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March 12, 2025

Stronger together: intact soil translocation increases the resilience of inoculated microbial communities

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Statement of authorship: SDP and MFB conceived and designed the research; SDP, SLK, AS collected the data; SDP, CCD, CL, MFB analysed the data; SDP wrote the first draft of the manuscript, and all authors contributed substantially to revisions.

Running Title: Intact soil translocation improves inoculation success

Keywords: ecosystem restoration, inoculation, microbiota, resilience, restoration ecology, soil translocation, translocation success

Abstract

Soil microbiota are fundamental ecosystem components capable of driving ecosystem recovery. However, their effective integration into ecosystem restoration efforts remains unrealised. Despite growing interest, there are limited experimental assessments on how to implement soil translocations to effectively inoculate whole microbial communities in restoration contexts. By embedding a soil translocation experiment into a restoration project in a global biodiversity hotspot, we show that retaining soil structural integrity through intact soil translocations is important in achieving successful inoculation. By contrast, surface spreading – the predominant method of soil translocation – saw microbial communities diverge away from the microbial profile of donor sites. Our findings suggest that the restoration sector should rethink its approach to microbial inoculations and consider the benefits of retaining structural integrity in translocated soils. Upscaling of investments and innovation are required to meet the increasing demand for soil translocations capable of effectively driving ecosystem recovery.

1 Introduction

Using soil microbiota directly has clear potential to improve ecosystem restoration outcomes (Coban *et al.* 2022; Robinson *et al.* 2023) as they are critical to ecological processes (e.g., nutrient cycling, soil formation). However, despite the recovery of soil microbiota increasingly being assessed following restoration interventions (Mohr *et al.* 2022; van der Heyde *et al.* 2022), soil microbiota are poorly integrated into ecosystem restoration. While post-restoration monitoring has identified patterns of soil microbiota recovery, large recovery debts can persist decades after restoration plantings (Watson *et al.* 2022). These persistent recovery debts highlight the need to improve restoration interventions that specifically target soil microbiota to improve restoration outcomes.

Soil translocation – the movement of topsoil from a donor to a recipient site – is increasingly used as a restoration intervention to inoculate entire microbial communities or select microbial taxa into restoration sites (van der Bij *et al.* 2018; Dadzie *et al.* 2024). These soil translocations can be effective in driving recovery of above- (e.g., vegetation) and below-ground (e.g., microbiota) ecosystem components in some contexts (Wubs *et al.* 2016; Han *et al.* 2022). However, there is a lack of research informing optimal soil translocation methods and further refinements are needed (Gerrits *et al.* 2023; Gomes *et al.* 2025).

The predominant soil translocation method used in restoration is surface spreading (Contos *et al.* 2021; Gerrits *et al.* 2023), where soil is collected from a donor site – ideally a nearby remnant site – transported to the recipient site and spread over the surface (Bullock 1998; Wubs *et al.* 2016). Recipient sites are often prepared by

removing existing topsoil, but sometimes donor soil is spread directly on top of existing surface soil. Inoculation effectiveness has been shown to improve with increasing soil volume due to a higher inoculation 'dose' (Han *et al.* 2022), however, this comes at the cost of increasing the volume of soil required from donor sites risking greater ecological impacts (Peddle *et al.* 2024b).

Surface spreading involves the mixing of distinct soil microhabitats, along with their corresponding microbiota, resulting in a homogeneous soil environment. This convergence of distinct microhabitats and microbial communities can drive compositional changes (West & Whitman 2022), affecting their likelihood of establishment. Microbial taxa vary in their response to disturbance of soil structure (van der Heyde *et al.* 2017). These varied responses can impact on predictions of community-level changes during the collection, transport, homogenisation and spreading of soil in translocations. For example, disrupting soil structural integrity by mixing can reduce bacterial richness, steering communities towards more homogenous compositions and favouring faster growing, generalist taxa (West & Whitman 2022). Therefore, preserving soil structural integrity during translocation may help retain donor communities and improve establishment of translocated microbiota, but there are no studies that assess the impact of varying soil disturbances during translocation.

As an alternative to surface spreading, intact soil translocation involves collecting intact sods, turfs or cores, and translocating these directly into the recipient restoration site (Bullock 1998; Gerrits *et al.* 2023). The structural arrangements of soil comprise of physical (e.g., aggregates and pores) and biological (e.g., soil

105 organic matter) legacies that have typically formed over decades and are key to soil
106 functioning (Rillig *et al.* 2017; Or *et al.* 2021). Thus, the objective of intact soil
107 translocation is to preserve this soil structural matrix, which should result in the
108 maintenance of the physical and biological legacies and their associated
109 microhabitats and functions (Boyer *et al.* 2011; Butt *et al.* 2022). Similarly to surface
110 spreading, studies of intact soil translocations have examined differing soil quantities
111 and depths, usually in the 1-2 m^2 range and soil depths of 10-30 cm. Most intact soil
112 translocation studies have focussed on vegetation (Kardol *et al.* 2009; Aradottir
113 2012; Cordier *et al.* 2019) or soil fauna (Moradi *et al.* 2018; Butt *et al.* 2022)
114 community responses, with mixed results. While intact soil translocations have led to
115 the recovery of soil microbial biomass and functional diversity (Waterhouse *et al.*
116 2014), their effectiveness compared directly to surface spreading remains untested.
117
118 Given that soil microbiota are sensitive to soil structural disturbance (West &
119 Whitman 2022), intact soil translocations could result in improved establishment of
120 soil microbiota compared with surface spreading. While scaling up intact
121 translocations presents logistical challenges, intact translocation sites could serve as
122 high-quality restoration nodes or soil biodiversity refuges. Over time, these nodes
123 may facilitate the dispersal of beneficial soil microbiota into surrounding soils,
124 creating a positive spillover effect. However, differences in abiotic factors such as
125 soil pH, moisture, and nutrient levels can limit microbial dispersal from translocated
126 soils to adjacent environments (Fierer 2017). Despite these barriers, mechanisms
127 such as water flow and active microbial motility can enable short-range dispersal,
128 suggesting some level of microbial exchange is possible (Chen *et al.* 2020; King &
129 Bell 2022). While microbial dispersal from translocated soil holds promise for the

wider restoration of soil biodiversity, dispersal remains largely unpredictable (Choudoir & DeAngelis 2022).

