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The impact of crop rotation on soil microbial diversity: A meta-analysis

Zander Samuel Venter^{a,*}, Karin Jacobs^b, Heidi-Jayne Hawkins^{c,d}

^a Department of Animal Sciences, Private Bag X1, Matieland 7602, Stellenbosch University, South Africa

^b Department of Microbiology, Private Bag X1, Matieland 7602, Stellenbosch University, South Africa

^c Conservation South Africa, Centre for Biodiversity Conservation, Kirstenbosch National Botanical Gardens, Private Bag X7 7735, South Africa

^d Department of Conservation Ecology and Entomology, Private Bag X1, Matieland 7602, Stellenbosch University, South Africa

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ABSTRACT

Agricultural intensification can involve the simplification of agroecosystems to crop monocultures while the practices of crop rotation, intercropping and companion planting maintain some crop diversity over time and space. It is generally postulated that reduced diversity can have an impact on ecosystem function. Here we determine whether decreased aboveground crop diversity affects belowground microbial biodiversity by conducting a meta-analysis of studies comparing monocultures and crop rotations. We based taxonomic richness and diversity indices on both molecular and biochemical fingerprinting methods. Soils under a higher diversity of crops in rotation produced higher microbial richness (+15.11%, $n = 26$) and diversity (+3.36%, $n = 43$) scores. This effect was significantly influenced by the proxy and methodology used where pyrosequencing produced higher richness scores compared to fingerprinting methods. Longer study trials produced larger increases in microbial richness, although the opposite was true for microbial diversity. The addition of legumes to rotation had no consistent effects on microbial diversity or richness. Whether the overall rotation-effect on microbial diversity promotes ecosystem functioning in terms of nutrient cycling and resilience to stress remains unclear. Future studies that incorporate next-generation sequencing techniques will help to elucidate complex microbial community structures and specific functional niches in agroecosystems.

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1. Introduction

The intensification of conventional agricultural practices is threatening ecosystem services and agroecosystem sustainability through soil erosion, agro-chemical pollution of groundwater,

* Corresponding author.

E-mail address: zander.v@hotmail.com (Z.S. Venter).

release of green-house gases and biodiversity loss (Tilman et al., 2001). This is causing a paradigm shift toward sustainability, characterized by practices and concepts such as organic agriculture (Badgley et al., 2007), agroecology (Rosset and Altieri, 1997; Thomas and Kevan, 1993), functional agrobiodiversity (Wood and Lenné, 1999) and conservation agriculture that includes crop rotation (Hobbs et al., 2008). The historical adoption of crop rotations was largely motivated by the associated yield increase in the cash crop (Bullock, 1992) attributed to enhanced agroecosystem function in terms of increased soil fertility (particularly when leguminous plants are used in rotation), maintenance of soil structure, disruption of pest cycles and weed suppression (Smith et al., 2008). These processes are mediated largely by soil microorganisms through their complex biochemical processes (Kennedy and Smith, 1995; Kennedy, 1999; Parkinson and Coleman, 1991).

Within the broader context of diversity-ecosystem function theory, an increase in plant species number within a crop rotation system may result in a concomitant increase in agroecosystem function (Smith et al., 2008; Vitousek and Hooper, 1994). However, the mechanism behind this positive effect may be attributed to singular key-stone species such as N₂-fixing legumes (the selection effect) or due to the facilitation and niche differentiation associated with a number of species (the complementarity effect) (Hooper et al., 2005). It is often the case that both these mechanisms are at work which gives support for the decoupling and coupling of biodiversity with function. It is less well known whether aboveground crop diversity is coupled to belowground microbial biodiversity and how this affects function.

The primary drivers of microbial diversity include edaphic variables such as soil pH and moisture, yet plant diversity is also now recognised as an important driver of soil microbial diversity (Berg and Smalla, 2009). It has been posited that management practices such as crop rotation, through increasing aboveground biodiversity, can result in corresponding increases in diversity belowground (Hooper et al., 2000). Soil microbial communities in the rhizosphere respond differently to root exudates associated with various crops (Costa et al., 2006; Wardle et al., 2004) and these rhizosphere communities can have an impact on the bulk soil microbiome (Kent and Triplett, 2002). In addition, crop rotations lead to a greater abundance and diversity of plant litter, which in turn can support a greater diversity of microbial decomposers (Kennedy, 1999). Thus increases in microbial diversity may not only be a direct effect of plant diversity, but due to the associated increases in soil organic matter caused by increased ground cover in rotations (Zak et al., 2003).

There is conflicting evidence in the literature addressing the relationship between above and belowground biodiversity in agroecosystems. A number of studies have shown microbial diversity to increase, not change, and decrease under crop rotations. For example, Lupwayi et al. (1998) discovered that microbial diversity, based on community level substrate utilisation, was significantly higher under a rotation of wheat and clover or field peas than under continuous wheat. However, Navarro-Noya et al. (2013) found that crop management (continuous maize versus maize-wheat rotation) had no effect on soil microbial diversity. Finally, Yin et al. (2010) found that incorporating soy beans into rotation with wheat decreased richness and Shannon's diversity indices in conventional tilled plots when compared to continuous wheat.

