Losses in microbial functional diversity reduce the rate of key soil processes.

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Abstract
The consequences of microbial functional diversity loss on key ecosystem processes remain debatable due to lack of firm evidence from observational or manipulative experiments for a link between microbial functional diversity and specialized ecosystem functions. Here, we conducted a microcosm experiment to test for a link between multiple microbial functional diversity (nitrifiers, methanotrophs and denitrifiers) and corresponding specialized soil functions (nitrate availability, methane, and nitrous oxide flux) using the dilution-to-extinction approach. We found that reductions in functional microbial diversity led to declines in the rates of specialized soil processes. Additionally, partial correlations provided statistical evidence that the correlations between microbial functional diversity and specialized functions were maintained after accounting for functional gene abundance (qPCR data) and substrate availability. Our analyses further suggested little redundancy in the relationship between microbial functional diversity and specialized ecosystem functions. Our work provides experimental evidence that microbial functional diversity is critical and directly linked to maintaining the rates of specialized soil processes in terrestrial ecosystems.

Key words: Microbial functional diversity; Specialized ecosystem functions; Nutrient cycling; Functional redundancy.
Experimental and observational approaches over the last twenty years have led to the conclusion that plant functional diversity is positively linked to ecosystem functioning (Hooper 2005, Diaz et al., 2007; Conti and Diaz 2013; Lavorel et al., 2013; Duffy et al., 2015). Much less is known on the relationship between microbial functional diversity and specialized soil processes in terrestrial ecosystems (van Elsas et al., 2012; Philippot et al., 2013; Vivant et al., 2013). Predictions of a decline in terrestrial functional biodiversity have raised substantial concerns over the consequences that losses in microbial functional diversity may have on key ecosystem processes and functions (Díaz et al., 2011; Conti and Díaz 2013; Lavorel et al., 2013). For example, Maestre et al., (2015) showed that increases in aridity might result in a significant decline in soil microbial diversity worldwide. Similarly, potential losses in microbial functional diversity along with changes in abiotic and biotic factors could potentially alter specialized ecosystem processes related to nutrient cycling and climate regulation (gases emissions) (Philippot et al., 2013; Colombo et al., 2016; Maron et al., 2018), but empirical evidence for this is lacking. Assessing the importance of soil microbial functional diversity in driving specialized soil processes (i.e., processes conducted for a highly specialized group of taxa; e.g., nitrifiers) is critical to fill the gaps between the theoretical framework of macroecology and microbial ecology (Barberan et al., 2014) leading to the formulation of improved sustainable management and conservation policies (Reed and Martiny 2007; Delgado-Baquerizo et al., 2016b).

Previous studies have found a significant and positive relationship between soil microbial functional and taxonomic diversity and ecosystem functions using observational correlative approaches (Levine et al., 2011; Singh et al., 2014; Ho et al., 2014; Powell et al., 2015; Trivedi et al., 2016). However, observational links have been questioned because of their inability to conclusively establish a cause-and-effect relationship between diversity and process outcomes (Rocca et al., 2015, Hall et al., 2018). Studies using manipulative experimental approaches to identify linkage between soil microbial functional diversity and key ecosystem processes provided inconclusive results. The lack of strong experimental support for the link between microbial functional diversity and specialized functions (BEF) is not solely due to a small number of studies, but also to apparently inconsistent results from those studies. For example, previous studies reported that soil microbial diversity promoted single ecosystem specialized functions (van Elsas et al., 2012; Philippot et al., 2013; Vivant et al., 2013), but others have reported weak relationships or lack of BEF correlations (Griffiths et al., 2000; 2001; Wertz et al., 2006). These contradictory results may have originated from two major limitations in previous studies including lack of consideration for (1) the role of microbial abundance and substrate availability in the interpretation of the microbial BEF results (Peter et al., 2011; Vivant et al., 2013), and (2) the lack of consideration for multiple functional gene markers and soil specialized processes simultaneously (Hector and Bagchi, 2007). Moreover, the importance of microbial functional diversity is commonly challenged by the concept of functional redundancy (Loreau 2004). However, specialized functions (Schimel and Schaeffer, 2012; Wood et al., 2015) are also expected to be highly sensitive to changes in diversity because they require a specific physiological pathway and/or are carried out by a small group of species possessing specialized functional genes (Schimel et al., 2005; Bodelier, 2011; Philippot et al., 2013; Delgado-Baquerizo et al., 2016a). Drawing on this theoretical knowledge, a proportional rather than redundant microbial BEF relationship would be expected for specialized functioning in terrestrial ecosystems.