Here, we conducted an experimental soil translocation field trial embedded in a restoration project situated within a global biodiversity hotspot in south-west, Western Australia. We compared three different soil translocation methods that aimed to isolate the effects of soil disturbance during translocation from the effects of establishment barriers at the recipient site (e.g., inoculation depth, abiotic legacies). Our treatments were (a) intact soil cores, (b) mixed soil cores and (c) surface spreading. Our first hypothesis was that reduced soil disturbance (i.e., the intact soil core treatment) would positively associate with the establishment of translocated soil microbiota due to soil microbiota being sensitive to structural disturbance and soil homogenisation alone being capable of driving divergence in microbial composition (West & Whitman 2022). Our second hypothesis was that if we saw improved establishment of microbiota in the intact cores stemming from the reduced soil disturbance, this would result in greater dispersal of soil microbiota from the intact cores into the surrounding recipient site soil.

2 Materials and Methods

2.1 Study Site

This study was conducted across two post-agricultural restoration sites, Monjebup North Reserve and Red Moort Reserve in southwest Western Australia (Fig 1). The sites reside within the southwest Australian floristic region – a global biodiversity hotspot with exceptional levels of plant species richness, endemism, and habitat fragmentation from land clearing (Myers *et al.* 2000). Restoration plantings occurred

in Monjebup in 2014 and Red Moort in 2015 (see Jonson (2010) and Peddle *et al.* (2024a) for further site and revegetation details). Previous soil biodiversity monitoring at these sites indicated a lack of bacterial community recovery (Peddle *et al.* 2024a), making them ideal for testing the effectiveness of soil translocations.

2.2 Experimental Design and T0 Sampling

Soil translocations and initial sampling (T0) occurred between 16-19 June 2022. At each site, two 20 m x 20 m plots were established; one in revegetated bushland that would receive the soil translocations (Recipient) and one in immediately adjacent uncleared remnant bushland where soil cores would be sourced for the translocations (Donor; Figure 1). Four parallel 18 m linear transects were marked out in each of the four plots. Along each transect, 18 independent experimental replicates were marked out (50 cm x 50 cm, n = 72 per site) and assigned a randomly selected translocation treatment. Along the transects in each donor plot, 54 soil cores were collected using 12.5 cm diameter x 20 cm deep stainless steel soil corers.

Soil samples (300 g) were collected from alongside every donor soil core for physicochemical and DNA analysis (detailed below). Each collected soil core then had one of three experimental translocation treatments applied: (1) Intact Core; 12.5 cm diameter x 20 cm deep soil cores kept intact during translocation; (2) Mixed Core; 12.5 cm diameter x 20 cm soil cores with the individual soil core broken-up and homogenised in a sterile plastic bag before translocation; and (3) Surface Spreading; 12.5 cm diameter x 20 cm cores that were individually homogenised identically to the mixed cores but spread in a 3 cm deep layer over a 30 cm x 30 cm area. To ensure

soil translocation treatments were randomly applied to the cores collected from the donor site, we used the same randomised order from the recipient sites. Samples were also collected from three donor controls along each transect (n = 12 per site).

In the recipient plots, individual translocation treatments or recipient controls were applied to the randomly assigned independent 50 cm x 50 cm replicates along the four transects. Recipient controls did not receive any soil translocation, and a 300 g soil sample was collected from each recipient control for DNA and physicochemical analyses. For the intact core and mixed core treatment replicates, the same soil corers were used to extract a soil core which was disposed of, and donor soil from the allocated translocation treatment was placed into the resulting hole. For surface spreading replicates, surface leaf litter was removed and the homogenised soil (identical soil volume as intact and mixed cores) from the donor site was spread evenly in a 3 cm depth over the surface (30 cm x 30 cm). Plastic corflute tree guards were placed over each replicate (including the controls) to reduce the risk of interference from foraging animals. Each of the two recipient plots received a total of 14 intact cores, 14 mixed cores, 14 surface spreading, and contained 18 recipient controls. The recipient plots were also paired with 12 donor controls per site. We collected a total of 144 soil samples (300 g) across the two sites (28 intact, 28 mixed, 28 surface spreading, 36 recipient controls, and 24 donor controls). From each soil sample, 30 mL was collected in a sterile falcon tube and frozen on site until DNA extraction and sequencing. The remaining soil was sent to CSBP labs (Perth, Western Australia) for soil physicochemical analysis.

2.3 T1 Sampling

Soil sampling was repeated between 28-30 May 2023 (T1) to assess both microbial establishment directly in the translocated soil as well as microbial dispersal into the surrounding soil matrix. We systematically chose half of all replicates at both sites to ensure an even resampling of the treatments and to leave enough replicates for future resampling. We also repeated sampling for the 12 donor controls in each site (i.e., n = 76 per site = 16 recipient controls, 16 intact, 16 mixed, 16 surface spreading and 12 donor controls). We collected two soil samples from each replicate: one directly from the soil translocated one year earlier to assess microbial establishment (hereafter referred to as establishment samples); and one from soil immediately surrounding the translocated soil to assess microbial dispersal (hereafter referred to as dispersal samples; n = 76 establishment, 76 dispersal).

