**Title:** Spatial Scaling Patterns of Community Composition are Context-Dependent for Microorganisms

**Authors:** Dave R. Clark, Graham J.C. Underwood, Terry J. McGenity, and Alex J. Dumbrell

**Addresses:** School of Biological Sciences, University of Essex, Wivenhoe Park, Colchester, Essex, CO4 3SQ, UK.

**Keywords:** Bacteria, Archaea, Eukarya, distance-decay of similarity, Mantel test, macroecology, dispersal limitation, meta-analysis

**Running title:** Meta-Analysis of Microbial Distance-Decay Relationships

**Abstract**

Ecological communities closer together in space and time, are generally more similar than those further apart, as defined by the distance-decay (d-d) of similarity relationship. Historically, microorganisms were assumed to defy this relationship due to their capacity for long distance, passive dispersal, and high population densities. Yet, recent studies have recorded highly variable d-d relationships in a range of microbial communities from disparate environments, using very different methods. The range of biological contexts incorporated by these studies may explain the differing distance-decay relationships reported as the dispersal of microorganisms may vary between different study systems, or spatial scales. Furthermore, methodological differences between studies will differ in their ability to detect rare species, thereby leading to contrasting estimates of compositional similarity between communities. Therefore, I sought to understand whether the variability in microbial d-d relationships is caused by different study methodologies, or biological contexts. To do this, I conducted an exhaustive meta-analysis and gathered data on 287 microbial d-d relationships. Given that most studies statistically test for d-d relationships using the Mantel correlation test, I used the Mantel correlation coefficient as a measure of effect size. I found that d-d relationships were weakly but significantly related to measures of community coverage, whilst different community quantification methods (e.g. community fingerprinting, high-throughput sequencing, morphological) only effected statistically-significant d-d relationships. The use of phylogenetic community similarity indices resulted in significantly weaker d-d relationships than compositional similarity metrics (e.g. Jaccard’s or Bray-Curtis index). Distance-decay relationships were significantly weaker in soils than other study systems, but significantly stronger in host-associated systems, potentially reflecting the ecological properties of the host taxon. The strength of the d-d relationships was also positively related to the spatial scale of the study but, against expectation, did not vary between different study taxa. I conclude that the microbial d-d relationship is dependent on biological context, but that methodological choices by the researcher can also strongly influence the strength of this relationship. I provide suggestions for selecting methods that will minimise methodological noise, and enhance ecological signal.

**Introduction**

The distance-decay (d-d) of community similarity is one of the most studied relationships in macroecology (Nekola & White 1999; Condit *et al.* 2002; Soininen *et al.* 2007). The relationship quantifies decrease in compositional similarity between communities with increasing geographic distance, such that proximate communities are more similar than distant communities. D-d relationships arise through several different, but often interacting ecological processes, and are hence of considerable interest to ecologists (Nekola & White 1999; Soininen *et al.,* 2007; Hanson *et al.,* 2012). Firstly, d-d relationships can form through spatially structured niche processes. Communities are often structured by the shared environmental niches of their component species. Consequently, in habitats where spatially structured environmental gradients occur, communities close together in space experience similar environmental conditions, thus selecting for similar communities. Alternatively, d-d relationships may occur through neutral processes. Dispersal limitation enhances d-d relationships by limiting the connectivity between communities, whilst drift contributes to d-d through the stochastic processes of speciation and extinction, thereby facilitating more compositionally dissimilar communities.

Distance-decay relationships have been documented in a multitude of “macro-organisms”, yet they are of particular interest to microbial ecologists, as microorganisms posess several characteristics that may defy classic d-d relationships. Firstly, their small size facilitates passive dispersal over large geographic distances by vectors such as wind, bio-aerosolisation, oceanic currents, migratory animals, and human activity (e.g. Bisson *et al.,* 2007; Favet *et al.,* 2013; Joung *et al.,* 2017). Additionally, microorganisms often maintain extremely high population densities in the environment leading to dispersal by via “mass effects”, whereby high dispersal rates from areas of increased population density maintain populations in less optimal environments (Shmida & Wilson, 1985). Finally, some microorganisms are able to enter vegetative states, such as cysts or spores, allowing them to survive in suboptimal environments until suitable habitat is reached. Combined, these properties make microorganisms effective dispersers, leading to the assumption that microorganisms should be globally dispersed, and that microbial communities would only show d-d relationships under niche processes in spatially autocorrelated environments (Baas Becking 1934; Finlay & Fenchel 2004).

However, the rapid development of molecular methods to study microbial communities has facilitated an explosion of studies empirically testing microbial distance-decay relationships. This research has yielded mixed results about the nature of microbial d-d relationships, with considerable variability across studies. Many studies find little or no correlation between microbial community composition and distance (Hazard *et al.* 2013; Kivlin *et al.* 2014), whilst others report relationships of varying steepness, across a range of spatial extents, study systems, and taxa (e.g. Dumbrell *et al.* 2010; Martiny *et al.* 2011; Barreto *et al.* 2014, Clark et al. 2017), even after accounting for the spatial structure of the environment (e.g. Green et al., 2004). Thus, few universal patterns have emerged regarding the spatial ecology of microorganisms.

