when exposed to exotic disturbance (Loreau *et al*., 2001; Nannipieri *et al*., 2003). Second, that functional redundancy is highly dependent on the diversity of the soil microbiome, as the same effect was not observed in the 10^{-6} dilution treatment. Third, redundancy is subjected to soil biotic and abiotic characteristics, given that the remaining three soils (LG, WZ and PL, respectively) were not functionally resilient. Moreover, for WZ soil, although resilience was, to a certain extent, observed at taxonomic level, it seems that from the functional perspective it was not reached.

While the genetic background (carrying different toxic genes) of the *E. coli* strains did not influence functional resilience, it did prompt different responses. In the one hand, the STEC strain invasion increased the niche of soil resident community by almost three times in the 10^{-6} dilution treatments. On the other hand, the non-virulent and ΔStx STEC strains showed different patterns, suggesting that the lack of shiga toxin in these strains might contribute to the shifts, either due to a direct effect of toxic compounds on the native soil microbiome – which can inhibit or kill sensitive strains or closely related species – or indirectly, due to the metabolic cost associated with toxin production (Czárán *et al*., 2002).

Long-term consequences of bacterial invasions

Collectively, we demonstrated that bacterial invasions have great impact on bacterial community diversity, structure and functionality, corroborating previous work that showed that microbial invasion could leave a legacy effect on the resident community even when the invasion was unsuccessful (Mallon *et al*., 2015b, 2018). Moreover, we provide evidence that legacy of invasions from diversity and structure perspective depends on invader's survival time in soil, which is linked to soil microbiome and soil type (Xing *et al*., 2019). From the functional standpoint, the impact of invasion was dependent on soil characteristics and initial diversity (dilution treatments). Although our experiment lasted for a relatively long period (120 days), it remains unclear whether the structure and function of soil microbiome have reached an alternative state of equilibrium or whether they will eventually return to the original state in an even longer run. More attention should be paid in soil microbial safety due to wide application of organic carbon, which can significantly prolong the *E. coli* invader survival time in the soil ecosystem (Franz *et al*., 2008). In Fig. 6, we summarize our short-term findings and provide a