For the establishment samples, we used a 23 mm diameter soil corer to extract 10 cm deep soil cores to collect 300 g from the intact, mixed, and both control replicates being careful to not sample surrounding soil. Due to the shallow 3 cm depth of the surface spreading replicates, a steel trowel was used to collect 300 g of soil from the top 2 cm, again avoiding any of the underlying non-translocated soil.

For the dispersal samples for intact, mixed and recipient controls, we used the 23 mm soil corers to collect 300 g of soil to a depth of 10 cm from 6 cm surrounding the translocated core avoiding any of the translocated soil. For the dispersal samples from the surface spreading replicates, we used the trowel to excavate the 3 cm layer of translocated soil and the first 3 cm of the underlying soil (to minimise contaminating the dispersal sample with translocated soil) before using the soil corer to collect 300 g of soil from under the cleared surface spreading treatment. From

each 300 g sample from both establishment and dispersal samples, 30 mL was collected in a sterile falcon tube for DNA analysis and frozen on site.

2.4 DNA Extraction, Sequencing and Bioinformatics

We used the Qiagen DNeasy PowerLyzer PowerSoil Kit for DNA extractions, following the manufacturer's instructions. DNA extractions were sent to the Australian Genome Research Facility (AGRF; Melbourne, Australia) for sequencing of the 16S rRNA V3-V4 region and internal transcribed spacer (ITS) region to characterise soil bacterial and fungal communities using established protocols (see Supplementary Methods section 1.1 for sequencing and bioinformatics details).

2.5 Statistics

Microbial establishment and inoculation success

We first assessed if translocated microbiota were successfully inoculated into recipient sites one year after translocation and whether there were any differences in inoculation success across our three translocation treatments. We define inoculation success as the retention of a high similarity to donor value relative to the donor to donor similarity, whereas inoculation failure is indicated by a shift away from the donor and an increased similarity to the recipient. Our 'establishment' samples (16S rRNA and ITS) were rarefied to an even read depth ensuring ASV richness was still well-represented at the chosen rarefaction levels (20,717 reads for 16S rRNA and 10,073 reads for ITS; Figs. S1, S2). Then, to assess inoculation success, we constructed a Bray-Curtis distance matrix, converted the values to a similarity (100%*(1-distance)), and plotted the similarity of each T1 treatment sample to the

mean similarity of the T1 recipient samples and the mean similarity of the T1 donor samples.

Bacterial and fungal community compositions were visualised with non-metric multidimensional scaling (NMDS) ordinations of Bray-Curtis distances. Differences in bacterial and fungal community compositions across translocation treatment, site and sample year were assessed with stratified permutation tests separately for bacteria and fungi (PERMANOVA) performing permutations within the levels of the specified strata (to account for each combination of site and sample year).

To assess the effect of translocation treatments on microbiota composition across sample years, we used Bray-Curtis similarities comparing each sample's similarity to all donor sample similarities. Kruskal-Wallis multiple comparison tests were used for each site/year combination to determine whether the similarity to donor values differed across treatments. Significant differences between translocation treatments were then identified using post-hoc Dunn tests with Bonferroni correction to adjust *p* values for multiple comparisons.

We assessed alpha diversity by calculating the effective number of ASVs for each sample separately for bacteria and fungi. We tested the effects of soil translocation treatment within each site and sample year combination on effective number of ASVs using ANOVAs with Tukey post hoc tests or, if assumptions were not met, Kruskal-Wallis and Dunn post hoc tests with Bonferroni correction.

<u>Microbial dispersal from translocated soils</u>

Next, we assessed if translocated microbiota had dispersed into surrounding soils one year after soil translocation and whether there was any differential dispersal across our treatments. To assess if soil translocation effected microbial community compositions in surrounding soils, we excluded all 'establishment' samples from the T1 sampling event, and rarefied all remaining data based on the rarefaction curves (20,717 for 16S rRNA and 10,073 for ITS) and, following methods identically to those outlined above for microbiota establishment, assessed community-level similarities in 'dispersal' samples using NMDS ordinations and similarity to donor boxplots.

To examine potential dispersal of microbial taxa in more detail, we used differential abundance analyses at the genus level using *ancombc2* (Lin & Peddada 2024) on unrarefied data from both establishment and dispersal samples. We ran pairwise differential analyses, comparing each soil translocation treatment – subset by either establishment samples or dispersal samples – to the recipient control samples (i.e., seven pairwise comparisons for each site, for both 16S rRNA and ITS). All genera with significant ($p < 0.05$) log fold changes in individual pairwise comparisons were visualised in a heatmap for each site.

<u>Soil physicochemical changes and associations</u>

Associations between bacterial and fungal community compositions and scaled (mean-centred and standardised) soil physicochemical variables were analysed separately for each site at T1 sampling using constrained correspondence analysis (CCA). Variables with high collinearity (>0.75) were removed and the remaining variables underwent automated model selection. Model-selected variables and their associations with bacterial and fungal composition were visualised in a CCA and

tested via permutated ANOVA with 999 permutations. To explore differences in soil physicochemical variables across sampling years, each variable was compared across years within each soil translocation treatment at both sites using paired t-tests.

3 Results

3.1 Microbial Establishment and Inoculation Success

Intact soil cores established the most donor-like communities for both bacteria (Figure 2b, Figure 3) and fungi (Figure 2c, Figure 4) at T1. At the time of soil translocation (T0), bacterial and fungal communities in donor controls and all soil translocation treatments differed to recipient controls. However, soil samples collected at T1 showed shifts in both bacterial and fungal communities, particularly the surface spreading treatment (Figure 3a, Table S1, T0: bacteria, PERMANOVA, $p = 0.001$ for soil treatment, site and sample year; Figure 3c, Table S2 T0: fungi, $p < 0.001$ for soil treatment, site and sample year).