The variation in reported d-d relationships could be due to different ecological contexts incorporated by studies. For instance, the study systems commonly of interest to microbial ecologists will vary by their connectivity, facilitating or hindering dispersal between communities. Well connected systems in which long distance dispersal is possible, such as oceanic waters, should show weaker d-d relationships than systems in which dispersal is limited, such as host-associated communities. Moreover, study systems differ in the environmental gradients they support. Soils for example, can support strong environmental gradients over distances of a few meters (e.g. Dumbrell *et al.,* 2010), leading to steep d-d relationships, whilst systems such as well-mixed ocean waters, may show weaker d-d relationships as environmental conditions will be less strongly spatially autocorrelated. Additionally, different study organisms are also likely to yield variable d-d relationships, particularly when we consider dispersal ability to be a trait mediated process. For example, small cell sizes lead to more efficient long distance dispersal (Wilkinson *et al.,* 2012; Norros *et al.,* 2014), thus organisms with smaller cell sizes, such as Bacteria and Archaea, should disperse further than microbial Eukarya, leading to weaker d-d relationships. Finally, it is well known that spatial extent can influence our perception of ecological relationships, and may contribute to variable d-d relationships. Studies that incorporate large spatial extents may be more likely to find strong d-d relationships as dispersal is assumed to be negatively related to geographic distance. Furthermore, large scale studies will likely incorporate greater environmental heterogeneity, and thus niche based processes may enhance d-d relationships (Martiny *et al.* 2011).

Whilst microbial d-d relationships may be context-specific, methodological differences between studies could also contribute

a real biological phenomenon, it could also be an artefact of the methodological differences between studies. Molecular methods have revolutionised microbial ecology, and have developed incredibly rapid. Consequently, the molecular methods utilised by microbial ecologists vary enormously in their ability to detect and resolve microbial taxa (Muyzer 1999; Glenn 2011). Community fingerprinting methods (e.g. denaturing gel gradient electrophoresis) offer limited community coverage, and are unable to resolve closely related taxa, meaning that communities may appear artificially similar in composition. In contrast, high-throughout sequencing platforms offer massively improved community coverage, enabling the detection of rarer taxa, as well as the ability to resolve closely related taxa, more accurately quantifying the compositional similarity between communities. In addition to the varying quantification methods used by microbial ecologists, analytical methods could also influence d-d relationships. In particular, the choice of similarity index may be important. There are an array of indices available to quantify the similarity in operational taxonomic unit (OTU; a sequence-similarity based pseudo-species definition) composition between communities, including qualitative (based on presence/absence of species e.g. Jaccard’s index), quantitative (based on composition and abundance of species e.g. Bray-Curtis), and phylogenetic indices (based on relatedness of communities e.g. Unifrac). These indices have different properties in terms of how they are influenced by sample sizes or species richness (Baselga 2012; Beck *et al**.* 2013), and especially in terms of what they quantify (e.g. phylogenetic similarity versus compositional similarity). For example, phylogenetic indices would be expected to yield weaker distance-decay relationships than other metrics, because communities can be phylogenetically closely related, yet dissimilar in OTU composition (e.g. Bryant *et al.,* 2008). On the other hand, quantitative indices are able to reflect more fine scale changes in community structure as they account for changes in the abundances of species, and thus should result in stronger d-d relationships as accounting for abundance changes should yield more dissimilar community comparisons.

Our perception of fundamental ecological relationships in microbial communities, such as the d-d relationship, are therefore vulnerable to several potential sources of variability that may be of ecological or artefactual origin. Despite this, the influence of such factors on ecological relationships, such as the d-d relationship, in microbial communities remains poorly quantified. Therefore, we sought to understand whether methodological or contextual differences between studies influence reported d-d relationships in microbial communities. To do this, we conduct a meta-analysis to synthesise available data on microbial d-d relationships, and test the effect of factors relating to methodology or ecological context on the strength of d-d relationships. Specifically, we test the following hypotheses:

* H1: Bacteria and Archaea will show weaker d-d relationships than other microbial taxa due to their smaller size and higher population densities in most environments.
* H2: Soils and host-associated study systems will show stronger d-d relationships than other systems, due to their ability to maintain steep physicochemical gradients or limited host range size respectively, whilst aquatic systems will have weaker d-d relationships due to the potential for increased connectivity between communities.
* H3: The spatial extent of a study will be positively related to the strength of a d-d relationship, as larger extent studies incorporate greater environmental heterogeneity and lower dispersal rates between communities.
* H4: Higher resolution community quantification methods, such as high-throughput sequencing, will yield stronger d-d relationships due to their ability to resolve closely related taxa.
* H5: Sampling depth (e.g. number of sequences, or number of individuals counted) will be positively related to the strength of d-d relationships as higher community coverage methods will capture more of the rare endemic taxa, thus decreasing community similarity.
* H6: Phylogenetic similarity metrics will result in weaker d-d relationships than other metrics as communities can be phylogenetically similar, yet different at the OTU level.

**Methods**

*Meta-Analysis*

In order to test our hypotheses, we first gathered all available data on microbial d-d relationships via a systematic literature search. To do this, five search terms were designed to detect relevant studies (Table 1). All literature searches were conducted using the Web of Science search portal on 08/06/2017, and search results published between 1900-2017 were retained. To further narrow down the dataset to studies suitable for testing our hypotheses, search results were downloaded and manually screened using the “metagear” (version 0.4; Lajeunesse 2016) package in R (version 3.4.1; R Development Core Team 2016). Here, “suitable studies” were those that tested the relationship between community similarity and geographic distance in microbial communities, and not studies of “macroorganisms”, or studies of strain-level genetic distance (e.g. using multi-locus sequence typing).

From these studies, we extracted the Mantel correlation coefficient as an effect size measure of the d-d relationship. The Mantel tests is a correlative test used to test for correlation between two distance matrices (i.e. community dissimilarity and geographic distance), and the Mantel correlation coefficient is an ideal effect size measure for several reasons. Firstly, the Mantel correlation test is the most frequently used method for testing the statistical significance of d-d relationships in microbial ecology (e.g. Ramette, 2007; Franklin & Mills, 2007). Secondly, as the Mantel coefficient is a standard correlation coefficient (i.e. is bound by -1 and 1), it provides an easily interpretable measure of effect size (Harrison, 2010), that is comparable across studies.

