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Intraspecies variation in a widely distributed tree species regulates the responses of soil microbiome to different temperature regimes

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Summary

Plant characteristics in different provenances within a single species may vary in response to climate change, which might alter soil microbial communities and ecosystem functions. We conducted a glasshouse experiment and grew seedlings of three provenances (temperate, subtropical and tropical origins) of a tree species (i.e., *Eucalyptus tereticornis*) at different growth temperatures (18, 21.5, 25, 28.5, 32 and 35.5°C) for 54 days. At the end of the experiment, bacterial and fungal community composition, diversity and abundance were characterized. Measured soil functions included surrogates of microbial respiration, enzyme activities and nutrient cycling. Using Permutation multivariate analysis of variance (PerMANOVA) and network analysis, we found that the identity of tree provenances regulated both structure and function of soil microbiomes. In some cases, tree provenances substantially affected the response of microbial communities to the temperature treatments. For example, we found significant interactions of temperature and tree provenance on bacterial community and relative abundances of *Chloroflexi* and *Zygomycota*, and inorganic nitrogen. Microbial abundance was altered in response to increasing temperature, but was not affected by tree provenances. Our study provides novel evidence that even a small variation in biotic components (i.e., intraspecies tree variation) can significantly influence the response of soil microbial community composition and specific soil functions to global warming.

Introduction

It is predicted that global mean temperature will increase 1.8–4.0°C by the end of 2100 (IPCC, 2013). In Australia, mean surface air temperature has already increased by 0.9°C since 1910 (CSIRO/BoM, 2014). Increases in temperature will alter the distribution of plant populations and ecosystem functioning in terrestrial ecosystems worldwide (Zhou *et al.*, 2011; Farrer *et al.*, 2015). Similarly, recent studies suggest that soil microbial communities and functions are highly vulnerable to climate change (Hayden *et al.*, 2012) and that global warming can directly alter soil microbial communities and structure (Pold and DeAngelis, 2013; Wu *et al.*, 2015). Such shifts in the microbial community could potentially have significant functional consequences because soil microbes play a vital role in ecosystem function including plant productivity, nutrient cycling and climate regulation (de Vries and Shade, 2013; Delgado-Baquerizo *et al.*, 2016b).

Aboveground biotic components could significantly influence both soil functions and microbes in terrestrial ecosystems (Garbeva *et al.*, 2008; Prober *et al.*, 2015). Also, recent studies provide evidence that two genotypes of the same species (domesticated plants and their wild relatives) can influence ecosystem function

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(Delgado-Baquerizo *et al*., 2016a) and microbial communities (Garcia-Palacios *et al*., 2013; Leff *et al*., 2017). Species identity of plants and lichens are known to play an important role in regulating responses of soil microbial community and ecosystem functions to global climate factors, including increasing temperature, nitrogen deposition and changes in water availability, mainly through root exudation or altered microclimate (Haichar *et al*., 2008; Liu *et al*., 2016; Yuan *et al*., 2016). Previous studies suggested that there was intraspecies variation in plant response to climate change including elevated CO_2 and warming via altered traits such as photosynthetic capacity, leaf area and growth (Drake *et al*., 2015; Huang *et al*., 2015). In theory, different provenances of the same species can differentially impact both microbial community and soil functions (Johnson *et al*., 2012; Leff *et al*., 2017). However, we lack experimental evidence for the role of plant provenances in regulating microbes and functions. Understanding and predicting whether plant intraspecific variation may influence the response of soil functions and microbial communities to climate change are critical to advance knowledge about ecological theory and for the development of effective adaptation strategies. If true, then even small variation in biotic components can have a large impact on the responses of soil microbes and functions to climate change.

Eucalyptus tereticornis is widely distributed along the eastern coast of Australia and exhibit intraspecies variation (provenances; Drake *et al*., 2017). Here, we evaluate the role of three provenances of *Eucalyptus tereticornis* in regulating the response of soil microbial abundance, diversity, community and soil functions to shifts of temperatures across wide-ranging thermal environments (18, 21.5, 25, 28.5, 32 and 35.5°C). Provenances were assigned to home temperature conditions based on climate records from a network of weather stations across Australia (Jeffrey *et al*., 2001). These provenances grow across a wide gradient of environmental conditions. For example, they grow in a range of summer temperatures from 18°C in temperate climates, to 25°C in subtropical climates and 28.5°C in tropical climates. We also evaluated how climate change-induced shifts in soil microbial communities under three tree provenances affected soil function. We hypothesized that: (i) intraspecies variation promotes different soil microbial communities; (ii) intraspecies variation regulates the response of microbial communities to changes in temperature: microbial communities from warmer origins will have less sensitivity to warming; and (iii) soil functions are linked to microbial community across the thermal range of the species.