At T1, intact cores retained the highest similarity to donor value across both sites for both bacteria and fungi. Bacterial communities in intact cores at both sites were as similar to donors as donor control samples were to each other (Figure 3b, Table S3. In contrast, fungal communities in intact cores at both sites had lower similarity to donor values than donor controls had to each other (Figure 3d, Table S4). The mixed core treatment had the second highest community similarity to donor for bacteria at both sites and fungi at Monjebup (surface spreading had the lowest). Bacterial communities in mixed cores at Red Moort did not differ in their similarity to donor value compared to the donor controls (Figure 3b, Table S3), although bacterial

compositions at Monjebup did differ as did fungal compositions at both sites (Figure 3d, Tables S3-S4). Bacterial and fungal similarity to donor in mixed cores from both sites were still different compared to the recipient control samples.

Bacterial and fungal communities from the surface spreading treatment both diverged away from donor controls in both sites (Figures 3b 3d; Tables S3-S4). Bacterial communities in surface spreading samples at Red Moort diverged so far that their similarity to donor values were equivalent to the recipient controls but retained difference at Monjebup (Table S3). Although fungal communities in surface spreading samples at both sites had the lowest similarity to donor value of all three translocation treatments, they were still different from those in recipient controls (Table S4).

At Monjebup at T0, bacterial alpha diversity in the surface spreading, intact core and donor control samples was higher than in the recipient controls (Figure S3; Table S5). Effective number of ASVs in mixed cores at T0 did not differ to any other treatment. At Monjebup at T1, effective number of ASVs did not differ between any translocation treatment (Figure S3; Table S5). At Red Moort at T0, effective number of bacterial ASVs did not differ across translocation treatment (Figure S3; Table S5). At T1, effective number of ASVs were lower in the donor controls than the recipient controls and mixed cores (Figure S3; Table S5) but were no different than intact cores or surface spreading treatments. Surface spreading and mixed cores also differed to each other (Figure S3; Table S5). Fungal alpha diversity (effective number of ASVs) at Monjebup at T0 did not differ across translocation treatments (Figure S4; Table S6) but was higher at T1 in intact cores than in surface spreading samples

(Figure S4; Table S6). Effective number of fungal ASVs in Red Moort at both T0 and T1 did not differ across all soil translocation treatments (Figure S4; Table S6,).

3.2 Microbial Dispersal from Translocated Soils

At the whole community level, we found no evidence that translocated soil microbiota dispersed into surrounding soil or altered soil microbial compositions at either site (Figures S5-8). At T1, bacterial and fungal mean similarity to donor values in soil surrounding the translocated cores and below the surface spreading did not differ from recipient controls but differed from donor controls (Figures S5-6; Dunn, $p < 0.05$ for donor control only). We also found no evidence at the whole community level of fungal dispersal into surrounding soils (Figures S7-8). For fungi however, mean similarity to donor values did differ between surface spreading and intact treatments at both sites (Figure S8; Monjebup surface spreading similarity to donor = 10.6 ± 3.42%, Monjebup intact similarity to donor = 13.6 ± 2.75%, Dunn $p < 0.05$; Red Moort surface spreading similarity to donor = 12.8 ± 4.44%, Red Moort intact similarity to donor = 15.3 ± 4.81%, Dunn $p < 0.05$), but all translocation treatments were similar to recipient controls and different to donor controls.

We only found evidence of differential abundances between recipient control samples and dispersal samples from each translocation treatment for a single fungal genus, *Cortinarius*, at one site (Figure 4d). This genus was higher in abundance in the surface spreading treatment. No bacterial genus was differentially abundant between the dispersal samples from any translocation treatment and the recipient controls (Figure 4).

3.3 Soil Physiochemical Changes and Associations

Bacterial communities at Monjebup associated with soil phosphorus, conductivity, sulphur and pH (Figure 5a). Increased phosphorus primarily associated with bacterial communities in recipient controls, as well as some mixed and surface spreading samples. Increased levels of pH associated with bacterial communities in mixed and surface spreading samples. Bacterial community compositions at Red Moort associated with organic carbon and pH, although patterns across specific soil treatments were less clear (Figure 5b). Fungal communities at Monjebup also associated with pH and phosphorus, as well as organic carbon (Figure 5c). Increases in both pH and phosphorus associated with fungal compositions in recipient controls as well as mixed and surface spreading samples. Fungal compositions at Red Moort associated with Sulphur and pH (Figure 5d). Although fungal communities in Donor controls largely associated with increased sulphur levels, similarly to bacterial communities at Red Moort, patterns across specific treatments were less clear than they were at Monjebup.

We found more differences in soil abiotic properties across sample years (i.e., T0 vs T1) in both mixed and surface spreading treatments than we did in either control or the intact treatment (Figures S9-10).

4 Discussion

We experimentally tested the effect of three soil translocation methods – intact cores, mixed cores and surface spreading – on inoculating desirable soil microbial communities in a restoration project within a global biodiversity hotspot. After one year under field conditions, microbiota translocated via intact soil cores established

most effectively, with bacterial communities in particular retaining similarity to donor controls. In contrast, surface spreading – the most common soil translocation method used in restoration – resulted in microbial communities that diverged away from donor sites, becoming more like those in recipient sites. Our study highlights the importance of preserving soil structure and microhabitats during translocation to affect successful microbial inoculations. We recommend that the restoration sector prioritises research and investment into scalable soil translocation techniques that preserve soil structure to enhance ecosystem recovery outcomes.

4.1 Soil Structural Integrity Improves Inoculation

We show that retaining soil structural integrity during soil translocation led to the establishment of whole microbial communities, supporting our first hypothesis. Our intact soil core treatment maintained the most donor-like bacterial and fungal compositions one year after translocation. While microbial communities in our mixed treatment did not diverge as far as those in the surface spreading treatment, they were generally less similar to donor controls than the intact treatment. This improved establishment of microbiota in intact cores likely reflects reduced disturbance during soil translocation. The difference between mixed and intact treatments in isolation underscores the impact of soil homogenisation on microbial communities. Our findings offer field-based evidence that homogenising heterogeneous soil microhabitats alters microbial communities and impacts inoculation capacity. Previous studies have shown that frequent soil mixing in microcosms increasingly diverges bacterial communities from unmixed controls (West & Whitman 2022), underscoring how soil disturbance can affect the establishment of inoculated microbiota.