Within the literature, community similarity is often quantified as a distance (e.g. dissimilarity) and therefore, any correlation coefficients based on similarity, instead of dissimilarity, were multiplied by -1 so that the direction of correlation was consistent across studies. For clarity, here a Mantel correlation coefficient of 1 indicates a strong d-d relationship, 0 indicates a lack of correlation between community (dis)similarity, and -1 indicates a strong negative correlation. It is possible that Mantel correlation coefficients can be negative in cases where more distant communities are more similar to each other than neighbouring communities. There are ecological reasons why this might occur (e.g. frequent long distance-dispersal, or environmental heterogeneity), and so they are included within our analysis. Partial Mantel statistics (which are able to test for correlation between two matrices whilst controlling for a third) were excluded as they are heavily influenced by which other variables are included in the test, and are therefore not easily comparable between studies.

In order to test our hypotheses, several variables relating to the ecological context and methodology of each d-d relationship were recorded. Details of these variables, and the relevant hypothesis each is used to address, are described in Box 1.

*Statistical Analyses*

In order to determine whether d-d relationships varied between categoric variables (as in hypotheses 1, 2, 4, and 6), we used ANOVA tests. In tests where significant differences between groups were found, Tukey’s Honest Significant Difference (HSD) tests were used to determine which groups were different. To test hypotheses 3 and 5, linear regressions were used to test relationships. The variables scale and sequence depth were log transformed prior to analysis to aid model fitting, as they spanned several orders of magnitude.

**Results**

The Web of Science searches resulted in 2,250 search hits (Table 1). Removal of duplicate hits (i.e. studies that appeared in multiple searches) and manual screening of abstracts further reduced this number to 547 studies that were deemed to be potentially suitable for use in this analysis. A total of 287 Mantel correlation coefficients were obtained from 108 studies, in 33 journals (Figs. 1, S1). Of the 439 “unsuitable” studies that were not inclusion in this analysis, most had not tested for correlation between geographic distance and community (dis)similarity (although the abstract still contained the search terms), whilst others had used different methods (e.g. multilocus sequence typing on individual species, or spatial eigenvector analysis). Reported Mantel correlation coefficients ranged from -0.24 to 0.95, with a mean of 0.27 (std. error = 0.014).

*Influence of Ecological Context on Distance-Decay Relationships*

In order to determine whether microbial d-d relationships depend on ecological context, we tested for differences in reported Mantel correlation coefficients (from d-d relationships) between different study organisms, study systems, and spatial scales. Within the dataset, the most frequently studied taxa were Bacteria, followed by Fungi, microbial Eukarya, and Archaea. In disagreement with our hypothesis (H1), Mantel correlation coefficients were not significantly different between study taxa (*F*5, 281 = 1.39, *P* = 0.23). Furthermore, when considering only statistically significant d-d relationships, only marginally significant differences emerged (*F*5, 172 = 2.51, *P* < 0.05), with studies jointly considering Bacteria and Fungi showing significantly weaker d-d relationships than studies of Archaea (Tukey HSD; *P* < 0.05). Finally, when only studies that had tested multiple taxa (n = 14 studies, 57 Mantel coefficients) were examined, there were still no significant differences between study taxa (*F* 4, 52 = 1.76, *P* = 0.15)..

To test our second hypothesis (H2), that the d-d relationship would vary between study system, the 287 recorded d-d relationships were classified into 20 different biomes. Of these, 11 biomes were represented by fewer than three d-d relationships, and were therefore excluded from biome analyses. The most frequently studied biomes were grasslands (*n* = 62), forest (*n* = 57), and lakes (*n =* 44). Mantel coefficients differed significantly between biomes (*F*8, 262 = 8.80, *P* < 0.001), in partial agreement with H2. Specifically, sponge associated communities displayed higher coefficients and therefore stronger d-d relationships than other biomes (Tukey HSD; *P* < 0.05 in all cases), and grassland communities had lower coefficients than most other biomes (forest, lake, ocean, river, sediment, and sponge. Tukey HSD; *P* < 0.05 in all cases). Additionally, when d-d relationships were classified into “micro-environments”, further significant differences were found (Fig. S3; *F*4, 280 = 7.35, *P* < 0.001). Against our expectation (H2), soils showed significantly lower coefficients (weaker d-d relationships) than host-associated, sediment, and water d-d relationships (Tukey HSD; *P* < 0.01 in all cases).

The spatial extent of the studies recorded here spanned between 10 cm and 18,700 km. As expected (H3), the strenth of d-d relationships was significantly and positively related to the (log) spatial extent of studies, albeit weakly (slope = 0.016, *P* < 0.001, adj-*R2* = 0.12). Furthermore, there was no co-correlation between spatial extent and sampling effort (Pearson’s ρ = 0.03, *P* = 0.64), confirming that the positive relationship described previously is not confounded by larger scale studies having greater sampling effort. Furthermore, when we included sampling effort alongside spatial extent as a model covariate, the fitted relationship between Mantel coefficients and spatial extent did not change (slope = 0.016, *P <* 0.001, adj-*R2* = 0.13).

*Influence of Methodology on Distance-Decay Relationships*

We also tested whether specific methodological differences (relating to community characterisation method, sampling depth, and community (dis)similarity index) between studies could explain variability in microbial d-d relationships. To test our hypothesis that high-resolution community quantification methods would result in stronger d-d relationships (H4), d-d relationships were classified into low (morphological), intermediate (fingerprinting), and high (sequencing) resolution. Our dataset revealed that the majority of d-d relationships were based on high-resolution sequencing (n = 197), whereas fewer were based on intermediate resolution fingerprinting approaches (n = 76), and low resolution morphological surveys (n = 14). In contrast to our hypothesis, we found no significant differences between different resolution approaches (F2, 284 = 0.47, *P* = 0.62; Fig. 2A). However, when only statistically significant d-d relationships were examined (Fig. 2B), near significant differences were found (F2, 175 = 2.73, *P* = 0.07), with high-resolution approaches yielding marginally significantly stronger d-d relationships than intermediate resolution methods (Tukey HSD; *P* = 0.06), but not low resolution methods (Tukey HSD; *P* = 0.99).