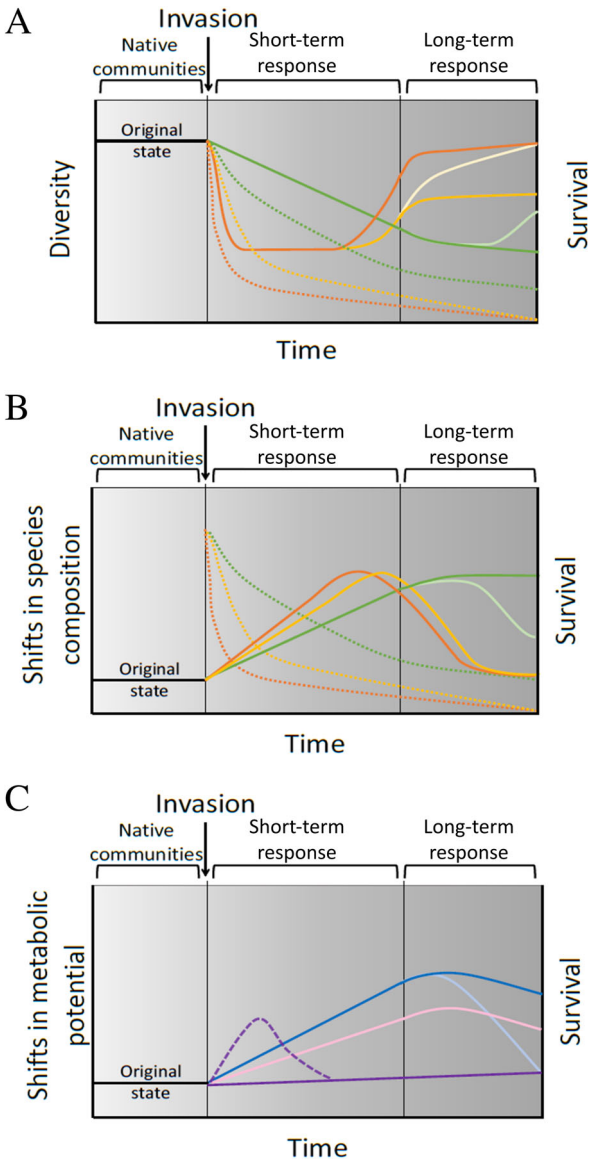

Fig 6. Short- and long-term changes in diversity (A), community structure (B) and functionality (C) of soil native communities upon invasion. Short-term changes reflect the timeframe of this experiment (120 days) and long-term responses represent hypotheses driven by the acquired data. The resilience of both diversity (A) and community structure (B) are associated with the survival time of the invader in the four different soils (dashed lines in A and B). Survival time has been previously shown to be dependent on both soil biotic and abiotic characteristics. Alternatively, resistance/resilience to functional changes (C) was related to native community (soil and diversity treatment; 10^{-1} and 10^{-6} dilution treatments). For soil NJ, the native communities belonging to the high diversity treatment were either resistant (full purple line) or resilient (dashed purple line) to invasion, whereas communities from the lower diversity treatment were not resilience in the short term (pink line). Blue line in (C) represents the remaining soils and diversity treatments, which might remain changed in the long term or return to the original state (light blue line). Respective survival levels are indicated by dashed lines (A and B; stain STEC, orange; ΔStx strain, yellow; non virulent strain, green). Survival is based on data provided by Xing *et al*. (2019). [Color figure can be viewed at wileyonlinelibrary.com]

conceptual representation of the potential long-term effects of *E. coli* invasion in soils in relation to the duration of the invasion process.

Upon invasion, the diversity of the native communities decreases, regardless of their initial diversity or soil type and community structure but is depended on the time the invasive species remains in the soil – the quicker the decline, the larger the impact (short-term response, Fig. 6A). According to our data, we propose that communities subjected to invasive species that are poor survivors tend to recover in diversity (full lines), indicating resilience in a long-term perspective (orange line). On the other hand, native communities under the effect of more persistent invaders might not recover, reaching an alternative stable state whose diversity is below the original (dark and light green lines). Invasive species that survive for intermediate periods of time might either be resilient or reach an alternative state of lower diversity (dark and light yellow lines). Respective survival levels are indicated by dashed lines (strain STEC: orange; ΔStx strain: yellow; non virulent strain: green). As showed in Fig. 6B, the turnover in composition of the native community increases, indicating that invasion leads to drastic changes in community composition, regardless of their initial diversity or soil type and community structure and that the impact is associated with the invasive strain. According to our data, we propose that the time needed by the native communities to experience a decline in turnover (full lines) will depend on the time the invasive species remain in the soil – the quicker the decline, the larger the turnover (short-term response) and the quicker the turnover returns to the original values (long-term response; orange, yellow and light green full lines). However, for the strain that survives well in soils, a reduction in turnover might be observed only after a very long period of time (green line). Respective survival levels are indicated by dashed lines (strain STEC: orange; ΔStxs train: yellow; non-virulent strain: green). Contrary to Fig. 6A and B, Fig. 6C shows that shifts in metabolic potential upon invasion are associated with the native community (soil and diversity treatment) rather than the invasive strain. The full lines indicate only the extreme responses: soil WZ (blue line), invasion lead to strong shifts in metabolic potential for both diversity treatments whereas for soil NJ, the native communities belonging to the high diversity treatment are either resistant (purple line) or resilient (dark pink line) to invasion, whereas communities from the lower diversity treatment showed no resistance and no resilience in the timeframe of the experiment (light pink line). It remains to be tested whether the non-resilient communities go back to their original metabolic potential in a longer time frame (long-term response). Survival levels are indicated by the black dashed lines, for each strain, according to the patterns drawn in Fig. 6A and B. Although it remains to be tested whether these communities go back to their original metabolic potential in a longer time frame (long-term response), the overall imprint lead by bacterial invasions in the soil native microbiome underlines the need to quantify the functional and taxonomic resilience of soils in response to microbial release.