Results and discussion

Response of bacterial and fungal community structure

We found that *Eucalyptus* provenance identity and changes in temperature regulate both bacterial and fungal community composition in our soils. We used a nonmetric multidimensional scaling (NMDS) approach to determine shifts in microbial community composition and found that bacterial and fungal community structure differed across different temperatures and tree provenances (Fig. 1; $P < 0.01$). Bacterial communities were separated by temperature along axis 2 (Fig. 1A) and fungal communities were separated by temperature along axis 1 (Fig. 1B). The cladograms identified microbial taxa that varied significantly between provenances and were responsible to explain differences between provenances (Supporting Information Fig. S1). Because our study was a relatively short-term experiment (54 days), we argue that impacts of provenance identity on microbial communities and functions should be related to differences in rhizodeposition (Smalla *et al*., 2001; Garbeva *et al*., 2008) rather than to other processes such as litter decomposition that would require a longer timeframe. Interestingly, our study also provides novel evidence that, in some cases (e.g., bacterial community composition) provenance identity can also regulate the shift in the microbial community composition in response to climate change. This result is supported by the significant interaction between these two factors (Temperature $\times$ Provenance interaction, $P = 0.03$). The capacity of intraspecies variation to regulate the response of bacterial community to changing temperature might be also related to differences in quality and quantity of intraspecies rhizodeposition, and to a strong linkage between aboveground plants and belowground bacteria as plant input into soil is the main carbon and energy source for soil bacteria (Li *et al*., 2011). It has been found that plant traits' vary significantly between provenances in response to warming, including photosynthetic capacity, leaf area and growth (Drake *et al*., 2015). Interestingly, provenance identity did not regulate the response of fungi to variable temperatures. The differential response of bacterial and fungal communities could be linked to the different carbon preferences from these two types of organisms (Rinnan and Baath, 2009; Clemmensen *et al*., 2013). Bacteria prefer more labile carbon sources released directly by plant roots (Kuzyakov *et al*., 2007); however, saprophytic fungi are well-known for their capacity to decompose complex and recalcitrant litter which are less available for bacteria (Xiong *et al*., 2014).

Network properties such as connectivity are often used to predict the responses of entire microbial communities to disturbance. The higher the connectivity, the higher the resistance to a given disturbance (e.g., climate change) (Jeanbille *et al*., 2016). Here, we generated correlation networks of bacteria and fungi using the microbial data

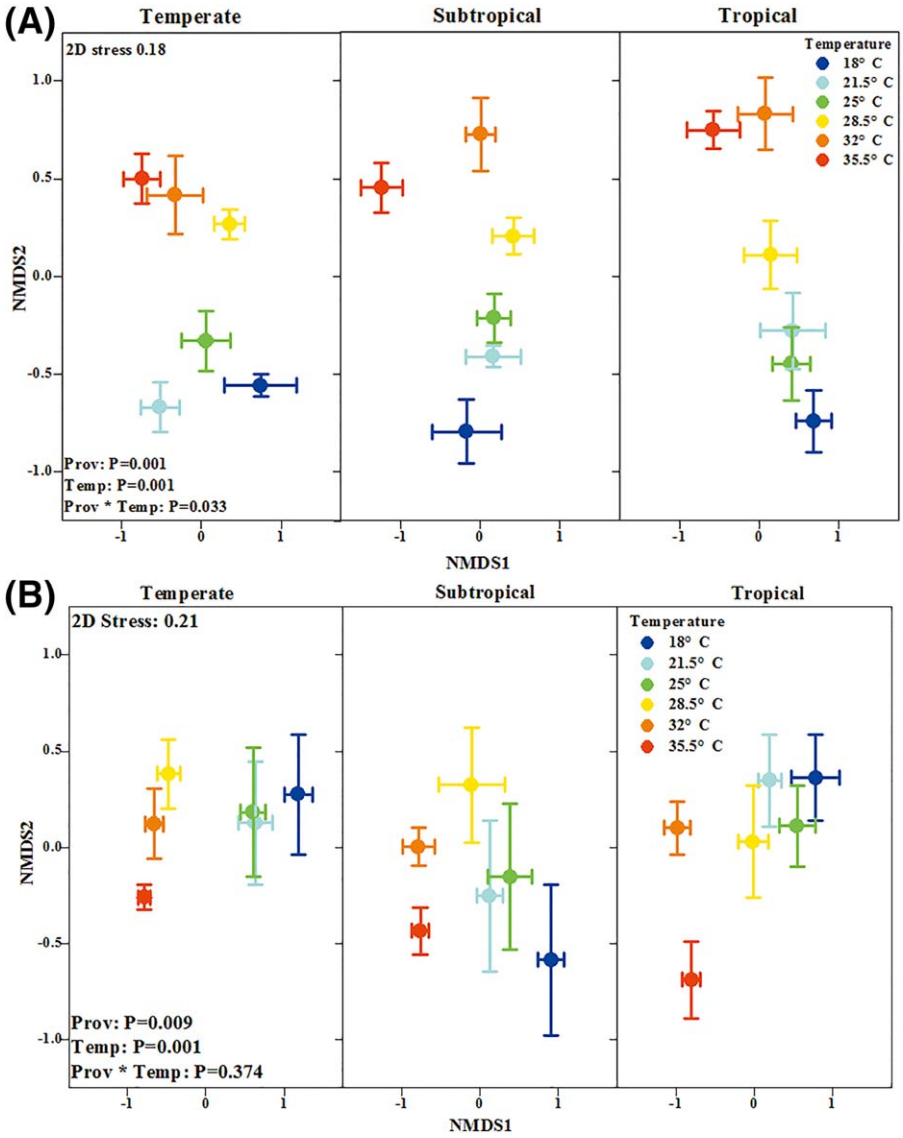

Fig. 1. NMDS ordinations derived from the Bray–Curtis dissimilarity matrices based on the 97% OTU level of the bacterial (A) and fungal (B) community compositions across different treatments. The stress values for both NMDS plots were lower than 0.25, indicating that these data were well represented by the two-dimensional ordinations. Labels: Prov = provenance, Temp = temperature.

along the temperature gradient within each provenance (Fig. 2). This analysis was aimed to identify the temperature-sensitive microbiome that may be specific to the tree provenance. The most connected nodes for bacteria in temperate, subtropical and tropical provenances belonged to phylum *Bacteroidetes*, *Acidobacteria* and *Chloroflexi* (Fig. 2A). Regarding fungal communities, the most connected nodes in temperate, subtropical and tropical provenances included those phylotypes within phylum *Ascomycota*, *Ascomycota* and *Basydiomycota* (Fig. 2B). Networks were compared across three provenances using topological properties (Table 1). For bacteria, network complexity decreased from temperate > tropical > subtropical provenance, as indicated by decreasing clustering coefficient and increasing path length. Temperate presented the highest node connectivity (2.74) compared with subtropical and tropical provenances, indicating temperate origin might be more resistant (less vulnerable) to changes in climate (Jeanbille *et al.*, 2016). For fungi, there were complex networks at subtropical than tropical and temperate provenances. The higher connectivity and clustering coefficient in fungal compared with bacterial networks suggest a higher resistance to shifts in temperatures for fungi versus bacteria. Therefore, the responses of bacterial and fungal networks to climate change might significantly depend on the provenance identity, with consequences for ecosystem functioning under global warming.