Fungal communities in our intact treatments diverged further from donor controls than bacterial communities. Fungi in natural soil systems rarely rely on sporulation and consist of extensive mycelia (Schnoor *et al.* 2011). These contrasting life history strategies in fungi likely explain the divergence from the donor soil composition observed in the intact translocation, as even intact core extractions will disrupt fungal organisms that are reliant on extended networks of mycelia.

We show that surface spreading was not effective in establishing donor microbial communities in the recipient plots after just one year. These results were likely driven by soil homogenisation (i.e., mixing many microhabitats and their constituent microbiota) and elevated exposure to environmental influences (e.g., due to surface spreading having a high surface area). Surface spreading is the predominant soil translocation method used in the restoration sector (Contos *et al.* 2021; Gerrits *et al.* 2023) and although surface spreading has previously been shown to be effective in inoculating some microbiota, our results support the finding that success is often site and context dependant (Gerrits *et al.* 2023). While our soil inoculation ‘dose’ is comparable to that used in other studies (Wubs *et al.* 2016; Han *et al.* 2022), surface spreading inoculations may be more effective on loamy soils (Gerrits *et al.* 2023) compared to the sandy soils in our study.

The homogenised soils in both mixed and surface spreading treatments appeared to be more susceptible to the soil abiotic legacies in the recipient site than the intact treatment. While we anticipated associations between soil microbiota and abiotic properties between our two controls, the associations between soil abiotic properties

and the surface spreading and mixed treatments after a single year were surprising. These associations may indicate elevated susceptibility of the translocated soils in these treatments to the abiotic legacies present in the surrounding soil at recipient sites. The features of pore space in soil (e.g., size, distribution, connectivity) are important for the biochemical processes of soil. Porosity, and the extent to which pores are saturated and connected, can affect abiotic and biotic conditions in soil (Six *et al.* 2004; Roger-Estrade *et al.* 2010). Here we found that the loss of physical structure in homogenised soils made them more susceptible to changes in abiotic properties. While there is strong evidence that abiotic properties and microbiota affect soil structure and aggregate formation (Rillig & Mummey 2006; Rillig *et al.* 2017; Or *et al.* 2021), further research is needed to improve our understanding of how disturbance to soil structure affects abiotic and biotic properties in soil and what this means for inoculation success across varied sites and contexts.

4.2 No Evidence of Microbial Dispersal from Translocations

We found no evidence to support our second hypothesis as none of our three translocation methods led to the dispersal of inoculated microbiota into the surrounding soil after one year. Successful dispersal of inoculated microbiota into surrounding soils is central to the ‘restoration island’ concept (Hulvey *et al.* 2017), where soil cores act as nodes of healthy soil biodiversity, cumulatively and positively affecting surrounding soil. While the lack of observed dispersal could simply be due to the short one-year period between re-sampling, both environmental filtering driven by the persistent agricultural land-use legacies in our sites (Peddle *et al.* 2024a) and limited dispersal capabilities of microbes in soil are likely barriers to dispersal (Chen *et al.* 2020; Walters *et al.* 2022; Liu & Salles 2024). Overcoming these land-use

legacies is a major challenge facing restoration in nutrient-limited ancient soils, like those in southwest Western Australia (Standish *et al.* 2006; Parkhurst *et al.* 2022). Restoration interventions like soil scraping and removal to address abiotic legacies are costly (Gibson-Roy *et al.* 2024) but may be warranted to facilitate successful inoculation. Furthermore, the relatively small soil volumes in our experiment may need to be increased across treatments to increase the propagule pressure needed for microbiota establishment and dispersal into surrounding soil. Further research with increased soil volumes will be beneficial to assess if intact soil translocations still outperform surface spreading. Longer term research might also investigate repeated surface spreading inoculation episodes at intervals that allow progressive development of a range of suitable microhabitats in recipient soils, to favour diverse requirements of the donor microbiota.

While our results indicate that intact soil translocation was the most effective method at inoculating soil microbiota, scaling up intact soil translocations to effect positive restoration outcomes faces numerous challenges. Sourcing soil for translocation impacts donor sites and projects need to carefully balance the benefits of soil translocation with the impacts to remnant ecosystems. Projects with existing remnant habitat already slated for clearing (e.g., surface strip mining) would be good candidates to consider large scale intact soil translocation. Additionally, restoration sites with abiotic soil legacies that differ strongly from restoration target conditions should reassess expectations from using surface spreading translocations. Strong physicochemical differences will present a barrier to establishment and dispersal of donor microbiota. Achieving positive outcomes in such situations may require extensive action to address the physicochemical limitation, and in extreme cases soil

removal and replacement in a manner that maintains soil structure during translocation.

Overall, our findings show that maintaining soil structural integrity via intact soil translocation is important to successfully establish whole soil microbial communities. In contrast, we show that surface spreading – a widely used method of inoculating soil microbiota in the restoration sector – was unsuccessful in establishing microbial communities in the recipient site after only one year. These results highlight the impact of soil homogenisation during translocation on the establishment of inoculated microbial communities. Furthermore, our findings suggest a need for the restoration sector to reconsider soil translocation approaches and invest in scalable applications that maintain the structural integrity of soil during translocation.