Here we assess whether increased crop diversity leads to increased soil microbial diversity and richness using a meta-analysis of the literature. A problem in reviewing independent studies is that differences between study conditions and abiotic factors may obscure the question of interest. The advantage of meta-analyses is that a relative measure of effect size (the

treatment relative to a control or other comparison) allows for comparisons across varying geographical regions, experimental and abiotic conditions. (Hedges et al., 1999). Although originally developed for medical and social science reviews, meta-analysis has been adapted for application to many ecological and agricultural datasets in the literature (e.g., Johnson and Curtis, 2001; McDaniel et al., 2014; Tonitto et al., 2006).

2. Methods and materials

2.1. Meta-analysis criteria

The analysis requires that a treatment and control are defined in order to calculate a response ratio. For the purposes of this analysis, the control was defined as any annual cash crop that is grown in monoculture every season. The experimental treatment/s were defined as any crop rotation including the same cash crop and at least one other rotation crop. Studies were included that recorded taxonomic (using genetic fingerprinting methods) or functional proxy measures (using biochemical fingerprinting methods) of soil (free-living) microbial (bacterial and/or fungal and/or archaeal) diversity, including the Shannon's diversity index (Shannon and Weaver, 1969) and/or richness (Hill, 1973). These are the most commonly used measures of soil microbial diversity in agricultural studies (Spellerberg and Fedor, 2003). Species richness is defined as the number of unique taxonomic sub-units, and diversity as the richness and relative abundance of these sub-units as calculated with the Shannon's diversity index. Many studies were excluded because they did not (1) contain the control, experimental treatment/s and diversity measures as listed above, (2) contain purely agricultural cash crops, or (3) metadata was not available upon request. From the rejected studies, those that reported some measure of microbial diversity were collated according to whether they detected significant increase, decrease or no change in microbial diversity with an increase in crop diversity over time.

2.2. Literature search and data extraction

The literature was searched using electronic databases, including Google Scholar, AGRIS, ScienceDirect, Elsevier and Wiley by entering the following search terms used in various combinations: crop rotation, microbial diversity, microbial richness. The Boolean operators 'AND' and 'OR' were used to combine two separate searches and include alternative search terms. The following criteria were used to select studies: (1) only peer reviewed articles in journals; and (2) studies with sufficient sample size to determine both a mean and standard error. In addition, studies were also identified through a 'snowballing' technique where reference lists of acquired studies were searched for additional relevant studies. For some studies, Data Thief[®] (Tummers, 2006) software was used to extract values from figures within studies. In cases where diversity measures or variances were not reported, the corresponding authors were contacted to request this information.

A total of 20 studies met the selection criteria and were entered into a database containing the following categorical moderating variables: microbial analysis method (biochemical fingerprinting, molecular fingerprinting and pyrosequencing), number of crops in rotation (2 or 3), use of legume (yes or no), use of cover crop (yes or no), and trial length (1–5, 6–15 and >15 years) (S-1 in Supplementary material). Here cover crop is defined as a crop that is not harvested but produced to enrich some aspect of soil health. To account for differences between next generation sequencing and other microbial analysis methods, studies using biochemical fingerprinting [fatty acid methyl ester analysis (FAME) analysis, community-level physiological profiling (CLPP) and plate

Table 1

Quality scoring system used in the Quality Effects model of the meta-analysis to assess the study design and possible bias. Questions and scores are adapted from epidemiology (Doi and Thalib, 2008) to be relevant to agronomy.

Question	Score
1. Did the experimental layout use randomization or another appropriate sampling strategy?	0 = No or not reported 0.5 = In part 1 = Yes
2. Were the groups being compared comparable at the baseline?	0 = No or not reported 0.5 = In part 1 = Yes
3. Were treatments clear and not confounded by e.g. soil type, cultivation history, tillage?	0 = No 0.5 = In part 1 = Yes
4. Was the trial conducted over an adequate time period to allow differences to emerge?	0 = 1–5 years 0.5 = 6–10 years 1 = 11–20 years 2 = >20 years
5. Was the analysis clearly reported and appropriate?	0 = No 0.5 = In part 1 = Yes
6. Were protocol deviations or losses during the study acceptable (< 20%)	0 = No or not reported 0.5 = In part 1 = Yes
Quality Score (Qi) = Sum of scores/7	

enumeration], molecular fingerprinting [denaturing gradient gel electrophoresis (DGGE), random amplified polymorphic DNA (RAPD) analysis, restriction fragment length polymorphism (RFLP)], and pyrosequencing were separated. ANOVA was used to determine which categorical moderating variables were significant predictors of the response variable in question. These were then used to perform meta-analyses with subgroups matching the levels of the moderating variables.