In partial support of our hypothesis that higher sampling depth studies would result in stronger d-d relationships (H5), a weak but significant relationship between (log) sampling depth and Mantel coefficients was detected (Fig. S2; slope = 0.02, *P* < 0.05, adj-*R2* = 0.02). As with our examination of spatial extent, we tested whether this relationship was independent of sampling effort. Sampling depth was not correlated with sampling effort (Pearson’s ρ = 0.03, *P* = 0.64), suggesting that these variables are not confounding. Furthermore, including sampling effort alongside sampling depth as a model covariate had little effect on the fitted relationship between Mantel coefficients and sample coverage (slope = 0.02, *P* < 0.05, adj-*R2* = 0.03).

Finally, we examined whether different community (dis)similarity indices resulted in consistently stronger or weaker Mantel coefficients (H6). The d-d relationships within our dataset featured 16 different similarity metrics, although 80% of these d-d relationships were calculated with only 4 metrics (Bray-Curtis, Unifrac, Sorensen, and Jaccard). In support of our hypothesis (H6), significant differences were detected between types of dissimilarity metrics (Fig. 3A; *F*2, 284 = 5.41, *P* < 0.01). As predicted, Tukey HSD tests showed that d-d relationships based on phylogenetic indices were significantly weaker than those based on quantitative (*P* < 0.01) or qualitative indices (*P* < 0.05). To further characterise differences between similarity indices, we analysed differences between specific indices. Several indices were excluded from this analysis as they had too few occurrences to calculate a reliable estimate of the central tendency (indices with < 4 occurrences were excluded). Again, significant differences were found between similarity indices (*F*14, 271 = 4.96, *P* < 0.001). Tukey HSD tests showed Mantel coefficients from Raup-Crick and Unifrac indices were significantly lower than Bray-Curtis (*P* < 0.01 in each case, Fig. 3A), whilst Sørensen based d-d relationships were higher than Euclidean, Raup-Crick, and Unifrac indices (*P* < 0.01 in all cases, Fig. 3A).

**Discussion**

Two decades of research into the spatial ecology of microbial communities has resulted in a highly variable impression of the microbial distance-decay (d-d) relationship. Our meta-analysis of 287 microbial d-d relationships has revealed two main findings. Firstly, d-d relationships may be influenced by methodological choices, including the sequencing depth used and the type of dissimilarity index. Secondly, as expected, the d-d relationship also appears to be dependent on various aspects of biological context, with different d-d relationships observed between different biomes and spatial scales.

The rapid development of methods in microbial ecology has improved our ability to detect and characterise ecological patterns in microbial communities, with high-throughput sequencing (HTS) platforms able to quantify microbial communities in ever increasing detail (Roesch *et al.* 2007; Caporaso *et al.* 2012). The tremendous sequencing depth of HTS platforms allows them to illuminate the “rare biosphere” (Caporaso et al., 2012), thus elevating them over other approaches such as “fingerprinting” which tend to capture a smaller proportion of the community. Initially, our results suggested that HTS-based approaches yielded similar strength d-d relationships to lower-resolution methods, such as fingerprinting and lower throughput methods, such as Sanger sequencing, suggesting that the massive sequencing depths offered by HTS platforms are not necessary to capture these ecological patterns (van Dorst *et al.* 2014). However, when we examined only statistically significant d-d relationships, the relationships derived from HTS approaches were stronger than other approaches. The ability of different methods to alter the strength of the d-d relationship is expected for two reasons. Firstly, fingerprinting and HTS approaches capture microbial diversity at different taxonomic resolutions. Comparative approaches have shown that fingerprinting approaches such as ARISA may be comparable to HTS data at the phylum level for instance (Gobet *et al.* 2014). Fingerprinting methods are therefore limited in that they may not detect compositional differences between communities at increasingly fine taxonomic resolutions (Ramette & Tiedje 2007; Bissett *et al.* 2010). This may weaken the d-d relationship in instances where communities are similar at the family level, but dissimilar at finer taxonomic levels. Secondly, fingerprinting methods are less able to sample from the “rare biosphere”, unlike HTS approaches. This is significant as, microbial communities often follow an occupancy-abundance relationship in which the most common organisms are also the most widespread, and the rarer organisms are the most restricted (Soininen & Heino 2005; Liu *et al.* 2015). Therefore, sampling only the most common, widespread organisms would flatten the d-d relationship by making communities appear artificially similar in composition. This is in contrast to recent studies which show that spatial turnover in communities is adequately reflected by “common species” (Heino & Soininen 2010). However, microbial communities are often enormously diverse and exhibit extremely “long tailed” species abundance distributions, such that the vast majority of microbial species in a community are “rare” (Hong *et al.* 2006; Galand *et al.* 2009; Locey & Lennon 2016). Therefore, it is likely that in microbial communities, common species alone may not adequately reflect patterns in spatial turnover (Galand *et al.* 2009).