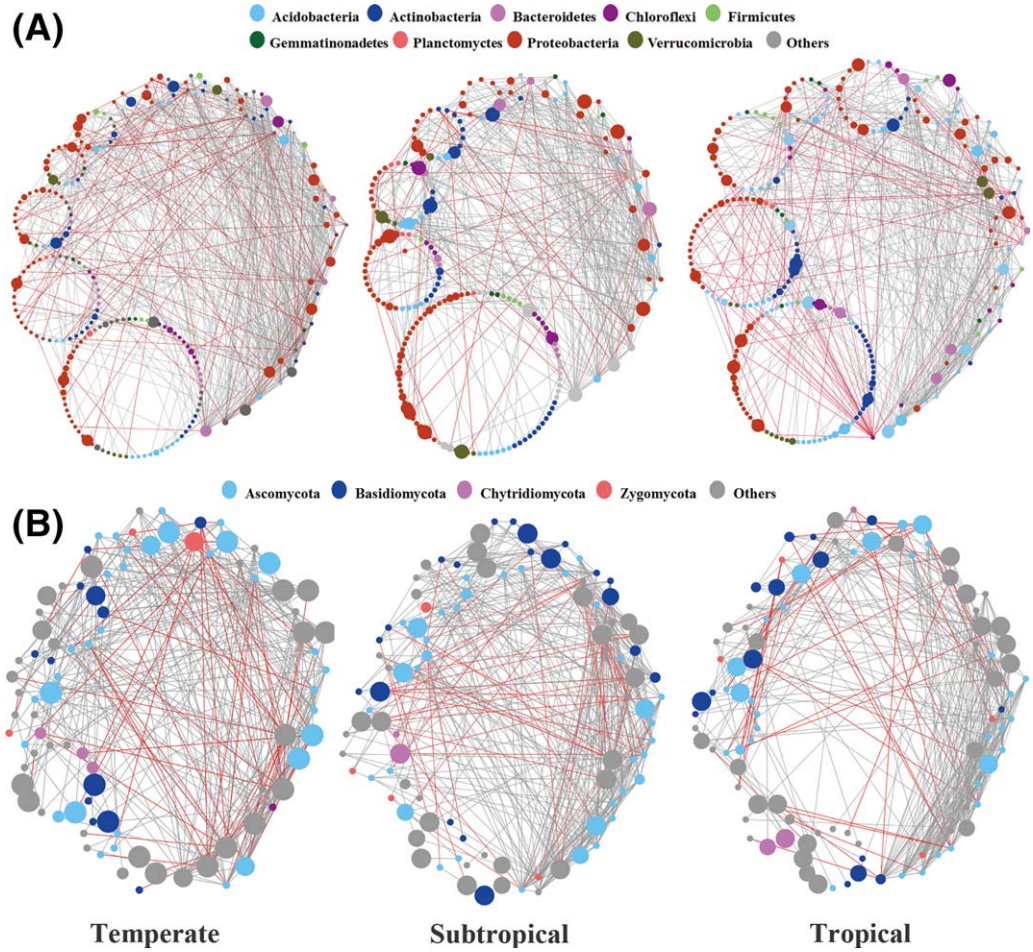

Fig. 2. Network analyses of bacterial (A) and fungal (B) communities. Nodes represent OTUs. Node size is proportional to average relative abundance for each genotype. Node colour denotes phylum level classification. The circular layout is ordered anticlockwise from the bottom based on degree of each node, starting with the highest degree. Lines connecting nodes (edges) represent strong and significant positive (grey) and negative (red) correlations.

Our study also provided strong evidence that temperature and tree provenance significantly and largely regulated the relative abundance of dominant bacterial and fungal taxa (Supporting Information Tables S1 and S2). Also, intraspecies variation modulated the response of relative abundance of *Chloroflexi* and *Zygomycota* to shifting temperatures (Temperature × Provenance interaction, $P = 0.044$ and 0.027 respectively). Increased temperature promoted

Table 1. Co-occurrence network topological properties of bacteria and fungi under three provenances.

	Bacteria			Fungi		
	Temperate	Subtropical	Tropical	Temperate	Subtropical	Tropical
Nodes(S)	239	217	255	90	97	96
Edges(L)	656	404	517	339	415	384
Connectivity (L/S)	2.74	1.86	2.03	3.77	4.28	4.00
Characteristic path length	4.22	5.86	5.77	2.82	2.88	2.97
Network diameter	13	15	16	6	6	7
Clustering coefficient	0.29	0.22	0.27	0.4	0.45	0.40

Nodes represent OTU with at least one correlation strong and significant.

Connect (L/S) indicates average degree or node connectivity.

Characteristic path length shows network distance between all pairs of nodes.

Network diameter shows longest distance between the nodes in the network.