From the selected studies, 43 individual Shannon diversity index comparisons and 26 individual richness comparisons were obtained. Many studies applied multiple treatments with different combinations of crops in rotation at varying lengths of time. We treated each unique rotation combination and rotation length as independent observations. In addition, where within-treatment effects such as tillage (e.g. Yin et al., 2010), fertilization (e.g. Reardon et al., 2014), length of rotation (Bucher and Lanyon, 2005), or analysis method (Yao et al., 2006) were recorded in split-plot designs, these factors were recorded as independent observations if they were significantly different. In cases where they were not significantly different they were averaged and entered as one observation.

2.3. The Fixed, Random and Quality Effects Models

Meta-analysis calculates the weighted average of a number of study results, and different models have been developed to assign individual study weights based on sample size, variance and other

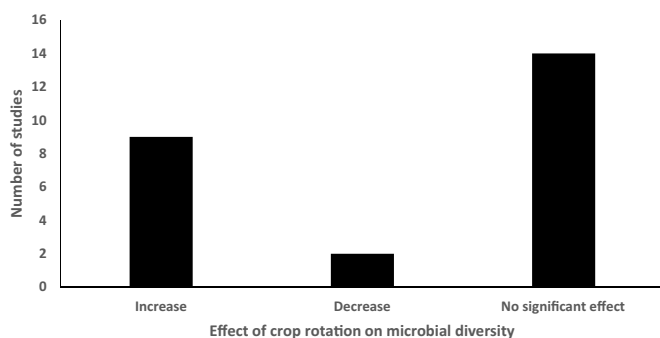


Fig. 1. Number of studies (including studies that did not meet the quality criteria for data extraction) that show a significant increase, decrease or neutral soil microbial diversity with an increase in crop diversity.

criteria related to study bias. The Fixed Effects model is most commonly used and is based on the assumption that differences between studies are simply a function of sampling error (Borenstein et al., 2010). The Random Effects model is also commonly used and assumes another source of variation from study level differences. Unlike the Quality Effects model, both these models do not account for bias that may be introduced due to study quality or publication bias (Doi and Thalib, 2008). For this reason, and because all three models gave similar results, we present the results of the Quality Effects model only, using MetaXL (v. 2.2, Epigear International).

In conducting the meta-analysis, an effect size estimate was calculated for each of the measurable variables to quantify the magnitude of the treatment effect (Osenberg et al., 1999). The effect size estimate used was the weighted mean difference (WMD), which is the difference in the mean response between the treatment (\bar{X}_t) and the control (\bar{X}_c). We calculated this as a percentage difference $(\bar{X}_t - \bar{X}_c / \bar{X}_c \times 100)$ so that positive values indicated an increase in microbial diversity with crop rotation/crop diversity relative to controls/monocultures. Within MetaXL, the Quality Effects model was used with effect size estimates weighted by variance, at 95% confidence intervals, as well as a quality score (Qi). The overall WMD is the average of all studies and is considered statistically significant at $P < 0.05$ when the confidence interval does not contain the null (Adams et al., 1997). Results were expressed as forest plots where the null is indicated as the line of 'no effect'. The Qi estimates the likelihood that results of a study are unbiased due to possible flaws in the experimental design (Doi and Thalib, 2008). The quality of studies included in the meta-analysis is important to quantify because if the quality of an experimental trial is inadequate, it may falsify the conclusions of the review. The Qi is calculated based on selected criteria with weighted scores representing low and high quality (Table 1).

Between-study heterogeneity is measured by the I^2 statistic ($I^2 = (Q - df / Q) \times 100\%$), where Q is the chi-square statistic and df is its degrees of freedom. Low to moderate heterogeneity is indicated by I^2 values at or below 60% (Higgins et al., 2003). Publication bias is a criticism of any review, and Funnel plots, which display the mean size effects of studies against standard error, are assessed for the resemblance to a symmetrical funnel. It is expected that there will be a spread of studies around the overall effect size, with more precise studies being closer to the overall weighted mean difference and less precise studies further away,