Another methodological choice that was found to influence the strength of the microbial d-d relationship is the choice of dissimilarity index. Dissimilarity indices can vary in the type of data they consider (quantitative vs qualitative), the type of distance they quantify (compositional vs. phylogenetic), and the weight they place on common, rare, or absent species (Anderson *et al.* 2011). Within our study, we found significant differences in the d-d relationship between different indices, and between different index types. In particular, d-d relationship using phylogenetic indices were significantly flatter than compositional indices, whereas there was no difference between binary (presence/absence) and abundance based indices. Phylogenetic dissimilarity metrics may result in lower Mantel correlation coefficients for the same reason that fingerprinting methods do; because communities predominantly differ at fine taxonomic resolutions. This means that whilst communities differ in exact species or OTU composition, they can still be phylogenetically closely related, as communities may be highly similar at higher taxonomic ranks. In contrast, community composition metrics give no weight to how related communities are at broader taxonomic levels. The result of this is that communities appear more similar when phylogenetic indices are used (Bryant et al., 2008), potentially resulting in flatter d-d relationships (and therefore lower Mantel coefficients). This effect might be exacerbated when all sampled communities are from environmentally similar sites, which select for particular taxonomic groups. For example, extremophilic habitats such as solar salterns, can be hihgly similar at broad taxonomic levels, yet distinct at the OTU/species level (Zhaxybayeva *et al.* 2013; Clark et al., 2017).

Surprisingly, no difference was observed between quantitative and qualitative dissimilarity indices. This suggests that qualitative compositional differences between communities drive d-d relationships rather than quantitative changes in species composition and abundance. In agreement with previous studies that have applied both binary and abundance based indices, these two measures of community similarity are likely to be highly correlated (Martiny *et al.* 2011), and result in similar estimations of d-d relationships (e.g. Green *et al*. 2004, Glassman *et al*. 2015). This analysis also revealed that classic dissimilarity metrics, such as Bray-Curtis or Jaccard's index, are overwhelmingly the most frequently used in studies of microbial d-d relationships. These indices are undoubtedly amongst the most frequently used, not only in microbial ecology, but also more widely in ecology. I to draw attention to several contemporary indices that may better suit the types of questions microbial ecologists ask. Classic metrics do not take into consideration co-occurrence information present within the data. To this end, a new family of metrics have been defined that account for species co-occurrence as well as shared taxa (Schmidt *et al.* 2017). Additionally, many indices rely on equal sample sizes, and are sensitive to differences in species richness (Green & Bohannan 2006), with potentially confounding effects on d-d relationships (Baselga 2007). Chao *et al*. (2005) therefore extended classic indices such as Jaccard and Sørensen to account for unobserved species, and to make them less sensitive to variable sample sizes, reducing the need for post-sequencing normalisation of sample sizes (McMurdie & Holmes 2014). Finally, many indices are known to merge true compositional turnover (replacement of species) and nestedness (whereby communities are subsets of one another). To combat this, modified versions of classic indices such as Jaccard, Sorensen, and Bray-Curtis have been developed, allowing the partitioning of community similarity metrics into their turnover and nestedness components. This should enable a more mechanistic understanding of the processes behind d-d relationships (Baselga 2010, 2013; Podani & Schmera 2011). I echo the call of Green and Bohanan (2006) for microbial ecologists to exercise more care in their choice of dissimilarity metrics, especially now that many are implemented in popular and freely accessible analysis software, such as R (e.g. Baselga and Orme 2012).

Whilst significant differences were found between different methodological approaches, we also found differences relating to the biological context of each study. Against our expectation, soil based studies had weaker d-d relationships than studies using other environmental materials. Soils are relatively stable habitats, in that they maintain physical structure and are therefore capable of maintaining significant environmental gradients over relatively small spatial scales. Therefore, we expected the combination of high habitat heterogeneity coupled with limited opportunity for dispersal to result in stronger d-d relationships than for example, oceanic waters, where physicochemical gradients are more diffuse. It is possible that the environmental gradients present in soils do not change linearly over geographic distance, for example if the similar environmental conditions are patchily distributed. Alternatively, soil microorganisms may be able to disperse more effectively than previously thought, perhaps via association with other soil organisms (Warmink *et al.* 2011), migratory species such as birds (Bisson et al., 2007), wind blown soil particles (Kellogg & Griffin 2006; Favet *et al.* 2013), or via bioaerosols (Joung *et al.* 2017).

Originally, we expected that studies of aquatic microbial communities may show the weakest d-d relationships as riverine or oceanic hydrology may provide an effective dispersal mechanism, thus homogenising microbial communities and presenting more diffuse environmental gradients over larger spatial scales. Contrarily, we found that aquatic communities actually showed stronger d-d relationships indicating increased spatial turnover in aquatic microbial communities. Soininen *et al*. (2007) recorded similar distance-decay rates between terrestrial, marine and aquatic ecosystems, showing that biome-dependent d-d relationships may be a feature of microbial communities. Host-associated communities showed relatively strong, but variable d-d relationships. We suggest that this is caused jointly by the ecology of the host species, in combination with the degree of host specificity with the associated microbial community. For example, if the host is not dispersal limited, and associates with a large variety of microorganisms, then the d-d relationship may be relatively flat. However, if the host is dispersal limited, and associates with a very specific microbiome, the d-d relationship might be steeper. To develop our understanding of the macroecology of host-associated microbial communities, an interesting approach would be to compare microbial d-d relationships of sessile and motile hosts (motile host-associated d-d relationships were excluded in this analysis), as incorporating the ecology of the host (e.g. movement, interactions, range size) would likely provide further explanatory power.