Clustering coefficient indicates how well a node is connected to its neighbors.

relative abundance of *Chloroflexi*, but only in the tropical provenance (Supporting Information Fig. S2). Relative abundance of *Zygomycota* was promoted by increased temperature in the temperate provenance (Supporting Information Fig. S3). The relative abundances of *Acidobacteria, Actinobacteria* and *Chloroflexi*, which were described to be heat-resistant and grow well in high temperature environments (Hayden *et al*., 2012; Yergeau *et al*., 2012; Zhang *et al*., 2013), were observed to increase with increasing air temperature. In contrast, the relative abundances of *Planctomycetes* and *Bacteroidetes* decreased (Supporting Information Fig. S2) perhaps because *Planctomycetes* lack peptidoglycan in cell walls and are mostly chemoheterotrophs (Fuerst and Sagulenko, 2011; Riah-Anglet *et al*., 2015). Elevated temperature increased the relative abundances of *Ascomycota*, but decreased those of *Basidiomycota* (Supporting Information Fig. S3). Free-living fungi generally respond slowly to changes in labile substrate caused by climate change (Chigineva *et al*., 2009; Andresen *et al*., 2014). Thus, the response of soil fungi may be related to other processes such as litter decomposition which have not been recorded here, explaining the low modulator effect of tree provenance on the responses of these organisms to climate change (Gulis and Suberkropp, 2003; Hättenschwiler *et al*., 2005). Overall, these analyses suggest that shift in temperature promotes different microbial communities.

Response of bacterial and fungal abundance and diversity

No significant interaction of tree provenance and temperature on microbial abundance and diversity were found, indicating intraspecies variation had small capacity to regulate the responses of diversity and abundance of soil microbes to climate change. However, we found a significant effect of increasing temperature on bacterial and fungal abundance (Fig. 3A and B; $P < 0.001$,

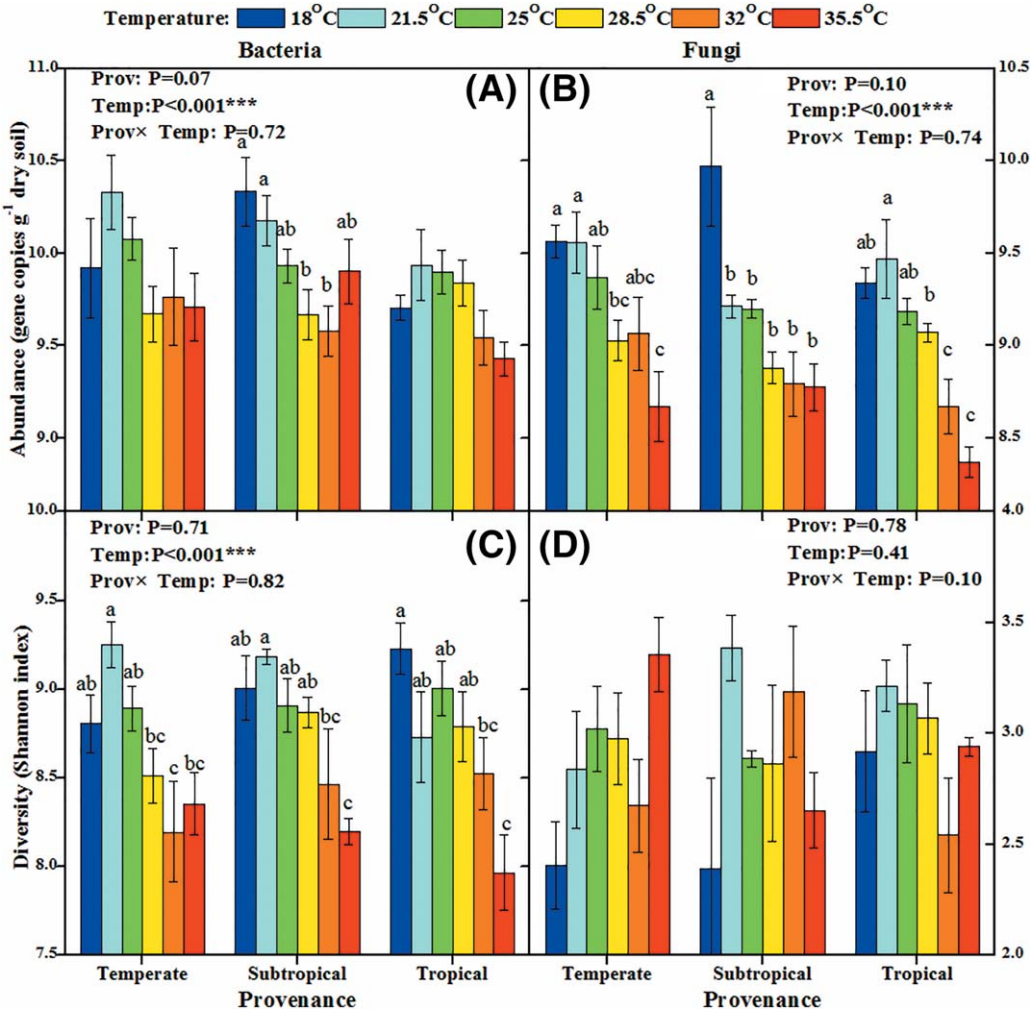

Fig. 3. Log-transformed abundance of the bacterial 16S rRNA (A) and fungal ITS (B) gene across treatment respectively. Variations of soil bacterial (C) and fungal (D) diversity (Shannon index) among different treatments. Error bars represent standard errors ($n = 5$). Labels: Prov = provenance, Temp = temperature. Significance was labelled as * $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$.