Acknowledgements

We thank Luisa Ducki, Kelsie Lambert, Emerson Lamond, Keely O'Connor, Daniel Shepherd, Nikki Maher, and Hannah Hall for their help with fieldwork, and Alex Hams and Lewi Marr for their help with on-site logistics. This research was supported by funding from The Holsworth Wildlife Research Endowment through the Ecological Society of Australia, a philanthropic donation from Peter and Maxine Wilshaw to Bush Heritage Australia, the Australian Research Council (grant numbers LP190100051, LP240100073), and the New Zealand Ministry of Business Innovation and Employment (grant UOWX2101).

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Figures

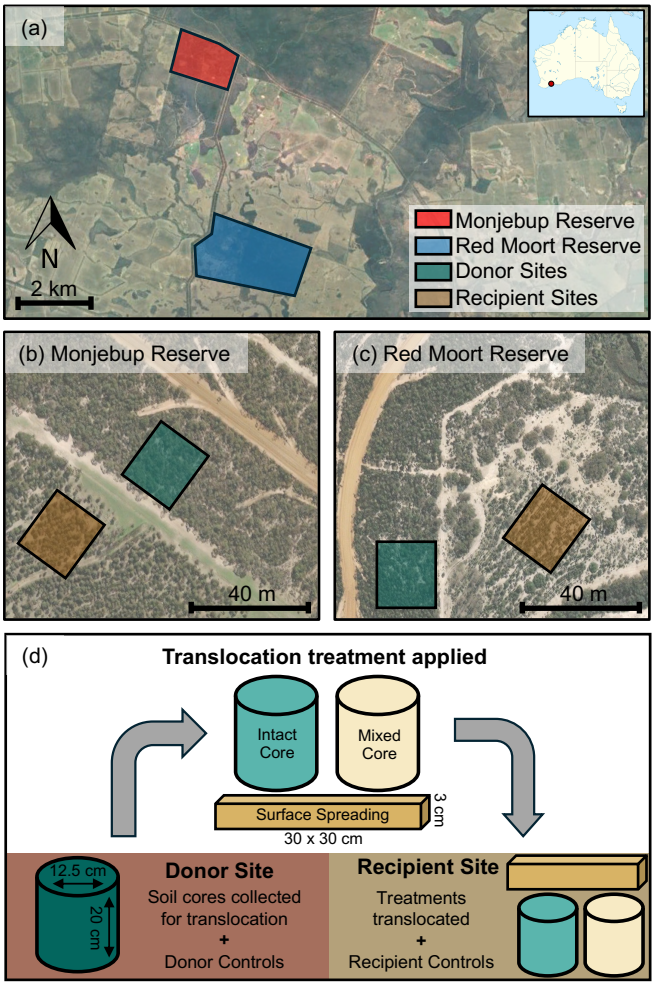
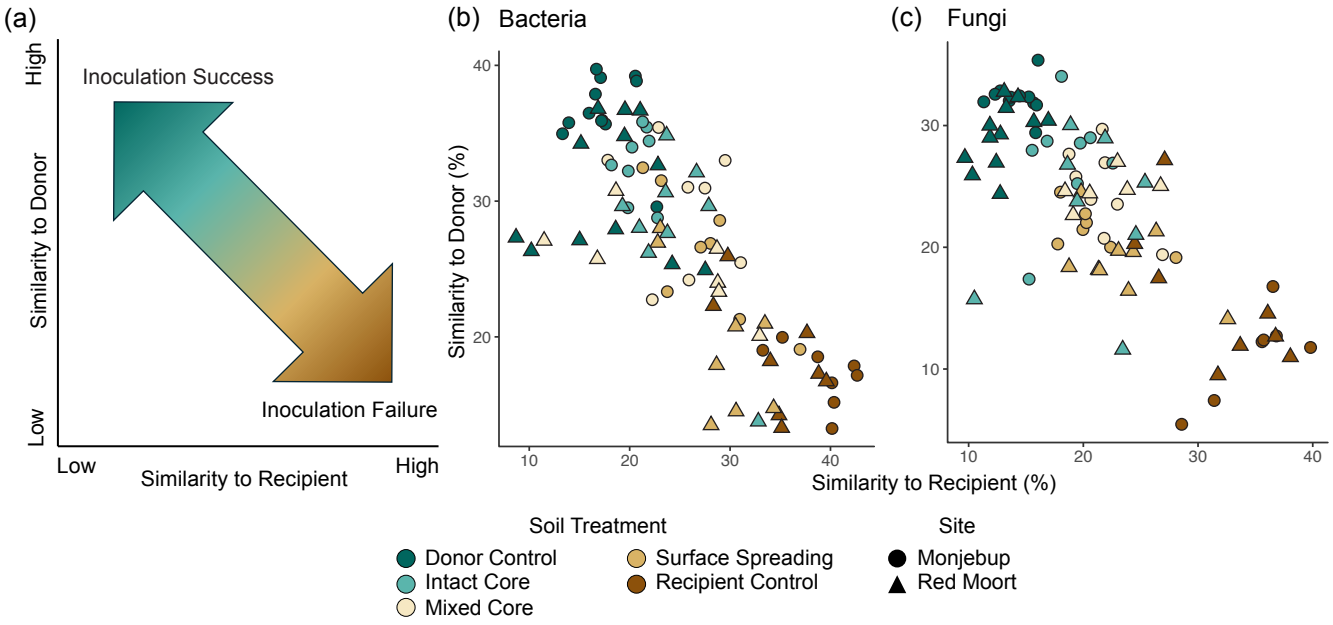


Figure 1. Map of the study locations in southwest Western Australia indicating (a) the locations of the two sites at Monjebup North Reserve and Red Moort Reserve in southwest Western Australia; the 20 m x 20 m donor plots in remnant bushland and the 20 m x 20 m recipient plots in revegetated areas at both (b) Monjebup North Reserve and (c) Red Moort Reserve. (d) graphical illustration of the experimental design showing the soil cores collected from donor sites, the experimental translocation treatments applied, and their translocation to the recipient sites.