Experimental procedures

Soil and microcosms

Four soils were sampled from natural forestry sites across eastern of China, and five soil cores were taken from a depth of 0–20 cm from the surface and then sieved through ≤ 2 mm mesh, homogenized and sterilized via gamma irradiation (50 kGy) in sealed plastic bags. Soil water content controlled at −33 kPa which was tested by pressure membrane apparatus (Soil Moisture Equipment Corp, Santa Barbara, CA, USA) as described by Kern (1995). Fifty grams of sterile soil was transferred aseptically into 100 ml sterile plastic bottles and the water content was adjusted to 100% of soil water holding capacity (WHC) at −33 kPa with sterile deionized water. Natural soils were stored at 4°C for further inoculation (see next section).

Dilution to extinction experimental setup

Each soil microcosm was inoculated with 1 ml natural soil solution, which prepared by serially diluting in sterile water (1:10; soil:water) up to 10^{-6} dilution. Dilutions 10^{-1} and 10^{-6} were inoculated in triplicates in sterile soils, establishing a gradient of diversity that consisted of three treatments: 10^{-1}, 10^{-6} and sterile soil (non-inoculated control where we added sterile water instead of cell suspensions). Microcosms were incubated for approximately 30 days in order to allow the inoculated communities to fully colonize the microcosms and reach the carrying capacity of the soils (Xing *et al*., 2019). Once all soil microcosms were fully colonized by the soil inoculum, *E. coli* EDL933 (ATCC43895), *E. coli* derivative strain T (Ritchie *et al*., 2003; Mallon *et al*., 2015b) and *E. coli* EDL933 (ATCC43895) Δstx_{1-2} mutant (Ma *et al*., 2011) were introduced at a density of 10^{-7} CFU g^{-1} (oven-dry weight). The soil moisture was adjusted to 100% of WHC at −33 kPa and inoculated microcosms were incubated at 25 ± 1°C for 120 days. We compensated for moisture loss every 5 days with sterilized water.

Polymerase chain reaction amplification and quantification of 16S rRNA gene

Polymerase chain reaction (PCR) reaction consisted of 20 μl containing 10 μl Premix Taq DNA Polymerase (TaKaRa, Dalian, China), 0.1 μl forward primer (100 μm), 0.1 μl reverse primer (100 μm), 8.8 μl double distilled water (ddH_2O) and 1 μl DNA temple. The cycling conditions were

1 cycle of denaturation at 95°C/5 min, followed by 40 cycles of amplification (94°C/30 s, 54°C/30 s, 72°C/40 s) and then completed by a final extension at 72°C/7 min. Quantitative PCR (qPCR) was performed with a real-time PCR detection system (Light Cycle 480; Roche). The bacterial quantified based on 16S rRNA gene using the primers 515F: GTGCCAGCMGCCGCGGTAA and 907R: CCGTCAATT CCTTTGAGTTT (Biddle *et al*., 2008). Each sample was prepared in three replicates and reaction consisted of 20 μl containing 10 μl SYBR Premix Mix (TaKaRa, Dalian, China), 0.2 μl (50 μm) forward and reverse primers, 1 μl DNA template and 8.6 μl ddH_2O. The cycling conditions were 1 cycle of denaturation at 95°C/5 min, followed by 45 cycles of amplification (95°C/10 s, 53°C/45 s, 72°C/45 s). Standard curves for qPCR were created using an up to 10-fold dilution series of PCR product containing a fragment with known 16S rRNA gene copy numbers.