$P < 0.001$ respectively). The impact of temperature on the bacterial diversity, assessed by Shannon's index, was also evident (Fig. 3C, $P < 0.001$), while its impact on fungal diversity was insignificant (Fig. 3D). There was only a marginal difference in abundance among the three tree provenances ($P = 0.07$, $P = 0.10$ respectively). The response of microbial abundance and diversity to temperatures was hump-shaped across three provenances. It can be argued that microbes are more efficient in a certain range of temperatures (Barcenas-Moreno *et al*., 2009) and beyond this range could have negative impact on microbial growth. The reduction in bacterial abundance and diversity by high temperatures was consistent with previous studies as temperature is the main

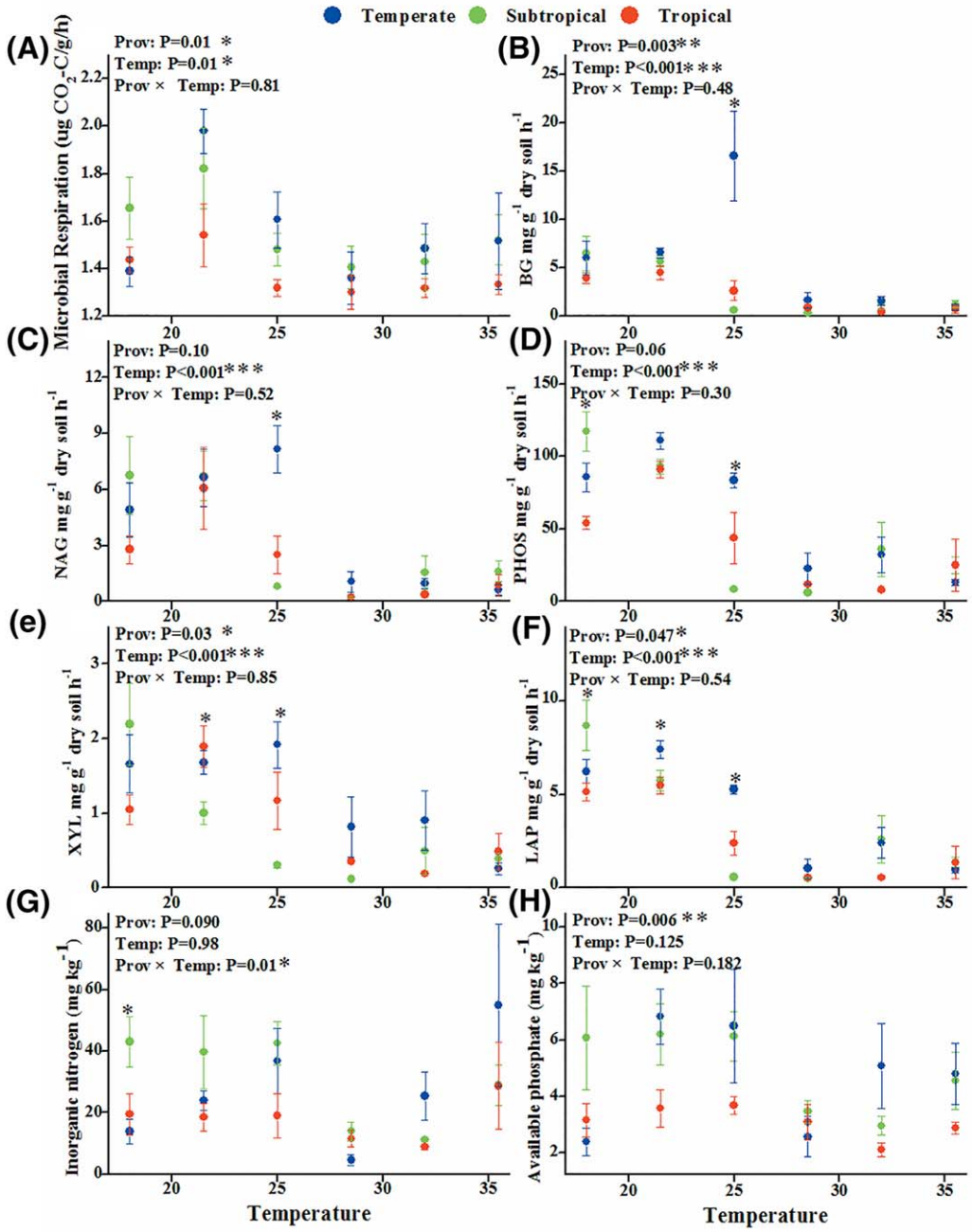

Fig. 4. Impacts of temperatures and provenances on (A) microbial respiration (Resp); (B) activities of β-glucosidase (BG); (C) activities of *N*-acetyl-glucosaminidase (NAG); (D) activities of phosphatase (PHOS); (E) activities of β-Xylosidase (XYL); (F) activities Leucine of aminopeptidase (LAP); (G) inorganic N and (H) available phosphate. Points and bars represent means ± SE ($n = 5$) and letters indicate significant differences ($P < 0.05$) between temperatures. An absence of letters indicates no significant treatment effects. Labels: Prov = provenance, Temp = temperature. Significance was labelled as * $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$.

climatic factor that influences bacteria (Allison and Treseder, 2008; Riah-Anglet *et al*., 2015; Wu *et al*., 2015). Considering home temperatures of three provenances, soil microbes linked to provenances from cooler origin (temperate provenance), were better acclimatized to warming than to soil microbes linked to provenances from warmer origin (subtropical and tropical provenance) which were probably approaching their thermal limits and may be negatively influenced by warming. Our results suggest that climate change may alter soil microbial diversity and abundance in terrestrial ecosystems, with little effect from provenance identity on these microbial attributes.