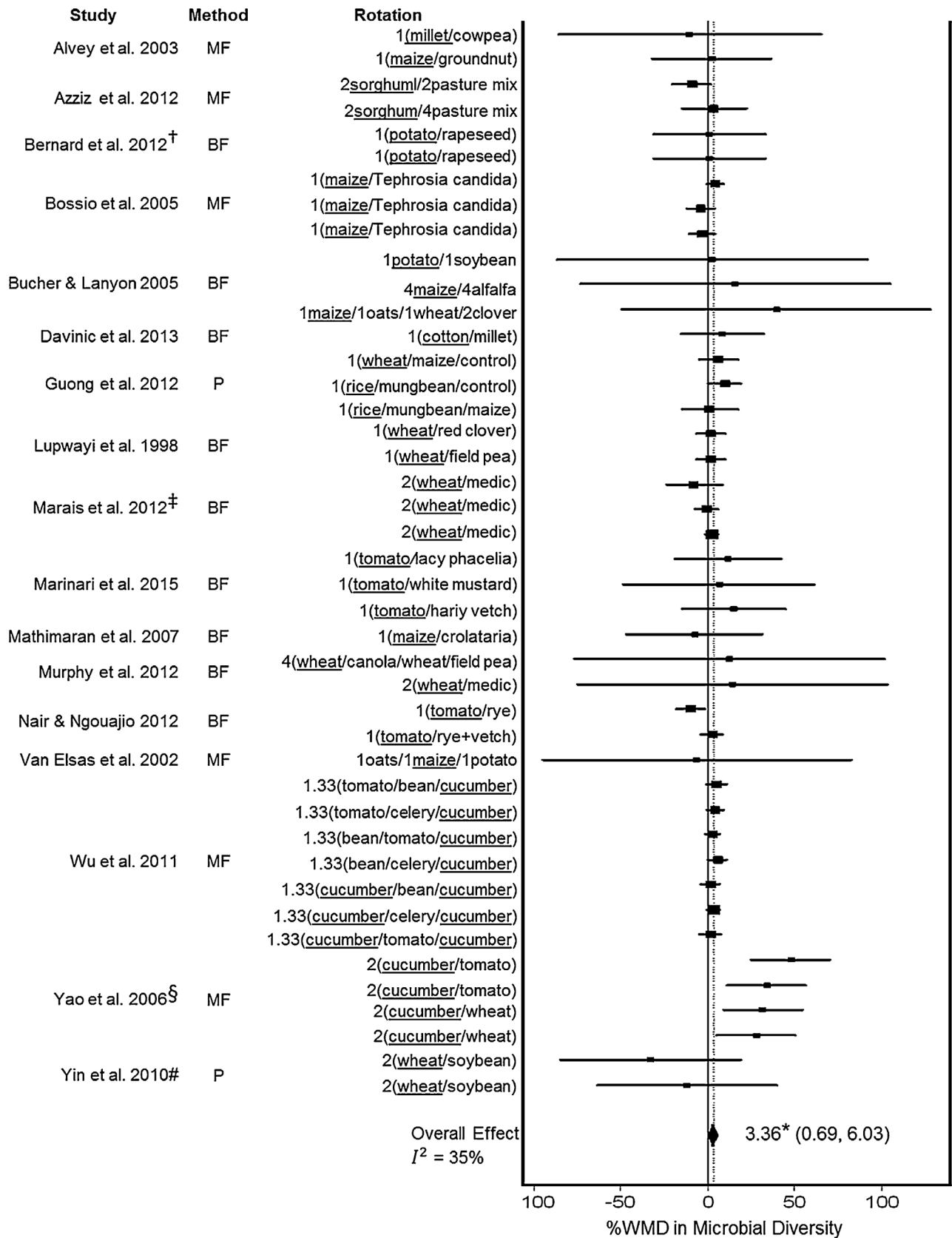


Fig. 2. Forest plot of percentage weighted mean difference (WMD) in soil microbial diversity (H') between crop monocultures (1 crop) and crop rotations (>1 crop) from 17 studies using the WMD method and Quality Effects model (Doi and Thalib, 2008) in MetaXL (v. 2.0, Epigear International). Studies are shown on the left with type of analysis method; biochemical fingerprinting (BF), molecular fingerprinting (MF) and pyrosequencing (P). Rotation combinations are listed with cash crop underlined and numbers signifying number of years. The Forest plot is shown on the right where studies >0 and <0 showed a positive or negative treatment effect, respectively. Treatment effect means (■) are weighted according to the weight (%) that the study was given based on the quality score (Table 1). Confidence intervals are indicated by horizontal lines for individual