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697 **Figure 2.** Success of microbial inoculations one year after soil translocation (T1). (a)
698 Conceptual illustration to visualise establishment of microbial inoculants after soil
699 translocations. We define inoculation success as the retention of a high similarity to
700 donor value relative to the donor to donor similarity, whereas inoculation failure is
701 indicated by a shift away from the donor and a high similarity to the recipient. (b)
702 Mean similarities of bacterial communities one-year after (T1) soil translocation to
703 both donor and recipient samples. (c) Mean similarities of fungal communities one-
704 year after soil translocation to both donor and recipient samples.

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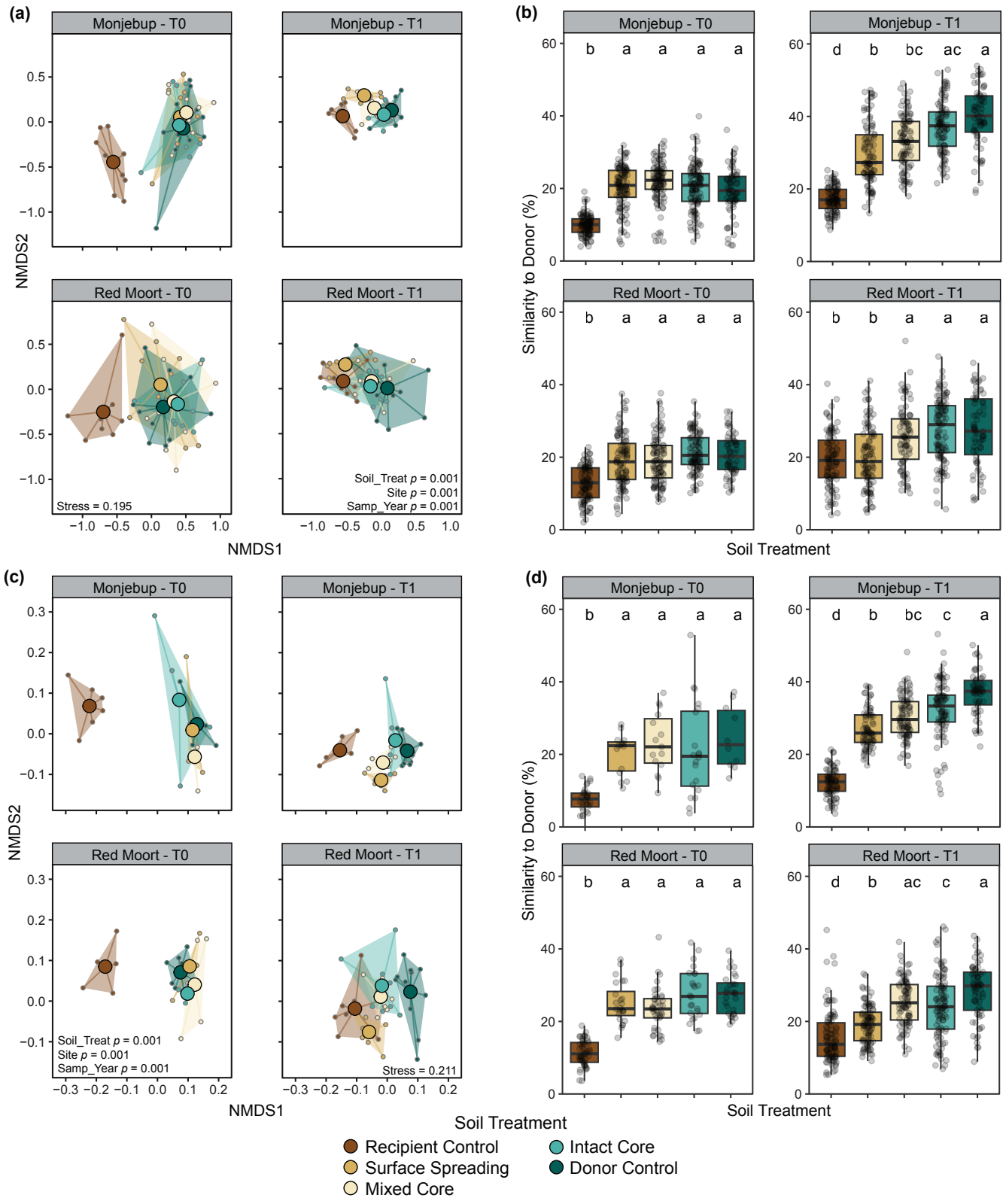

Figure 3. Community composition and similarities to Donor Controls at the time of translocation (T0) and one year post-translocation (T1). Non-metric multidimensional scaling (NMDS) ordinations for (a) bacteria and (c) fungi both faceted by site and

sample year visualising changes in microbial community composition across the three translocation treatments and two controls. Statistics and stress values refer to all panels within a series. Similarity to donor boxplots for (b) bacteria and (d) fungi at both sites visualising the similarities (Bray-Curtis) of the three translocation treatments and recipient controls to the donor controls. Groups not sharing a letter are significantly different ($p < 0.05$, Kruskal-Wallis and Dunn post-hoc).