Response of soil functions to temperature across different provenances

Our results suggested some regulatory capacity of provenance identity in driving the responses of soil functions to climate change. For example, intraspecies variation modulated the responses of inorganic nitrogen to changing temperatures (Temperature × Provenance interaction, $P = 0.01$). Inorganic nitrogen in the temperate provenance showed a positive response to increasing temperature; in the subtropical provenance, it showed a negative response (Fig. 4 and Supporting Information Fig. S4). The effects of tree provenance on soil functions were only evident at lower temperatures (18, 21.5 and 25°C) and not at higher temperatures. Intraspecies effects could be attributed to its impact on microbial community composition which in turn responds to differences in root exudates (Waldrop and Firestone, 2006). It was reported that soil respiration and phosphatase enzyme activity were influenced by vegetation types (Chen *et al*., 2000) and this study indicated that intraspecies variation can also influence the rate of soil functions. We observed decoupling between home temperatures and functioning temperatures. Home temperatures for temperate, tropical and subtropical provenances are 18, 28.5 and 21.5°C respectively. The activities were significantly higher at 25°C than 18°C in the temperate provenance, indicating warming increased soil activities in the cool-origin trees. However, extracellular enzyme activities (EEA) were higher at 21.5°C than 28.5°C in the tropical provenance, and EEA were higher at 18°C than 21.5°C in the subtropical provenance, suggesting decreasing temperature enhanced microbial activities under warm-origin trees. Soil functional response to warming was different across three provenances, suggesting plant-soil feedback (Knelman *et al*., 2014).

Assessing the link between soil microbial communities and functions

Correlation analyses were performed to examine the relationships between the soil functions and microbial abundance, diversity and communities (Table 2). Spearman's rank analysis found that microbial respiration, *N*-acetyl-glucosaminidase (NAG) and available phosphate were significantly and positively correlated with bacterial and fungal abundance. Activities of β-glucosidase (BG), Phosphatase (PHOS), β-Xylosidase (XYL) and leucine aminopeptidase (LAP) were positively correlated with fungal abundance, suggesting that the decline in microbial abundance might generate reduced EEA at high temperatures. Soil functions were not significantly correlated with bacterial diversity and fungal diversity, suggesting that at this scale, we did not have enough spatial resolution to detect such a pattern. Bacterial and fungal communities were strongly correlated with variation in ecosystem function. Significantly positive correlations were found between soil functions and relative abundances of *Bacteroidetes*, *Planctomycetes, Proteobacteria* and negative correlations between soil functions and those of *Acidobacteria, Actinobacteria* and *Verrumicrobia*

Table 2. Correlation coefficients (Spearman's rho) between soil functions with microbial community and abundance.

	Bac abun	Bac div	Bac PCO1	Bac PCO2	Fun abun	Fun div	Fun PCO1	Fun PCO2
Resp	0.66***	0.25	−0.5***	−0.43**	0.5***	−0.12	0.13	0.32
BG	0.39	0.24	−0.02	−0.4*	0.47***	−0.02	0.42*	0.05
NAG	0.45***	0.32	−0.05	−0.54***	0.61***	−0.11	0.43**	0.01
PHOS	0.37	0.25	0.15	−0.52***	0.58***	−0.07	0.54***	−0.03
XYL	0.36	0.21	0.11	−0.47***	0.62***	−0.12	0.51***	−0.05
LAP	0.39	0.31	0.14	−0.58***	0.61***	−0.1	0.57***	−0.04
IN	0.35	0.05	−0.35	−0.13	0.24	−0.04	0	0.3
AP	0.46***	0.22	−0.33	−0.32	0.44**	−0.13	0.12	0.21

Values' labels indicate significance levels examined by multiple tests (* $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$).

Row headings: Resp = Microbial respiration, BG = β-Glucosidase, NAG = *N*-acetyl-Glucosaminidase, PHOS = Phosphatase, XYL = β-Xylosidase, LAP = Leucine of aminopeptidase, IN = Inorganic N, AP = Available P.

Column headings: Bac/Fun abun = bacterial/fungal abundance, Bac/Fun div = bacterial/fungal diversity (Shannon index), Bac/Fun PCO1 and Bac/Fun PCO2 are the first and second axis of bacterial/fungal principal coordinate.

(Supporting Information Table S3), indicating the shift in microbial communities linked to provenance and climate change could have functional consequences (Garcia-Palacios *et al*., 2015). Therefore, even small changes in microbial community linked to provenance identity might have significant consequences for the functioning of terrestrial ecosystems.

Conclusions

Here, we provide novel evidence that tree intraspecies variation can significantly regulate the response of microbial communities and ecosystem functions to variable temperatures. For example, we found strong and statistically significant interactions between temperature and tree provenance for bacterial community composition and for the relative abundances of dominant taxa (e.g., *Chloroflexi* and *Zygomycota*), and specific surrogates of soil functions (N cycling). Soil bacterial communities linked to temperate provenances presented the highest node connectivity, suggesting higher resistance to climate change. There were significant correlations between soil function and microbial community composition and abundance, suggesting that microbial responses play an important role in understanding the mechanisms by which temperature and tree provenance impact ecosystem functions. Our results provide novel evidence that even small changes in biotic components (intraspecies variation) can have a large impact on the response of microbial communities and soil functions to rising temperature with implications for terrestrial ecosystem functioning under global warming.