Finally, we also found a relationship between the strength of the d-d relationship and the spatial scale over which the study was conducted. Scale-dependent d-d relationships have previously been reported (Bissett *et al.* 2010; Martiny *et al.* 2011; Soininen *et al.* 2011), albeit with contrasting results. Our results are comparable to those of Martiny *et al*. (2011) and Soininen *et al*. (2011) who reported that d-d relationships for various microbial communities were generally steeper as greater spatial scales were incorporated. The scale dependence of this relationship may be explained by greater environmental heterogeneity in large scale studies, thus communities are subjected to different environmental filters, resulting in more dissimilar communities. In combination with this, communities separated by very large geographic distances should have minimal dispersal between them, assuming connectivity is linearly related to geographic distance. Alternatively, this observation may be a statistical artefact, caused by studies with very large spatial extents incorporating many zero similarity community comparisons (i.e. communities with no species in common), therefore biasing our quantification of the d-d relationship (Millar *et al.* 2011; Steinbauer *et al.* 2012). This point highlights that careful consideration is required in the statistical analysis of d-d relationships, especially when incorporating large geographic extents or highly dissimilar communities.

Despite its common use in the literature as evidence for neutral processes in microbial ecology, the d-d relationship alone does not provide evidence for neutral processes acting on microbial communities. As discussed previously, d-d relationships can arise from spatially autocorrelated environmental gradients as well as dispersal limitation (Nekola & White 1999). Furthermore, dispersal limitation itself is not solely a property of ecological neutrality. Dispersal limitation may be stochastic as predicted by neutral theory (Chave 2004), but also by asymmetric dispersal abilities between organisms (Salomon *et al.* 2010; Liu & Zhou 2011), thus violating the central tenet of neutral theory; that organisms are ecologically equivalent (Hubbell 2001). Thus we suggest caution in attributing distance-decay relationships to either niche or neutral processes without further evidence, for example from examining species-abundance distributions (e.g. Dumbrell *et al*. 2010). However, this is not to say that examining distance-decay relationships is futile as the relationship jointly reflects species turnover due to historical, environmental, and spatial factors, all of which are important factors to consider in studying biodiversity (Nekola & White 1999).

Moving beyond distance-decay relationships, focussing on other factors that influence the compositional similarity of microbial communities should provide interesting results. For example, quantifying the extent to which microorganisms differ in their dispersal abilities, and what traits are responsible for these differences may help to provide information on the biogeography of microorganisms at the population level, and given appropriate statistical approaches may allow us to predict the range size and habitat occupancy of different microbes. Furthermore, it is commonly assumed that the connectivity between communities is linearly related to the spatial distance between communities. However, given that different dispersal vectors may disperse microorganisms over differing geographic distances, this assumption may not be valid. Therefore, the growing movement towards examining the role of connectivity *per se* *(*Declerck et al. 2013; Vannette et al. 2016), rather than using geographical distance as a proxy, will likely provide a fruitful direction for spatial microbial ecology. By modeling the dispersal process itself and accounting connectivity, a more mechanistic understanding of the spatial ecology of microbial communities could be gained.

**References**

Anderson MJ, Crist TO, Chase JM *et al.* (2011) Navigating the multiple meanings of β diversity: A roadmap for the practicing ecologist. *Ecology Letters*, **14**, 19–28.

Baas Becking LGM (1934) *Geobiologie of inleiding tot de milieukunde*. W.P. Van Stockum & Zoon, The Hague, Netherlands.

Barreto DP, Conrad R, Klose M, Claus P, Enrich-Prast A (2014) Distance-decay and taxa-area relationships for bacteria, archaea and methanogenic archaea in a tropical lake sediment (J Waldenström, Ed,). *PLoS ONE*, **9**, e110128.

Baselga A (2007) Disentangling Distance Decay of Similarity from Richness Gradients: Response to Soininen *et al*. 2007. *Ecography*, **30**, 838–841.

Baselga A (2010) Partitioning the turnover and nestedness components of beta diversity. *Global Ecology and Biogeography*, **19**, 134–143.

Baselga A (2012) The relationship between species replacement, dissimilarity derived from nestedness, and nestedness. *Global Ecology and Biogeography*, **21**, 1223–1232.

Baselga A (2013) Separating the two components of abundance-based dissimilarity: Balanced changes in abundance vs. abundance. *Methods in Ecology and Evolution*, **4**, 552–557.

Baselga A, Orme CDL (2012) betapart: an R package for the study of beta diversity. *Methods in Ecology and Evolution*, **3**, 808–812.

Beck J, Holloway JD, Schwanghart W (2013) Undersampling and the measurement of beta diversity. *Methods in Ecology and Evolution*, **4**, 370–382.

Bissett A, Richardson AE, Baker G, Wakelin S, Thrall PH (2010) Life history determines biogeographical patterns of soil bacterial communities over multiple spatial scales. *Molecular Ecology*, **19**, 4315–4327.

Bisson IA, Marra PP, Burtt EH, Sikaroodi M, Gillevet PM (2007) A molecular comparison of plumage and soil bacteria across biogeographic, ecological, and taxonomic scales. *Microbial Ecology*, **54**, 65-81.

Bryant JA, Lamanna C, Morlon H, Kerkhoff AJ, Enquist BJ, Green JL (2008) Microbes on mountainsides: contrasting elevational patterns of bacterial and plant diversity. *Proceedings of the National Academy of Sciences USA*, **105** (Supplement 1), 11505-11511.

Caporaso JG, Lauber CL, Walters W a *et al.* (2012) Ultra-high-throughput microbial community analysis on the Illumina HiSeq and MiSeq platforms. *The ISME Journal*, **6**, 1621–1624.

Chao A, Chazdon RL, Colwell RK, Shen T-J (2005) A new statistical approach for assessing similarity of species composition with incidence and abundance data. *Ecology Letters*, **8**, 148–159.

Chave J (2004) Neutral theory and community ecology. *Ecology Letters*, **7**, 1–39.