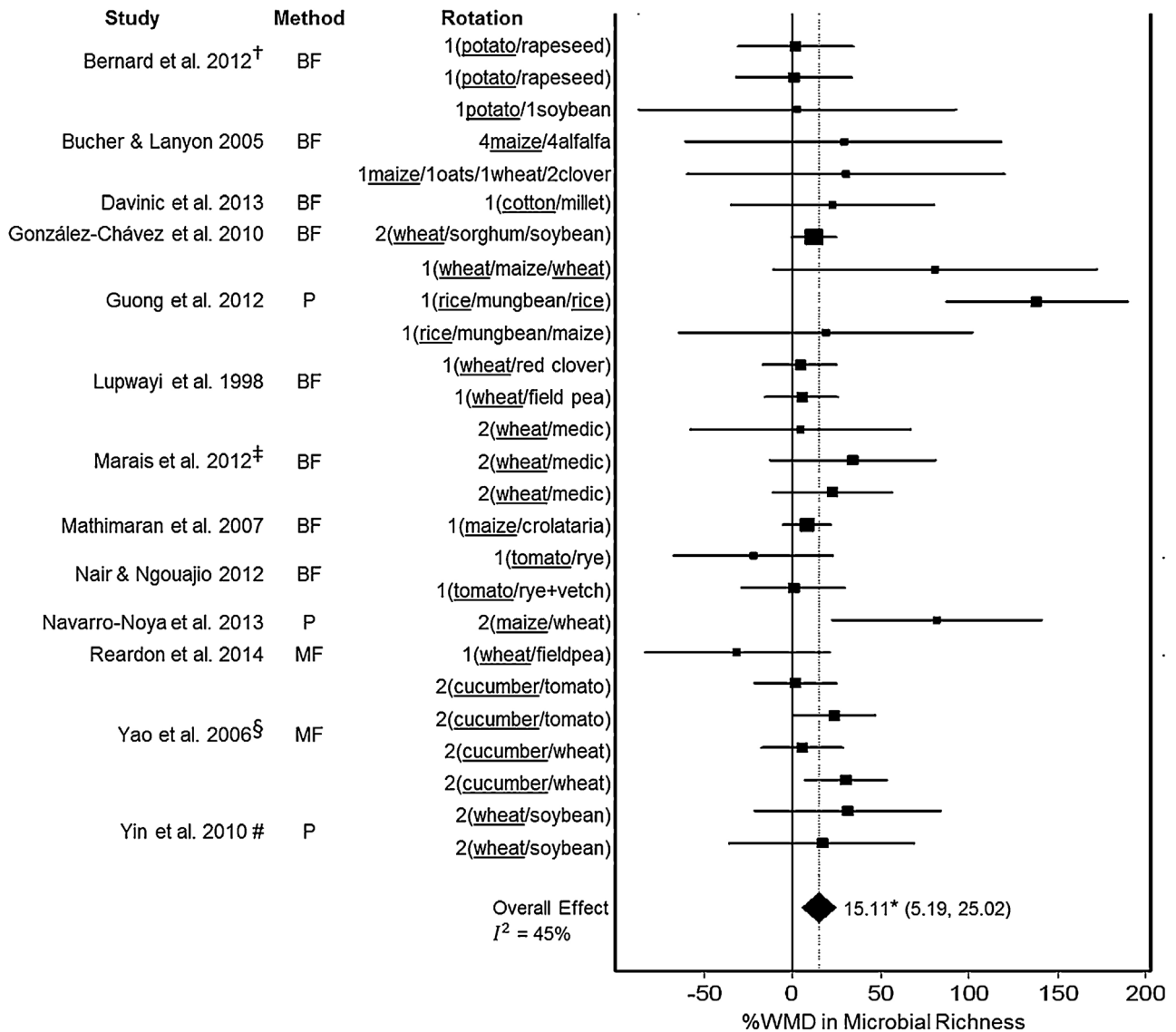


Fig. 3. Forest plot of percentage weighted mean difference (WMD) in soil microbial richness between crop monocultures (1 crop) and crop rotations (>1 crop) from 13 studies using the weighted mean difference (WMD) method and Quality Effects model (Doi and Thalib, 2008) in MetaXL (v. 2.0, Epigear International). The plot can be interpreted as explained in Fig. 2 (González-Chávez et al., 2010; Marais et al., 2012; Nair and Nguouajio, 2012).

forming a funnel. A criticism of Funnel plots is that there are other reasons beside publication bias that can result in an asymmetrical plot, e.g. small study effects. For that reason, the distribution of studies within the Funnel plots were assessed to see if there is a 'gap' in the area of non-significance (Higgins and Thompson, 2011).

A few studies (7/20 studies) did not report any measures of variance and metadata could not be obtained upon request. Thus the within-study variances that were available were used to calculate a pooled variance for the set of studies. This was used to calculate confidence intervals for studies lacking variance under the assumption that the variances of the responses within these studies were homogenous. The studies which did not calculate variance were not discriminated against in the scoring of Qi.

3. Results

We found 27 studies that reported some measure of microbial diversity in response to a change in crop diversity. Of the 27 studies, 14 reported a neutral effect of rotation on microbial diversity, while 9 studies reported a positive effect (Fig. 1). Of the initial 27 studies, 7 had to be rejected for meta-analysis because they did not (1) report Shannon's diversity index or richness scores (Chen et al., 2012; Ferreira et al., 2000; Larkin, 2008; Li et al., 2010; Souza et al., 2013), or (2) increase crop diversity over time (Dorr de Quadros et al., 2012), or (3) measure free-living microbial diversity (Herrmann et al., 2014).

WMDs, and by the width of the diamond in the overall effect size (◆) with vertical dotted line. Where these confidence intervals cross the 'line of no effect' (midline) the overall result indicates non-significance at the P=0.05 level. I² measures between-study heterogeneity. *Overall effect size with confidence intervals in parenthesis. † Treatments separated because of two study sites. ‡ Treatments separated based on year. § Treatments separated based on analysis method (CLPP/RAPD). # Treatments separated based on tillage (till/no-till) (Alvey et al., 2003; Azziz et al., 2012; Bernard et al., 2012; Bossio et al., 2005; Van Elsas et al., 2002; Wu et al., 2011).