Here, we used the dilution-to-extinction (e.g. Salonius, 1981; Peter et al., 2011; Philippot et al., 2013; Delgado-Baquerizo et al., 2016a) experimental approach on soil from two independent sites to explore the relationship between microbial functional diversity and functional redundancy (Loreau 2004). However, specialized functions (Schimel and Schaeffer, 2012; Wood et al., 2015) are also expected to be highly sensitive to changes in diversity because they require a specific physiological pathway and/or are carried out by a small group of species possessing specialized functional genes (Schimel et al., 2005; Bodelier, 2011; Philippot et al., 2013; Delgado-Baquerizo et al., 2016a). Drawing on this theoretical knowledge, a proportional rather than redundant microbial BEF relationship would be expected for specialized functioning in terrestrial ecosystems.
specialized soil processes in terrestrial ecosystems. In this study, we explicitly examine the links between microbial functional diversity (e.g., nitrifiers, methanotrophs and denitrifiers) and the rates of specialized functions (e.g. CH$_4$ flux, NO$_3$ production, and N$_2$O flux). All these specialized functions require specific genes to encode enzymes capable of performing these functions which are limited to relatively few microbial species. We chose these functional groups because they are ubiquitous across the globe; functional genes that catalyse processes are well characterised and studied, and their exact role and mechanisms in carrying out processes are well established. This provides a strong theoretical framework to test the linkages between microbial functional diversity and specialized functions. Additionally, activities of these functional microbial communities play key roles in climate regulation (e.g. greenhouse gas emission and mitigation) and nutrient (N) cycling. We aim to experimentally test the hypothesis that reduction in the microbial functional diversity has proportional impact on the specialized processes in terrestrial ecosystems. We hypothesized that: (a) experimental losses in microbial functional diversity will lead to reductions in specialized soil processes; and (b) given the expected importance of soil microbial functional diversity for key soil processes, the microbial BEF relationship should show little redundancy.

Materials and methods

Site description

We collected soil samples from two sites in Australia with contrasting precipitation regimes – an important environmental factor which often leads to contrasting microbial communities and soil attributes (Maestre et al., 2015). Soil sampling was carried out in March 2014. Soil samples were collected from the top 10 cm from Goolgowi mallee (site A; NSW 33.9667° S, 145.7000° E) and Warraderry State Forest (site B; NSW, 33.7035° S, 148.2612° E), New South Wales, Australia; both of them dominated by Eucalyptus spp. Site characteristics and soil properties for both soils are presented in Table 1.

Microcosm preparation

Soil samples from each site were sieved to < 2mm and divided in two portions: (1) soil for sterilization, and (2) soil for microbial inoculum and experimental controls (non-sterilized original soils). The first portion was sterilised using a double dose of gamma radiation (50kGy each) at ANSTO Life Sciences facilities, Sydney. Gamma radiation was used as it is known to cause minimal change to the physical and chemical properties of soils when compared with other methods of sterilisation such as autoclaving (Wolf et al., 1989; Lotrario et al., 1995). The dilution-to-extinction approach was used to prepare soil microcosms (Salonius, 1981; Peter et al., 2011; Philippot et al., 2013; Delgado-Baquerizo et al., 2016a). A parent inoculum suspension was prepared by mixing 25 g soil in 180 ml of sterilized Phosphate buffer saline (PBS). The mixture was vortexed on high speed for 5 min to mix the contents. The sediment was then allowed to settle for 1 min and serial dilutions were prepared from the suspension. For each soil (soils A and B), 5 dilutions were used as the microbial inoculum (20 ml of inoculum for each microcosm) to create a diversity gradient; these dilutions were undiluted ($10^0$); 1/10 dilution (D1); 1/10$^3$ dilution (D3); 1/10$^6$ dilution (D6); and 1/10$^{10}$ dilution (D10). Microcosms with non-sterilized soil served as references but not included in our statistical analyses. A total of 50 microcosms (500g each; 5 dilutions x 5 replicates x 2 soil types) were prepared. Additionally, we had five replicates of original (no dilution) control samples for each soil type. The moisture content in these microcosms were adjusted to 50% water holding capacity to allow microbial activities to be maintained (by adding sterile water if needed) during the incubation period. These microcosms were established under sterile conditions; aseptic techniques were used throughout the experiment to avoid contamination.
Soil microcosms were incubated at 20°C for 6 weeks for microbial colonization and biomass recovery as described in Delgado-Baquerizo et al., (2016a). This is critical for the dilution-to extinction method (Delgado-Baquerizo et al., 2016a); microcosms with the highest dilution are expected to have the lowest microbial biomass initially, which may affect any interpretation regarding the relationship between microbial diversity and ecosystem functioning. Biomass recovery is needed to properly address the link between microbial diversity and ecosystem functioning by controlling for biomass interferences. Thus, we started measuring microbial diversity and functions only after the abundance of functional gene had recovered similar levels to those in undiluted treatments.