DNA extraction and 16S rRNA gene sequencing

The DNA from soil samples collected at 0, 4, 40 and 120 days after inoculation was extracted by taking approximately 0.5 g soil from each flask, which stored at −20°C. Extraction was performed using FastDNA SPIN Kit (Qbiogene, Carlsbad, CA, USA) according to the protocol of manufacturer. The purity and concentration of the DNA extracts were measured by Nanodrop spectrophotometer (Thermo Fisher Scientific, Wilmington, DE, USA). The V4-5 region of the 16S rRNA gene was amplified using the primers 515F: GTGCCAGCMGCCGCGGTAA and 907R: CCGTCAATT CCTTTGAGTTT (Biddle *et al*., 2008) and the sequencing was performed at Novogene (Beijing, China). Briefly, PCR products were mixed in equi-density ratios and then purified with GeneJET Gel Extraction Kit (Thermo Scientific). Sequencing libraries were generated using TruSeq DNA PCR-free Library Prep Kit for Illumina (NEB, USA) following the recommendations of manufacturer and index codes were added. The library quality was assessed on the Qubit 2.0 Fluorometer (Thermo Scientific) and Agilent Bioanalyzer 2100 system. At last, the library was sequenced on an Illumina HiSeq platform and 250 bp paired-end reads were generated.

Sequences were analysed using UPARSE software to create the operational taxonomic unit (OTU) table. Sequences with ≥ 97% similarity were assigned to the same OTUs. A *de novo* chimera removal was performed using UCHIME (Edgar *et al*., 2011). Barcodes will be removed for further analysis if lengths of less than 200 bp. The phylogenetic tree was built by FastTree.

Carbon sources utilization and quantification of community niche

Five grams of soil samples (oven-dry weight) before (day 0) and at 120 day after inoculation were mixed with 50 ml of physiological saline (0.85%, w/v) and shaken for 1 h at 200 rpm at 25 ± 1°C on an orbital shaker, followed by standing for 30 min. One millilitre of the upper soil suspension was transferred into 9 ml sterile water and then serially diluted to 10^{-3}. Each well of the BIOLOG ECO plate™ (BIOLOG, Hayward, CA, USA) – which consisted of 31 different carbon sources and the blank controls, in triplicate – were inoculated with 150 μl dilutions multichannel pipette. After inoculation, the BIOLOG ECO plate was placed in an incubator at 25 ± 1°C, and colour development in the wells was measured at 4, 24, 48, 72, 96, 120, 144, 168 and 192 h using a BIOLOG reader (BIOTEK Instruments, Winooski, VT, USA) at wavelength of 590 nm. Raw data were normalized by the maximum observed value across all wells and used to calculate community niche according to Salles *et al*. (2009) and Mallon *et al*. (2015b). The community niche was calculated from the sum of observed highest level of use of each carbon sources. The use efficiency of different carbon sources was determined by integrating average well colour development (AWCD) over incubation time from 0 to 196 h, namely, the intensity of carbon substrate metabolism (*S*), estimated by the area underneath AWCD and *t*, using the following formula:

$$\mathrm{AWCD} = \sum_{i=1}^{31} (C_i - r)/n$$

$$S = \sum \left(\frac{v_i + v_{i-1}}{2} \times (t_i - t_{i-1}) \right)$$

where C_i was the absorption of the substrate, r was the comparable absorption of blank control, n was the number of substrates involved, v_i was the AWCD at time $t = t_i$. $\sum$ represents summation for the entire incubation period (Hackett and Griffiths, 1997; Wang *et al*., 2011). These data were used to generate the niche breadth of each community (number of carbons source used by them) as well as the niche structure (quality and quantity of sources consumed by the communities in the ECO plates; Mallon *et al*., 2018).

Statistical analyses

Univariate analysis of covariance was conducted using SPSS 19.0 for windows (SPSS, Chicago, IL, USA). The relevant relative abundances were calculated and all of the sequencing figures were drawn by R studio where packages, for example, phyloseq, Deseq2 and ggplot2 were used (Wickham, 2009; McMurdie and Holmes, 2013). The averages, standard deviations and community niche were processed using Excel 2013 (Microsoft Corporation, Redmond, WA, USA) then figures were visualized using ggplot2 package (Northampton, MA, USA). AMOVA was

calculated to evaluate the significant differences in bacterial community structures among the day 0 and day 120.

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Conflict of interest

The authors declare no conflict of interest.

Availability of data and materials

The data set supporting the conclusions of this article is available in the NCBI Sequence Read Archive under Bioprojects (PRJNA533459).

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Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Appendix S1: Supporting information