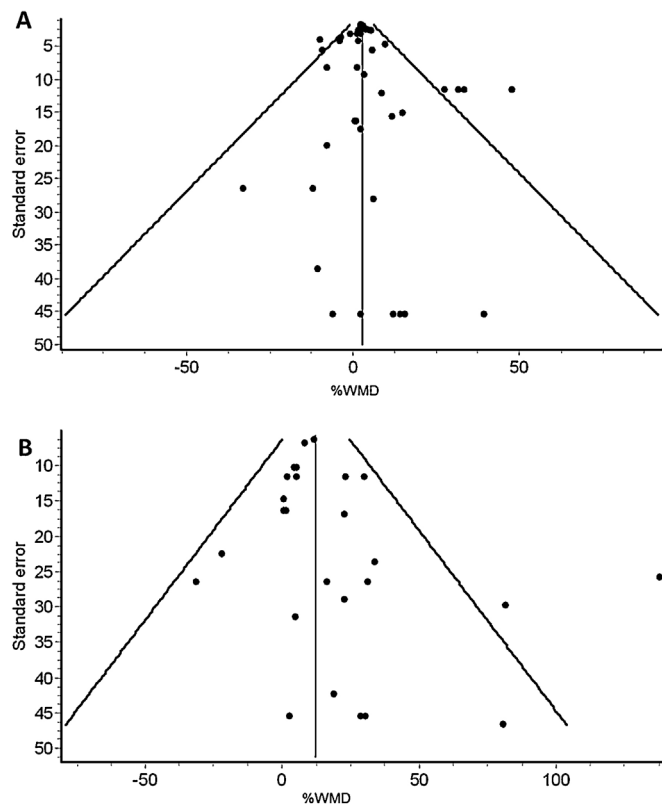


Fig. 4. Funnel plots indicating percentage weighted mean differences (%WMD) against standard errors for individual studies within meta-analyses assessing the difference in soil microbial diversity (A) and richness (B) between crop monocultures and crop rotations.

3.1. Diversity and richness

There were 17 studies retrieved that measured the effect of increasing crop diversity on soil microbial diversity using Shannon’s diversity index (H') and from these, 43 individual WMDs (effect sizes) were calculated. The meta-analysis revealed that there was on average an increase of 3.36% in microbial H' with increasing crop diversity ($p < 0.05$, Fig. 2). There were 13 studies retrieved that measured the effect of increasing crop diversity on

soil microbial richness and from these, 26 individual WMDs were calculated. The meta-analysis revealed that increasing crop diversity increased soil microbial richness on average by 15.11% ($p < 0.05$, Fig. 3). There were moderate levels of between-study heterogeneity with an I^2 of 35% for microbial H' and 45% for richness (Higgins et al., 2003). Funnel plots produced moderately symmetrical funnels for diversity and richness, with a few outlier studies, indicating that publication bias was unlikely (Fig. 4). There were several studies with both large and small standard errors that lay in the ‘null effect’ area, confirming that studies showing no effect were not excluded in the literature (Fig. 4).

3.2. Categorical moderating variables

Significant categorical moderating variables determined using ANOVA (S-2 in Supplementary material) included microbial analysis method, legume presence, and trial length. Pyrosequencing studies reported significant increases in microbial richness ($p < 0.05$) whereas this was not the case for diversity (Fig. 5). Molecular fingerprinting studies appeared more sensitive to increases in microbial diversity than microbial richness and the opposite was true for biochemical fingerprinting studies. The addition of a legume to rotations did not appear to make a difference to soil microbial richness or diversity. Shorter study trials produced significant increases in microbial diversity, while longer studies resulted in increases in microbial richness ($p < 0.05$).

4. Discussion

The overall effect sizes indicate that increasing crop diversity has a positive effect on soil microbial diversity and richness. The results from the meta-analysis confirms what other broader ecological studies report about positive relationships between above and belowground biodiversity (Hooper et al., 2005; Zak et al., 2003). Of the 20 studies included, 18 measured the effects on total microbial diversity (bacterial, fungal and archaeal), with only two studies (Mathimaran et al., 2007; Davinic et al., 2013) measuring fungal diversity, both of which recorded no significant change. Although out of the meta-analysis scope, the crop rotation effect on microbial diversity may be scalable to macrofaunal soil biodiversity. Sileshi et al. (2008) found an increase in soil macrofaunal richness and abundance in maize-legume rotations when compared to continuously cropped maize.