**Microbial community analysis and quantification**

**DNA extraction**

Total genomic DNA was extracted from 0.25 g of soil using the MoBio PowerSoil DNA Isolation Kit (MoBio Laboratories, Carlsbad, CA, USA) as per the manufacturer’s instructions, with a slight modification in that a FastPrep bead beating system (Bio-101, Vista, CA, USA) at a speed of 5.5 m s⁻¹ for 60 s was used at the initial cell-lysis step. The quantity and quality of extracted DNA were checked photometrically using a NanoDrop® ND-2000c UV-Vis spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA).

**Abundance of functional genes**

The abundances of ammonia-oxidizing archaea (using amoA gene), N₂O reducing bacteria (using nosZ gene), and methanotrophs (using pmoA gene) were quantified on a CFX-96 thermocycler (Biorad, USA) using primers and conditions described in Table S1. Standard curves were generated using ten-fold serial dilutions of plasmids containing the correct insert of each respective gene. The 10 μl reaction mixture contained 5 μl SensiMix SYBR No-ROX reagent (Bioline, Sydney, Australia), 0.3 μl of each primer (20 mM), 0.4 μl BSA (20 mM), and 1 μl of diluted template DNA (1-10 ng). Melt curve analyses were conducted following each assay to verify the specificity of the amplification products, and the PCR efficiency for different assays ranged between 86-95%, 92-98%, and 96-99% for amoA, nosZ, and pmoA genes, respectively. Amplified products were run on a 2% agarose gel to confirm product size and specificity. Note that the AOB (ammonia-oxidizing bacteria) community was not included in our study because of low AOB abundance according to our results from qPCR. Thus, the PCR products did not satisfy the requirements for T-RFLP (next section). The same problem has been reported in a previous study including samples from a region near our sampling locations (Liu et al., 2016).

**Diversity of functional genes**

Terminal restriction fragment polymorphism (T-RFLP) for pmoA, amoA (only for ammonia oxidising archaea), and nosZ were performed using fluorescent labelled primer pairs A189F/Mb650R (Bourne et al., 2001), CrenamoA23F/CrenamoA616R (Touma et al., 2008), and nosZ1211f/nosZ1917R (Scala&Kerkof, 1998), respectively. More details regarding primer sets used in this study can be found in Table S1. The PCR reactions in a 50 μl mixture contained 2.5 U of BioTaq DNA polymerase (Bioline, Sydney, Australia), 0.5 μl of each primer (20 mM), 1 μl dNTP mix (20 mM), 5 μl 10xNH₄ reaction buffer, 2 μl BSA (20 mM), 2 μl MgCl₂ solution (50 mM), 2 μl of five-fold diluted template DNA (1-10 ng). Thermal-cycling conditions for each gene are provided in Table S1. The PCR products were purified using the Wizard SV Gel and PCR Clean-Up System (Promega, San Louis, CA, USA). The concentrations of PCR products were fluorometrically quantified using the NanoDrop® ND-2000c UV-Vis spectrophotometer. PCR products obtained from individual reactions were digested separately.
with *Hhal* (for *amoA*), *MspI* (for *nosZ*), *Rsal* (for *pmoA*) restriction enzymes in 10 μl volume containing approximately 200 ng purified PCR products, 20U of the restriction enzymes (BioLabs, Sydney, Australia), 0.1 μl BSA and 1 μl of 10 × NE Buffer. Digests were incubated at 37°C for 3 h, followed by 95°C for 10 min to deactivate the restriction enzyme. Terminal restriction fragments (TRFs) were resolved on an ABI PRISM 3500 Genetic analyzer (Applied Biosystems, CA, USA). During the fragment analyses, we were unable to successfully resolve all replicates for each treatment. Therefore, only successful replicates were used for downstream analyses.