Clark DR, Mathieu M, Mourot L, Dufossé L, Underwood JC, Dumbrell AJ, McGenity TJ (2017) Biogeography at the Limits of Life: Do Extremophilic Microbial Communities Show Biogeographic Regionalisation? *Global Ecology and Biogeography,* In press.

Condit R, Pitman N, Leigh EG *et al.* (2002) Beta-diversity in tropical forest trees. *Science*, **295**, 666–669.

Declerck SAJ, Winter C, Shurin JB, Suttle CA, Matthews B (2013) Effects of patch connectivity and heterogeneity on metacommunity structure of planktonic bacteria and viruses. *The ISME Journal*, **7**, 533–42.

van Dorst J, Bissett A, Palmer AS *et al.* (2014) Community fingerprinting in a sequencing world. *FEMS Microbiology Ecology*, **89**, 316–330.

Dumbrell AJ, Nelson M, Helgason T, Dytham C, Fitter AH (2010) Relative roles of niche and neutral processes in structuring a soil microbial community. *The ISME Journal*, **4**, 337–345.

Favet J, Lapanje A, Giongo A *et al.* (2013) Microbial hitchhikers on intercontinental dust: catching a lift in Chad. *The ISME Journal*, **7**, 850–867.

Finlay BJ (2002) Global dispersal of free-living microbial eukaryote species. *Science*, **296**, 1061–1063.

Finlay BJ, Fenchel T (2004) Cosmopolitan Metapopulations of Free-Living Microbial Eukaryotes. *Protist*, **155**, 237–244.

Galand PE, Casamayor EO, Kirchman DL, Lovejoy C (2009) Ecology of the rare microbial biosphere of the Arctic Ocean. *Proceedings of the National Academy of Sciences USA*, **106**, 22427–22432.

Glassman SI, Peay KG, Talbot JM *et al.* (2015) A continental view of pine-associated ectomycorrhizal fungal spore banks: A quiescent functional guild with a strong biogeographic pattern. *New Phytologist*, **205**, 1619–1631.

Glenn TC (2011) Field guide to next-generation DNA sequencers. *Molecular Ecology Resources*, **11**, 759–769.

Gobet A, Boetius A, Ramette A (2014) Ecological coherence of diversity patterns derived from classical fingerprinting and Next Generation Sequencing techniques. *Environmental Microbiology*, **16**, 2672–2681.

Green J, Bohannan BJM (2006) Spatial scaling of microbial biodiversity. *Trends in Ecology and Evolution*, **21**, 501–507.

Green JL, Holmes AJ, Westoby M *et al.* (2004) Spatial scaling of microbial eukaryote diversity. *Nature*, **432**, 747–750.

Harrison F (2011) Getting started with meta‐analysis. *Methods in Ecology and Evolution*, **2**, 1-10.

Hazard C, Gosling P, van der Gast CJ *et al.* (2013) The role of local environment and geographical distance in determining community composition of arbuscular mycorrhizal fungi at the landscape scale. *The ISME Journal*, **7**, 498–508.

Heino J, Soininen J (2010) Are common species sufficient in describing turnover in aquatic metacommunities along environmental and spatial gradients? *Limnology and Oceanography*, **55**, 2397–2402.

Holt RD (2009) Bringing the Hutchinsonian niche into the 21st century: ecological and evolutionary perspectives. *Proceedings of the National Academy of Sciences USA*, **106** (Supplement 2), 19659-19665.

Hong SH, Bunge J, Jeon SO, Epstein SS (2006) Predicting microbial species richness. *Proceedings of the National Academy of Sciences USA*, **103**, 117–122.

Hubbell SP (2001) *The Unified Neutral Theory of Biodiversity and Biogeography*. Princeton University Press. Princeton, NJ, USA.

Joung YS, Ge Z, Buie CR (2017) Bioaerosol generation by raindrops on soil. *Nature Communications*, **8**, 14668.

Kellogg CA, Griffin DW (2006) Aerobiology and the global transport of desert dust. *Trends in Ecology and Evolution*, **21**, 638–644.

Kivlin SN, Winston GC, Goulden ML, Treseder KK (2014) Environmental filtering affects soil fungal community composition more than dispersal limitation at regional scales. *Fungal Ecology*, **12**, 14–25.

Lajeunesse MJ (2016) Facilitating systematic reviews, data extraction and meta-analysis with the metagear package for R. *Methods in Ecology and Evolution*, **7**, 323–330.

Legendre P, Fortin M-J, Borcard D (2015) Should the Mantel test be used in spatial analysis?. *Methods in Ecology and Evolution*, **6**, 1239–1247.

Lisboa FJG, Peres-Neto PR, Chaer GM, da Conceição Jesus E, Mitchell RJ, Chapman SJ, Berbara RLL (2014) Much beyond mantel: bringing procrustes association metric to the plant and soil ecologist’s toolbox. *PloS ONE*, ***9***, e101238.

Liu L, Yang J, Yu Z, Wilkinson DM (2015) The biogeography of abundant and rare bacterioplankton in lakes and reservoirs of China. *The ISME Journal*, **9**, 2068–2077.

Liu J, Zhou S (2011) Asymmetry in species regional dispersal ability and the neutral theory. *PLoS ONE*, **6**, e24128.

Locey KJ, Lennon JT (2016) Scaling laws predict global microbial diversity. *Proceedings of the National Academy of Sciences USA*, 201521291.

Martiny JBH, Eisen JA, Penn K, Allison SD, Horner-Devine MC (2011) Drivers of bacterial β-diversity depend on spatial scale. *Proceedings of the National Academy of Sciences USA*, **108**, 7850–7854.

McMurdie PJ, Holmes S (2014) Waste not, want not: why rarefying microbiome data is inadmissible. *PLoS Computational Biology*, **10**, e1003531.