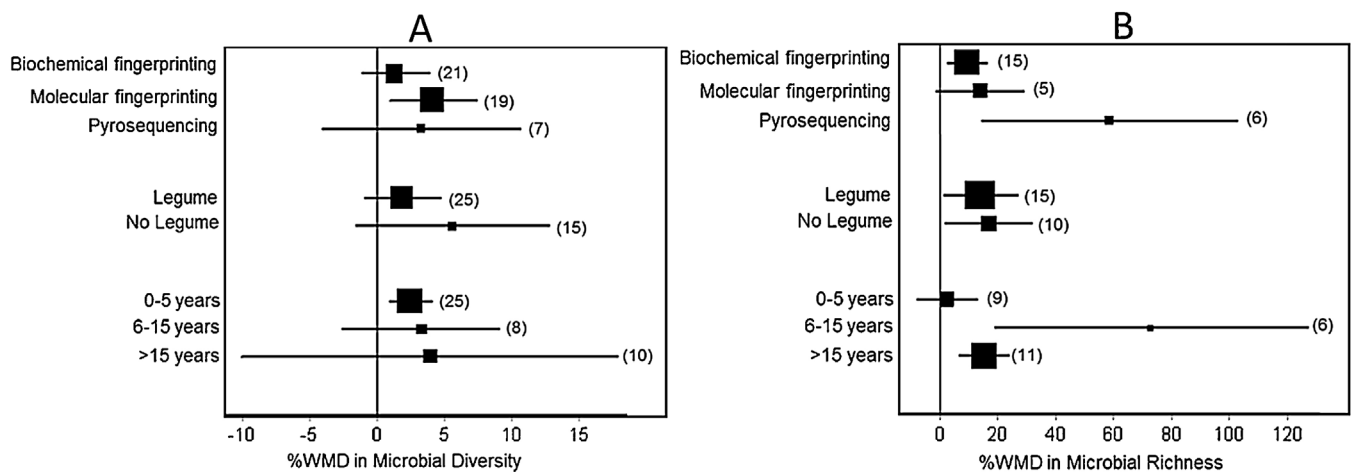


Fig. 5. Forest plot of percentage weighted mean differences (WMD) in soil microbial diversity (A) and richness (B) between crop monocultures (1 crop) and crop rotations (>1 crop). WMDs are averaged across studies separated based on significant categorical moderating variables using the Quality Effects model (Doi and Thalib, 2008) in MetaXL (v. 2.0, Epigear International). Treatment effect means (■) are weighted according to the weight (%) that the studies were given based on the quality scores (Table 1). Where confidence intervals (indicated by solid horizontal lines) cross the ‘line of no effect’ (midline) the overall result indicates non-significance at the $P = 0.05$ level.

The causal mechanisms behind this increase in belowground microbial diversity could involve physico-chemical changes in the soil brought about by increased crop diversity (Dias et al., 2015). Crop rotations are known to influence the physical structure of the soil and enhance soil water-use efficiency and stabilize temperature fluctuations through increased ground cover and soil organic matter content (Kennedy, 1999). The differential root action and niche exploitation from successive crops can allow for the proliferation of microbes to a larger extent in the bulk soil. These physical changes can create a favourable microclimate for soil microbes to thrive. The chemical changes in soil mediated by rotations are caused predominantly by build-up of residual root exudates and plant litter from preceding crops (Garbeva et al., 2004). These provide a greater diversity of residual carbon (C) substrates in the bulk soil which can support the growth of diverse microorganisms. Other studies have shown the host-specificity of bacterial and fungal groups in agricultural systems (Berg and Smalla, 2009; Smalla et al., 2001; Wardle et al., 2004). For example, Costa et al. (2006) detected plant specificity in the rhizosphere by bacterial, fungal and group-specific denaturing DGGE profiles for strawberry and oilseed rape crop. These plant-specific microbial species are predominantly of soil origin, as studies have shown that rhizosphere communities are more similar to bulk soil communities than to endophytic communities (Kent and Triplett, 2002). Thus, rather than introducing new species into the soil system, rotation crops stimulate the growth of specific microbial communities that are latent in the bulk soil. These residual rhizosphere communities from antecedent crop can have an impact on the bulk soil microbiome.

Thus, changes in microbial communities may not be due to increased plant diversity per se. Zak et al. (2003) found that microbial communities responded to increased plant production of detritus and C substrates associated with higher plant diversity rather than to the diversity itself. In addition, adding legumes to a rotation has been shown to increase bulk soil C pools, supporting a greater abundance of microbiota (Carranca et al., 2009; Drinkwater et al., 1998). Leguminous plants also harbour host-specific symbiotic bacteria (*Rhizobium*) which cannot exist without their hosts. These physico-chemical factors can have positive feedbacks into microbial growth and abundance and, as the meta-analysis results suggest, even diversity.

With an increase in soil microbial diversity, there may be beneficial effects on agroecosystem functioning and sustainability (Brussaard et al., 2007; Hooper et al., 2005). The major benefits expected to result from microbial diversity are functional redundancy, facilitation and niche differentiation (Giller et al., 1997; Hooper et al., 2000). High microbial diversity acts as an insurance against ecosystem malfunctioning due to multiple taxonomic sub-units that can perform the same function and have variable tolerances to stress (Griffiths et al., 2000). In addition, microorganisms facilitate ecosystem processes such as nutrient cycling. In a long-term ecological trial on grassland and savanna vegetation, increasing plant diversity (1–16 species) was positively correlated with more rapid N mineralization (Zak et al., 2003). In agricultural systems, there are examples of the association between high microbial diversity and higher soil N content for crops grown in rotation (Marinari et al., 2015; Murphy et al., 2011), although a systematic review of such studies has not yet been performed. Further, the relationship between microbial diversity and soil nutrient levels is not unidirectional, but is reciprocal and non-linear. The future use of next generation sequencing and other methods that link taxonomic units to their ecological functional niches within agroecosystems may elicit further clarity.

Adopting crop rotations to enhance microbial diversity may require the use of specific crop combinations that are expected to affect soil microbial diversity more than others due to plant-

specific root exudates and C sources. Within the present meta-analysis, two grain-legume rotations produced the greatest increase in microbial richness (rice/mung bean and maize/wheat), however a grain-legume combination also produced the greatest decrease in microbial richness (wheat/field pea). For Shannon's diversity index scores, the crop combinations enhancing microbial diversity the most were of very different varieties (cucumber/tomato and rice/mung bean). Thus, the present data does not present any specific crop combinations that could enhance soil microbial diversity.