**Functional measurements**

Soil gas flux for nitrous oxide (N\textsubscript{2}O) and methane (CH\textsubscript{4}) were monitored by placing 20 g of soil from each microcosm in a glass jar (12 cm depth, 75 cm diameter, Ball, USA), and then sealing with a gas-tight lid, which had a rubber stopper in the middle. Gas samples (12 ml) were collected in 15 ml gas-tight syringes at 0, 30 and 60 min after sealing. Gases were measured in an Agilent-7890a gas chromatograph equipped with a flame ionization detector (FID) and an electron capture detector (ECD) (Agilent Technologies, Wilmington, DE, USA).

A linear model was then applied to estimate the gas flux rate inside the jar headspace (Matthias *et al.*, 1980; Martins *et al.*, 2017) and expressed as micrograms of N\textsubscript{2}O-N/CH\textsubscript{4}-C - (μg N\textsubscript{2}O-N/CH\textsubscript{4}-C g\textsuperscript{-1} soil h\textsuperscript{-1}). Note that one of the limitations of our work is that measuring the absolute consumption of N\textsubscript{2}O and CH\textsubscript{4} in soil (functions driven by *nosZ* and *pmoA*) is extremely challenging. Instead, in our study, we related the diversity of these genes with the flux of N\textsubscript{2}O and CH\textsubscript{4}. Nitrate availability after incubation (our surrogate of nitrification) was measured from K\textsubscript{2}SO\textsubscript{4} extracts as explained in Delgado-Baquerizo *et al.*, (2013). Dissolved organic C (DOC) was measured -as described by Jones and Willett (2006).

**Data analysis: diversity of functional genes**

Raw T-RFLP data were analysed using the GeneMapper v5 software (Applied Biosystems) with the advanced peak detection algorithm. A GeneScan 600-LIZ internal size standard was applied to each sample. The T-RFLP profiles were analyzed using a local southern size calling method (peaks between 50 and 650 bp in size) and a peak amplitude threshold setting of 50, using Genemapper version 40 (Applied Biosystems). TRF peaks that differed by less than 1 bp were binned into the same fragment. The relative fluorescence abundances of all TRFs were exported for microbial community analysis. A binary table of peak presence/absence was generated and exported for further statistical analysis for determining the Shannon diversity index (Singh *et al.*, 2006).

**Testing the relationship between microbial diversity and specialised soil functions.**

We used two independent approaches to analyse our dataset (a P-value and a non P-value approach). First, we tested for differences in functional diversity and key processes across dilution treatments using non-parametric PERMANOVA analyses (PRIMER-E Ltd., Plymouth Marine Laboratory, UK), with dilution as a fixed factor (Anderson 2001). We then used the distance based linear model (distlm function, McArdle & Anderson 2001) to evaluate the correlation between the diversity (Shannon) within functional gene and specialized functions. This is a non-parametric method. As we did not transform our data, we used the Bray-Curtis distance matrix for these analyses –to reduce the influence of extreme values. Additionally, as an alternative statistical approach, we also used Spearman’s correlation analysis to evaluate the correlations between microbial functional diversity and specialized functions. We conducted partial correlation analysis to evaluate any potential influence of abundance of functional gene
(qPCR data) and substrate availability (dissolved organic carbon (DOC) content) in our conclusions (see Delgado-Baquerizo et al., 2016a for a similar approach).

We then used a non P value dependent approach to evaluate two potential fits for the relationship between microbial functional diversity [ammonia-oxidizing archaea (using amoA gene), N₂O reducing bacteria (using nosZ gene), and methanotrophs (using pmoA gene)] and their corresponding specialized functions at the two sites using two characteristic functionally redundant (logarithmic model) vs. non-functional redundancy (linear model describing at least proportional losses) models. Best model fits were selected by Akaike information criteria (AICc; Burnham & Anderson 2002) where a lower AICc value represents a model with a better fit. AICc is a corrected version of AIC that is highly recommended when dealing with small sample sizes, as in our case (Burnham and Anderson 2002). We further used a difference in AICc values of 2 (ΔAICc > 2) to determine substantial differences between models (Burnham and Anderson 2002; Burnham et.al., 2011). The analysis was performed using R package (https://www.r-project.org/). We used the lm functions from R to conduct these analyses. For the logarithmic model we used this command: lm(y ~ log(x)). Information on the AICc index was obtained using the package MuMIn from R (Barton 2018).