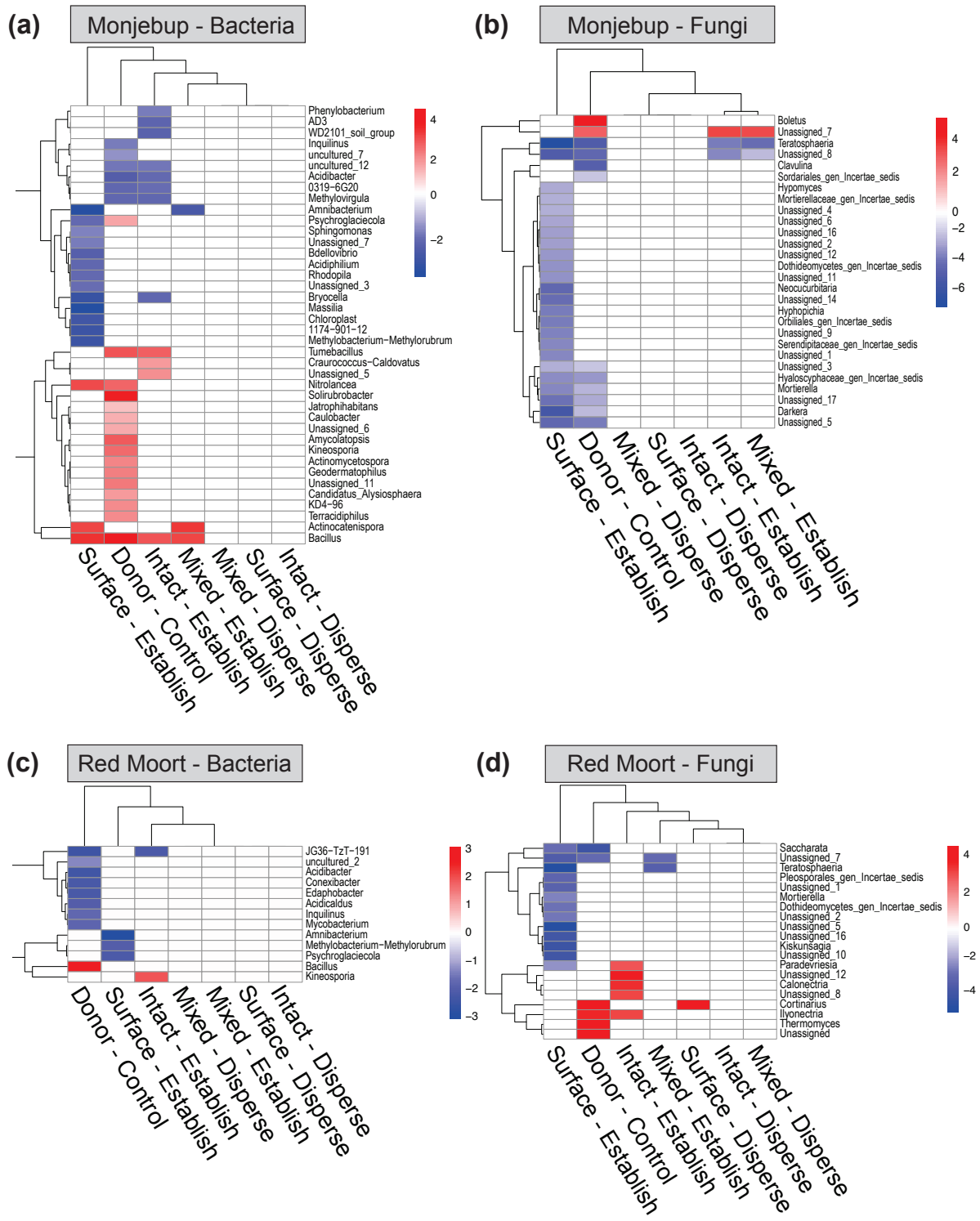

Figure 4. Heatmaps of significant differential abundance (log fold change $p < 0.05$) in bacterial (a, c) and fungal (b, d) genera at Monjebup Reserve (a, b) and Red Moort Reserve (c, d) assessing microbial dispersal from translocated soil into the surrounding soil. The three translocation treatment levels (Intact Cores, Mixed Cores

and Surface Spreading) are split by dispersal (samples collected 6 cm away from translocated soil) and establishment (samples collected from translocated soil) levels and log fold changes across all levels including the donor control are compared to the recipient controls one year (T1) after translocation. Only a single fungal genus, *Cortinarius*, showed evidence of dispersal from the translocated soil into the surrounding soil and only at Red Moort.

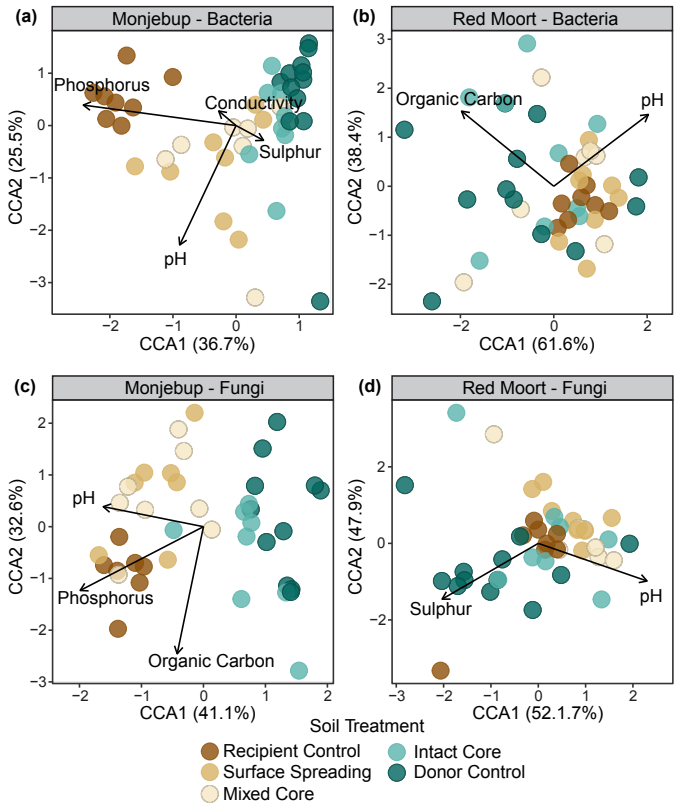

Figure 5. Constrained correspondence analysis (CCA) plots indicating associations between model-selected soil physicochemical properties and bacterial (a, b) and fungal (c, d) community compositions at Monjebup (a, c) and Red Moort (b, d).