Experimental procedures

Experimental design and soil sample

The experiment was conducted in a sunlit, environmentally-controlled glasshouse on the campus of Western Sydney University, Richmond, New South Wales, Australia (33°36′40″S, 150°44′26.5″E). We used material representing three provenances of *Eucalyptus tereticornis*, a widely distributed tree species across a latitudinal gradient in eastern Australia. Three provenances originated from different latitudinal regions in Australia: the temperate provenance originated from southern New South Wales (35°23′60″S, 150°4′12″E), the subtropical provenance originated from southern Queensland (26°34′12″S, 152°0′36″E) and the tropical provenance originated from northern Queensland (15°30′0″S, 145°8′24″E). Seeds from three provenances were germinated simultaneously for 8 weeks in a glasshouse, after which seedlings were transplanted to the lab individually in 7 L pots. The tree seedlings were then grown at six temperatures (18.0, 21.5, 25.0, 28.5, 32.0 and 35.5°C) using a series of climate-controlled glasshouse bays. These temperature set-points were designed to span the entire range of average summer temperatures experienced by *E. tereticornis* in its native range; 32 and 35.5°C were the two high temperatures outside its native range, considering the possible global warming effect. Based on spatially interpolated climate records from a network of weather stations across Australia (Jeffrey *et al*., 2001), the average summer temperatures for temperate, subtropical and tropical origin are 18, 21.5 and 28.5°C respectively. The temperature-controlled glasshouse bays was set on the same plants as described previously (Drake *et al*., 2017). The air temperature of each bay was controlled over a 24h period at three set-points (i.e., night-time from 20:00 to 06:00, mid-day from 10:00 to 16:00 and from 06:00–10:00 to 16:00–20:00 h) to finish a day-night cycle with an average temperature range of 9°C.

We used a sandy clay loam collected from the surface horizon of a local dry sclerophyllous forest in Menangle, NSW, Australia. Soil was moderately fertile and basic characteristics are as follows: pH 4.4 (0.01 M $CaCl_2$), total organic carbon 1.21%, total N 520 mg kg^{-1} and total P 230 mg kg^{-1}. Nine kilograms of soil was added to each of 90 cylindrical pots (5 replicates $\times$ 3 provenances $\times$ 6 temperatures; PVC pipes, 15 cm diameter by 40 cm height). Tree seedlings were grown for 54 days (from 7 January 2016 to 29 February 2016). After harvesting the aboveground portion of the trees, soil samples were collected at 0–10 cm depth near tree roots with a 5 cm diameter cylinder at the end of the experiment (2nd March of 2016). Soil samples were separated into two sets. One sample set was stored at −80°C before DNA extraction and the other set was stored at 4°C before physicochemical analyses. Plant roots were carefully removed from soil samples before DNA extraction and functional analyses

Soil functions

We measured 10 surrogates of soil function, including seven extracellular enzyme activities (EEA), basal respiration and available nitrogen and phosphorus. The seven extracellular enzymes included C-cycling, N-cycling and P-cycling enzymes. β-glucosidase (BG) catalyzes one of the later steps of cellulose degradation. β-D-cellubiosidase (CB) degrades cellulose. β-Xylosidase (XYL) is involved in hemicellulose degradation. α-Glucosidase (AG) cleaves terminal α-glucose residues to release a single α-glucose molecule. *N*-acetyl-glucosaminidase (NAG) is involved in chitin and fungal cell wall breakdown. Leucine aminopeptidase (LAP) breaks down polypeptides involved in the mineralization of N

from the substrates with polypeptide. Phosphatase (PHOS) is involved in the phosphorus mineralization.

The EEA were measured as previously described (Bell *et al*., 2013). Briefly, field moist soil (1 g) was mixed with 33 ml of 0.05 M sodium acetate buffer (pH 5.6). After shaking for 20 min at 185 r.p.m., soil slurry (800 μl) was added to 96-well microplates. 200 μl of appropriate 200 μM substrates were added to the plates and the plates were incubated in the dark at 35°C for 1.5 h. Enzyme activities were assayed spectrophotometrically using *p*-nitrophenol linked substrates. Microbial basal respiration was measured using MicroResp ™ (Campbell *et al*., 2003). Soil sample (0.4 g) was weighed and put into 25 μl water in a 96-well deep well plate. Another plate containing the CO_2 detection gel was connected with the deep well plate by a rubber mat. We incubated soil at 26°C for 6 h. After incubation, fluorescence was determined on CLARIOstar plate reader (BMG LABTECH GmbH, Germany). Enzyme activities were expressed as mg paranitrophenol formed g^{-1} dry soil h^{-1}. Microbial basal respiration was expressed as μg CO_2-C g^{-1} dry soil h^{-1}. Soil ammonium and nitrate were extracted from fresh soils (5 g) with 50 ml of 2 M KCl by shaking at 180 r.p.m. for 60 min. Soil phosphate was extracted from fresh soils (1 g) with 20 ml of 0.5 M $NaHCO_3$ by shaking at 180 r.p.m. for 60 min. The filtered solution was determined by a SEALAQ2 Analyzer (SEAL Analytical, Maquon, WI, USA).

DNA extraction and quantitative polymerase chain reaction

Soil DNA was extracted from 0.3 g soil with the MoBio UltraCleanTM soil DNA isolation kit (Mo Bio Laboratories, San Diego, CA, USA) according to the manufacturer's instructions. The quantity and quality of the extracted DNA were examined using NanoDrop® ND-2000c UV-Vis spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA). The DNA samples were stored at −20°C and used for later molecular analysis.

Abundance of the bacterial 16S rRNA gene and fungal ITS gene were quantified by quantitative polymerase chain reaction on an iCycler iQ 5 thermocycler (BioRad Laboratories, Hercules, CA, USA) using the primer pairs 338f/518r (Øvreås and Torsvik, 1998) and ITS1/5.8S (Evans and Wallenstein, 2012). The 10 μl reaction mixture contained 5 μl SensiFast SYBR No-ROX reagent (Bioline, Sydney, NSW, Australia), 0.25 μl of each primer (20 μM), 0.25 μl of BSA (20 mM) and 1 μl of diluted DNA template (1–10 ng). Amplification conditions were as follows: 95°C for 3 min, bacteria: 40 cycles of 10 s at 95°C, 10 s at 60°C and 20 s at 72°C, fungi: 40 cycles of 15 s at 95°C, 15 s at 53°C and 15 s at 72°C, followed by melt curve from 65°C to 95°C at 0.5°C increment. Standard curves were developed using tenfold serial dilutions of plasmid containing correct insert of the bacterial 16S rRNA gene and fungal ITS gene. Triplicate reactions were produced for each DNA sample. PCR efficiency for different assays ranged between 80% and 100% and R^2 of 0.99.