**Results**

**Recovery of microbial abundance**

After a six-week incubation, we measured the abundance of functional genes (N₂O reducing bacteria using nosZ gene; methanotrophs using pmoA gene and ammonia oxidising archaea and bacteria using amoA) using qPCR - as a proxy for biomass of functional groups in our soil microcosms from two different sites (Soil A and B). Our results showed that microbial abundance had successfully recovered in all diversity dilution microcosms. As such, we did not detect significant differences for microbial abundance levels across different dilution treatments (PERMANOVA P > 0.05; Fig. 1).

On the contrary, T-RFLP analysis showed significant differences in the diversity (Shannon) of N₂O reducing bacteria (using nosZ gene); methanotrophs (using pmoA gene); and ammonia oxidising archaea (using amoA) for both sites (PERMANOVA P < 0.05; Fig. 2). Shannon diversity for these functional genes was always positively and significantly related to richness in both soils (P < 0.05).

**Links between functional diversity and specialized functions**

We observed significant correlations between the diversity of functional groups and their specialized functions for both soil types using all three models tested (Fig. 3). The values of specialized functions across different dilutions are shown in Fig. S1. These correlations were maintained after using an alternative non-parametric approach (Spearman; Table S2).

To account for the influence of functional gene abundance and substrate availability on the functional diversity- specialized function relationship, we conducted partial correlations using microbial functional diversity as a predictor of soil specialized functions and accounting for functional gene abundance (qPCR data) and substrate availability (DOC) (Table S3; Fig. S2). In general, the results were similar to those observed in Fig 3 where functional diversity was significantly correlated to specialized functions. As NO₃⁻ concentration is also known to regulate N₂O production we conducted further partial correlation analysis using nosZ functional diversity as a predictor of N₂O flux controlled by NO₃⁻ concentrations. Our results showed significant correlations of functional diversity of denitrifiers with N₂O flux even after accounting for nitrate production (Table S4).

Overall, statistical modelling did not demonstrate functional redundancy in the relationship between microbial functional diversity and soil processes (Table 2). In fact, we
observed little functional redundancy in our results. Thus, the redundant (logarithmic) relationships were observed only in two cases including the relationship between functional diversity and N₂O flux and NO₃ production at site A (Table 2). In the rest of the cases - 4 out of 6 a proportional loss or not clear functional redundancy was detected (Table 2).

Discussion

Our findings provide experimental evidence that microbial functional diversity positively relates to three important specialized ecosystem functions (nitrification, denitrification and methane flux) in terrestrial ecosystems. As such, our findings provide experimental support to previous observational studies linking microbial functional diversity with ecosystem functions. These results were maintained after accounting for potential effects of functional gene abundance and substrate availability. Moreover, further analyses provided evidence for little functional redundancy in the relationship between microbial functional diversity and specialized functions. This knowledge is essential for developing a predictive understanding of functional consequences for microbial community responses to environmental perturbations (Girvan et al., 2005; Singh et al., 2014; Blaser et al., 2016).

A positive correlation was observed in this study between CH₄ flux, NO₃ production, and N₂O flux and the functional diversity of pmoA genes (for methanotrophs), amoA genes (for ammonia oxidisers) and nosZ genes (for denitrifiers). Thus, any reductions in the diversity of amoA genes derived from biotic or abiotic changes might largely reduce the availability of nitrate in terrestrial ecosystems -h (Robertson & Groffman, et al., 2007). Moreover, reductions in the diversity of methanotrophs (pmoA genes) and denitrifiers (nosZ genes) could have potential negative consequences for climate regulation on Earth by increasing the amount of methane and N₂O released to the atmosphere. Both methanotrophs (pmoA gene) and denitrifiers (nosZ genes) are essential microbial communities in terrestrial ecosystems as they constitute the ultimate barriers that reduce the release of potent greenhouse gases CH₄ and N₂O gasses - from deeper soil layers to the atmosphere (Smith et al., 2003; Heimann and Reichstein, 2008). This finding is supported by previous experimental work in water (Peter et al., 2011; Delgado-Baquerizo et al., 2016b) and soil (Phillipot et al., 2013) that have reported positive relationships between microbial diversity of specialized microbes with highly specialized functions.