Despite the overall positive effect of rotation on microbial diversity and richness, there were studies which revealed negative relationships. Yin et al. (2010) found that wheat in rotation with soybean resulted in a decrease in microbial diversity when compared to continuous wheat (Fig. 2). This may have been caused by the rise in pH associated with leguminous rotations which may negatively affect microbial diversity (Berg and Smalla, 2009). Another study on West African soils also found that grain-legume rotations increase pH in the bulk and rhizosphere soils (Alvey et al., 2001). Reardon et al. (2014) found that wheat in rotation with field pea negatively affected microbial richness (Fig. 3). This value exhibited large error margins which may have been attributed to year-to-year variability in the richness scores possibly driven by climatic variables. There was no common trend in crop type among studies exhibiting negative relationships to suggest host specificity.

Meta-analysis can be limited by publication biases, inherent problems in the design of studies, and the subjectivity related to pooling similar studies (Garg et al., 2008). The Quality Effects model accounted for some of these problems, however a number of outlier studies were identified through the diversity (Yao et al., 2006; Yin et al., 2010) and richness (Navarro-Noya et al., 2013; Guong et al., 2012) funnel plots. When removed from the meta-analysis model, these studies did not affect the direction or significance of the overall effect size. It is expected for studies with small sample sizes to produce less-significant size effects and thus produce asymmetrical funnel plots (Sterne et al., 2001), however this was not the case with the present meta-analysis. Intermediate I^2 values and slightly asymmetrical funnel plots identified moderate levels of between-study heterogeneity and bias within our meta-analysis. This was addressed by assessing the differences in significant categorical moderating variables that could explain between-study heterogeneity.

Results from pyrosequencing studies differed to those from fingerprinting studies for richness scores. This may be due to the higher accuracy of pyrosequencing compared with fingerprinting methods, or due to other study-specific variables. Pyrosequencing revealed an average decrease in microbial diversity in response to rotations, yet an increase in richness. This suggests that although new species may emerge with crop rotations, the relative abundance of microorganisms within species remains homogeneous between species. It also reveals the sensitivity of next generation sequencing techniques to unique taxonomic units that may go unidentified with biochemical methods. The low number of studies using pyrosequencing to assess crop rotation may explain the discrepancy between diversity and richness scores and this highlights the need for further application of next generation sequencing within agroecological studies.

Study trials longer than 15 years produced increases in microbial richness, yet trials shorter than 5 years produced increases in diversity. This may indicate that species evenness is variable over the short term, while the introduction of new microbial species to a crop rotation agroecosystem is more likely over time spans greater than 15 years. This has been shown in an ecological grassland experiment where the effect of plant diversity on soil microorganisms was most pronounced after a lag period of four years (Eisenhauer et al., 2010). This may have links to a build-

up of a diversity of C substrates in the soil over time. Many studies show that incorporating cover crops into rotations can boost soil C and thus support greater microbial abundance and diversity (McDaniel et al., 2014).

The presence or absence of a legume in rotation did not have any consistent effect on microbial richness or diversity. This is not surprising since the host-specific microbes associated with legume crops such as *Rhizobia* are low in diversity relative to free-living species. Indeed, studies have shown that key functional plant groups like grasses and legumes have inconsistent effects on soil microbial functioning and diversity (Eisenhauer et al., 2010).

5. Conclusions

In a comprehensive meta-analysis review of the literature, we found that increasing the diversity of crops in rotation has a positive impact on soil microbial richness (+15.11%) and diversity (+3.36%). This effect was true predominantly for total (fungal, bacterial and archaeal) free-living microorganisms in the bulk soil. Although there was moderate heterogeneity between studies, the direction and significance of the overall effect size was not affected within the meta-analysis. The results suggest that the adoption of crop rotation as a management practice is beneficial for soil microbial diversity, although there were no identifiable specific crop combinations or varieties that are more beneficial than others. The increase in microbial diversity was significantly influenced by the microbial analysis method used, where pyrosequencing produced higher richness scores compared to fingerprinting methods. This highlights the sensitivity of next generation sequencing to unique taxonomic units and the need for further application of these methods within agroecological studies. The increased response in microbial richness scores to crop rotations established for longer periods was not mirrored by diversity scores. The addition of legumes in rotation made no difference to total soil microbial diversity.

Finally, although microbial diversity may increase with crop rotation, the assumption that this is beneficial for agroecosystem functioning needs to be questioned. The relationship between biodiversity and agroecosystem functioning is complicated and future research on the link between above and belowground diversity would do well to measure the associated effects on components of agroecosystem function (e.g. soil N fertility) as well as the spatial and temporal changes therein. In addition, the use of next generation sequencing techniques can aid in understanding the role of specific functional groups in the yield increase of crop rotation systems.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.pedobi.2016.04.001>.

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