Sequencing and bioinformatics processing

For amplicon sequencing, bacterial 16S rRNA gene and fungal ITS fragments were amplified using the primers 341F/805R (Herlemann *et al*., 2011) and FITS7/ITS4 (Ihrmark *et al*., 2012) respectively. Amplified products were sequenced on the MiSeq platform (Illumina, San Diego, CA, USA) at Western Sydney University, Australia. Across the 90 samples examined, the high-throughput sequencing yielded 3,406,093 bacterial 16S rRNA gene sequences and 7,527,208 fungal ITS gene sequences in total, and the minimum sequence numbers for individual sample were 16,033 and 20,906 respectively.

Raw sequences were processed following the Quantitative Insights into Microbial Ecology (QIIME) 1.7.0 (Caporaso *et al*., 2010b) to join the paired ends. After removing chimeras, uclust was used to pick operational taxonomic units (OTUs) at 97% identity (Edgar, 2010). Representative sequences of each OTU were then aligned using PyNAST (Caporaso *et al*., 2010a) and assigned using ribosomal database project (RDP) Classifier (Wang *et al*., 2007) based on the Greengenes database. Resampling across all samples according to the minimum sequence numbers was performed before the downstream analyses.

Network analysis and visualization

To explore ecologically meaningful microbial associations, networks of co-occurring bacterial and fungal OTUs were performed individually using the Cytoscape plug-in CoNet (Smoot *et al*., 2010; Faust *et al*., 2012). Poorly represented OTUs (total number across all the samples in a provenance less than 10) were removed to reduce ambiguous correlations. Correlation scores were calculated using a Brown combination of *P* value of Spearman correlation, Pearson correlation, Kullback-Leibler dissimilarity and Bray–Curtis dissimilarity. Strong ($r > 0.6$) and significant ($P < 0.01$) correlations were shown in the resulting networks. Networks were visualized with Cytoscape 3.0 and the topology of networks was calculated using Cytoscape plug-in Network Analyzer (Assenov *et al*., 2008).

Statistical analysis

The 16S rRNA and ITS gene copies were log-transformed prior to statistical analysis to satisfy the

normality assumptions. Analyses of variance (ANOVA) were conducted to compare the microbial abundance, diversity and soil functions across treatments. Here, temperature was considered as a continuous factor and 'lm' function in R was used. Then we used ANOVA *post hoc* analyses to examine the temperature and tree provenance effect respectively. Spearman's rank test was used to assess the correlations between soil functions with the bacterial/fungal community, abundance, diversity and relative abundance of some taxa groups. All statistical analyses were performed using R.3.3.2 (http://www.r-projext.org). Nonmetric multidimensional scaling (NMDS) based on the Bray–Curtis dissimilarity matrices was completed to visualize shifts in the microbial community compositions based on the 97% OTU level across different treatments. All data were used in ordinations and shown separately under three provenances for the quality of figures. Permutation multivariate analysis of variance (PerMANOVA) was performed to examine treatments' effects on the Bray–Curtis dissimilarity matrices. NMDS plots and PerMANOVA were performed using Primer 7. Bacterial and fungal taxonomies that varied significantly among the three provenances were explored using the linear discriminant analysis (LDA) effect size (Segata *et al*., 2011), with a 0.05 α value for Kruskal–Wallis test and using provenance as the main feature class while temperate gradient as the subclass. Cladograms with significant taxa coloured accordingly were generated using the Galaxy workflow framework provided by the Huttenhower lab (Goecks *et al*., 2010).

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Authors' Contribution

All authors contributed to the experimental design. C.Z. carried out most of laboratory work and anlaysed data with help from M.D.-B. J.W. carried out network and cladogram analyses. C.Z. wrote paper with help from all co-authors.

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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:

Table S1. Summary of results (*F* value and *P* value) of analysis of variance (ANOVA) showing the effect of temperature and provenance on dominant bacterial classes. Values are probability with significant results ($P < 0.05$) shown in bold.

Table S2. Summary of results (*F* value and *P* value) of analysis of variance (ANOVA) showing the effect of temperature and provenance on dominant fungal classes. Values are probability with significant results ($P < 0.05$) shown in bold.

Table S3. Correlation coefficients (Spearman's rho) between soil functions with relative abundance of bacterial/fungal taxa. Values' labels indicate significance levels examined by correlation tests (* $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$).

Fig. S1. Difference in bacterial (a) and fungal (b) groups linked to three provenances. Green bars represent temperate groups, red bars represent subtropical groups and blue bars represent tropical groups.

Fig. S2. The relative abundances of 8 dominant bacterial taxa in response to a range of temperatures across three provenances. Two-way ANOVA was used to test the treatment effects ($P < 0.05$). Labels: Prov = provenance, Temp = temperature. Significance was labelled as * $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$.

Fig. S3. The relative abundances of 3 dominant fungal taxa in response to a range of temperatures across three provenances. Two-way ANOVA was used to test the treatment effects ($P < 0.05$). Labels: Prov = provenance, Temp = temperature. Significance was labelled as * $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$.

Fig. S4. Impacts of temperatures and species varieties on (a) activities of β-D-cellubiosidase (CB) and (b) activities of α-Glucosidase (AG). Points and bars represent means s varieties on (a))abProv = provenance, Temp = temperature. Significance was labelled as * $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$.