In accordance with our hypothesis, the results demonstrate that specialized functions (Levine et al., 2011; Hu et al., 2015a, Phillipot et al., 2013) are highly sensitive to losses in functional diversity because they require a specific physiological pathway and/or are carried out by a phylogenetically clustered group of organisms (Schimel and Schaeffer, 2012; Wood et al., 2015). In particular, we found little functional redundancy in the relationship between microbial functional diversity and specialized functions (only 2 out of 6 cases were statistically identified as functionally redundant). This is also in agreement with previous experimental assays in freshwater ecosystems (Delgado-Baquerizo et al., 2016b) and ultimately indicates that specialised functions are potentially sensitive to losses of microbial diversity in natural settings. Identifying the reasons for the reported differences in the shape of the relationship between functional diversity and specialized functions using two single soils is challenging, an out of the scope of this paper, however, our results suggest that the shape of the microbial functional BEF relationship seems to be consistent for different types of processes in N cycle and the same model was selected for denitrification and nitrification rates in soils A (logarithmic) and B (linear; Table 2).

It can be argued that functional gene abundance and substrate availability can influence the relationship between biodiversity and functions in our results. Our results provide evidence that the significant relationship between microbial functional diversity and specialized functions is maintained after statistically controlling for effects of functional gene abundance.
and substrate availability. Also, we would like to highlight that we do not expect any effect on our conclusions by the use of T-RFLP analyses in our study. Despite low resolution, T-RFLP has been used to determine the diversity-function relationships in several studies (Korhonen et al., 2011; Delgado-Baquerizo et al., 2016a). Recent studies have provided evidence that T-RFLP and next generation sequencing (including 454 pyrosequencing and MiSeq) provide similar results in terms of diversity estimation (Van Dorst et al., 2014; Delgado-Baquerizo et al., 2016a). This technique is especially efficient for determining the diversity and composition of specialized microbial groups using functional genes wherein the diversity is low, and the groups represent only a minor fraction of the overall microbial community (Stralis et al., 2004; Singh et al., 2007; Hu et al., 2015b). Overall, we were able to create strong functional diversity gradients in our microcosms and these provided us with an appropriate system to explore the functional responses of changes in microbial diversity and the consequences of these changes for the specialized functioning of three important functions for the soil ecosystem.

Together, our study provides experimental evidence that, similar to what has been reported for plant functional diversity, microbial functional diversity largely influence important soil processes associated with the production of NO₃⁻ and fluxes of N₂O and CH₄. We also provide evidence that the correlation between functional diversity and specialized functions is robust to any effects from functional gene abundance and substrate availability. Our results further suggest that there is little functional redundancy in the relationship between microbial functional diversity and associated specialized processes. Together, our study indicate that loss of soil microbial functional diversity associated with changes in biotic and abiotic environmental factors could have important consequences for specialized soil functions in terrestrial ecosystems.

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Authors’ contributions
BKS, MD-B and PBR designed this study. CT collected data with help from MD-B. CT and MD-B analysed data. M.D-B. and C.T. led the writing of the manuscript with help from all co-authors.

Data accessibility
The primary data used in this paper have been deposited in figshare: https://figshare.com/s/305d6bb9b2570f0f45f8f (DOI: 10.6084/m9.figshare.8020391).

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Fig 1. Abundance [mean number of gene copies g⁻¹ soil (± SE)] of functional microbial communities. DC represents the original soil (not included in statistical analyses). DX to D10 represent dilutions from 10⁰ to 10⁻¹⁰.
Fig 2. Mean (± SE) values for microbial functional diversity (Shannon). DC represents the original soil (not included in statistical analyses). DX to D10 represent dilutions from $10^0$ to $10^{-10}$.
Fig 3. Correlations between microbial functional diversity [as determined by T-RFLP analysis of functional genes pmoA, amoA and nosZ] and their specialized functions. Different colours represent different dilutions darker to light (DX-D10). DC represents the original soil (not included in statistical analyses). Potential regression fits are available in Table 2.
**Table 1.** Environmental characteristics, location and soil properties of sampling sites.

| Environmental variables | Site A (Goolgowei mallee) | Site B (Warraderry state forest) |
|-------------------------|---------------------------|----------------------------------|
| Rainfall (mm year\(^{-1}\)) | 400                       | 657                             |
| Latitude                | -34.99803                 | -33.72992                       |
| Longitude               | 145.72637                 | 148.20335                       |
| Soil texture            | Clay loam                 | Sandy clay                      |
| Clay %                  | 32                        | 37                              |
| pH (H\(_2\)O)           | 6.01                      | 5.68                            |
| Total carbon (%)\(^a\)  | 1.73                      | 1.84                            |
| Total nitrogen (%)\(^b\)| 0.13                      | 0.15                            |
| NH\(_4\)\(^+\) - N (mg kg\(^{-1}\))\(^b\) | 5.23                      | 4.90                            |
| Olsen P (mg kg\(^{-1}\))\(^c\) | 9.58                      | 6.93                            |
| MB-P (mg kg\(^{-1}\))\(^c\) | 21.64                      | 22.8                            |

\(^{a}\) Measured with a CN analyzer (Leco CHN628 Series, LECO Corporation, St Joseph, MI, USA) following the Dumas combustion method.

\(^{b}\) Analysed colorimetrically (Sims et al., 1995) from K\(_2\)SO\(_4\) 0.5 M soil extracts using a 1:5 soil:extract ratio as described in Jones and Willett (2006).

\(^{c}\) Measured by NaHCO\(_3\) extracts of the Olsen method (Watanabe & Olsen, 1965).
**Table 2.** Model fit statistics and AICc index describing the relationship between microbial diversity and ecosystem functions. AICc measures the relative goodness of fit of a given model; the lower its value, the more likely it is that this model is correct. Two models with a ΔAICc value > 2 are considered to be substantially different. Logarithmic: \( Y = a + b \cdot \log(X) \); Linear: \( Y = a + b \cdot X \).

| Gene  | Function            | Site | Model       | R2  | Formula                                      | AICc  | DeltaAICc | Selected Model(s) |
|-------|---------------------|------|-------------|-----|----------------------------------------------|-------|------------|-------------------|
| amoA  | Nitrate production  | A    | Logarithmic | 0.80| \( Y = 0.0699 + \log (0.1581X) \)           | -94.38| 0.00       | ✓                 |
|       |                     |      | Linear      | 0.75| \( Y = -0.0506 + 0.1203X \)                  | -89.73| 4.65      |                   |
| amoA  | Nitrate production  | B    | Logarithmic | 0.81| \( Y = 0.1196 + \log (0.1522X) \)           | -120.38| 4.67      |                   |
|       |                     |      | Linear      | 0.85| \( Y = -0.0235 + 0.1395X \)                  | -125.05| 0.00      | ✓                 |
| nosZ  | \( \text{N}_2\text{O} \) flux | A    | Logarithmic | 0.58| \( Y = 0.0026 + \log (-0.0028X) \)         | -197.85| 0.00      | ✓                 |
|       |                     |      | Linear      | 0.49| \( Y = 0.0047 - 0.0018X \)                  | -193.91| 3.94      |                   |
| nosZ  | \( \text{N}_2\text{O} \) flux | B    | Logarithmic | 0.65| \( Y = 0.0051 + \log (-0.0045X) \)         | -237.04| 2.85      |                   |
|       |                     |      | Linear      | 0.69| \( Y = 0.0078 - 0.0027X \)                  | -239.89| 0.00      | ✓                 |
| pmoA  | Methane flux        | A    | Logarithmic | 0.05| \( Y = -1.609e-04 + \log (-2.536e-05X) \)  | -322.11| 0.00      | ✓                 |
|       |                     |      | Linear      | 0.04| \( Y = -1.444e-04 - 1.502e-05X \)         | -321.99| 0.12      | ✓                 |
| pmoA  | Methane flux        | B    | Logarithmic | 0.60| \( Y = -1.841e-04 + \log (-8.459e-05X) \)  | -410.16| 5.07      |                   |
|       |                     |      | Linear      | 0.67| \( Y = -9.603e-05 - 7.439e-05X \)          | -415.23| 0.00      | ✓                 |