Millar RB, Anderson MJ, Tolimieri N (2011) Much ado about nothings: Using zero similarity points in distance-decay curves. *Ecology*, **92**, 1717–1722.

Muyzer G (1999) DGGE/TGGE a method for identifying genes from natural ecosystems. *Current Opinion in Microbiology*, **2**, 317–322.

Nekola JC, White PS (1999) The distance decay of similarity in biogeography and ecology. *Journal of Biogeography*, **26**, 867–878.

Podani J, Schmera D (2011) A new conceptual and methodological framework for exploring and explaining pattern in presence - absence data. *Oikos*, **120**, 1625–1638.

R Developement Core Team (2016) R: A Language and Environment for Statistical Computing. *R Foundation for Statistical Computing, Vienna, Austria*.

Ramette A (2007) Multivariate analyses in microbial ecology. *FEMS Microbiology Ecology*, **62**, 142-160.

Ramette A, Tiedje JM (2007) Biogeography: an emerging cornerstone for understanding prokaryotic diversity, ecology, and evolution. *Microbial Ecology*, **53**, 197–207.

Roesch LFW, Fulthorpe RR, Riva A *et al.* (2007) Pyrosequencing enumerates and contrasts soil microbial diversity. *The ISME Journal*, **1**, 283–90.

Salomon Y, Connolly SR, Bode L (2010) Effects of asymmetric dispersal on the coexistence of competing species. *Ecology Letters*, **13**, 432–441.

Shmida AVI, Wilson MV (1985) Biological determinants of species diversity. *Journal of Biogeography*, **12** 1-20.

Schmidt TSB, Matias Rodrigues JF, von Mering C (2017) A family of interaction-adjusted indices of community similarity. *The ISME Journal*, **11**, 791–807.

Sogin ML, Morrison HG, Huber JA *et al.* (2006) Microbial diversity in the deep sea and the underexplored “rare biosphere.” *Proceedings of the National Academy of Sciences USA*, **103**, 12115–12120.

Soininen J, Heino J (2005) Relationships between local population persistence, local abundance and regional occupancy of species: Distribution patterns of diatoms in boreal streams. *Journal of Biogeography*, **32**, 1971–1978.

Soininen J, Korhonen JJ, Karhu J, Vetterli A (2011) Disentangling the spatial patterns in community composition of prokaryotic and eukaryotic lake plankton. *Limnology and Oceanography*, **56**, 508–520.

Soininen J, Korhonen JJ, Luoto M (2013) Stochastic species distributions are driven by organism size. *Ecology*, **94**, 660–670.

Soininen J, McDonald R, Hillebrand H (2007) The distance decay of similarity in ecological communities. *Ecography*, **30**, 3–12.

Steinbauer MJ, Dolos K, Reineking B, Beierkuhnlein C (2012) Current measures for distance decay in similarity of species composition are influenced by study extent and grain size. *Global Ecology and Biogeography*, **21**, 1203–1212.

Vannette RL, Leopold DR, Fukami T (2016) Forest area and connectivity influence root-associated fungal communities in a fragmented landscape. *Ecology*, **97**, 2374–2383.

Warmink JA, Nazir R, Corten B, van Elsas . JD (2011) Hitchhikers on the fungal highway: The helper effect for bacterial migration via fungal hyphae. *Soil Biology and Biochemistry*, **43**, 760–765.

Wilkinson DM, Koumoutsaris S, Mitchell EAD, Bey I (2012) Modelling the effect of size on the aerial dispersal of microorganisms. *Journal of Biogeography*, **39**, 89–97.

Zhaxybayeva O, Stepanauskas R, Mohan NR, Papke RT (2013) Cell sorting analysis of geographically separated hypersaline environments. *Extremophiles*, **17**, 265–275.

**Tables and Boxes**

Table 1. Details of the five Web of Science search terms and, the number of hits. A Web of Science search history file is provided in the Supplementary Material.

|  |  |  |
| --- | --- | --- |
| Search | Search terms | Number of hits |
| 1 | TS = (biogeograph\*) AND TS = (bacteria\* OR archaea\* OR microb\* OR microorganism\*) | 1,872 |
| 2 | TS = (macroecolog\*) AND TS = (bacteria\* OR archaea\* OR microb\* OR microorganism\*) | 85 |
| 3 | TS = ("everything is everywhere") AND TS = (bacteria\* OR archaea\* OR microb\* OR microorganism\*) | 53 |
| 4 | TS = ("geographic distance") AND TS = (bacteria\* OR archaea\* OR microb\* OR microorganism\*) | 133 |
| 5 | TS = ("distance decay") AND TS = (bacteria\* OR archaea\* OR microb\* OR microorganism\*) | 107 |
| \* is used as a wildcard to allow searches to match multiple terms, e.g. microb\* could match “microbiome”, “microbial”, and “microbe” | | |

Box 1. Details of the explanatory variables extracted from each study.

|  |
| --- |
| *Community characterisation method*  This refers to the method used to quantify the species present in their sample and their abundances (if applicable). Each d-d relationship was categorised into either high-throughput sequencing (HTS; Pyrosequencing, Illumina, Ion Torrent, Pac-Bio), community fingerprinting (ARISA, TRFLP, DGGE, PhyloChip), or other (Sanger sequencing, morphological identification).  *Sampling depth*  This refers to the sequencing depth in sequencing based studies, or number of individuals counted in morphological based studies. For sequencing studies, we recorded the number of sequences after rarefaction, or if this was not given, the average number of sequences per sample. As it is hard to quantify the sampling depth of fingerprinting approaches, we recorded these as NA and excluded them from analyses involving sampling depth.  *Sampling effort*  This variable represents the number of individual communities/samples used to formulate the d-d relationship.  *Dissimilarity index*  We recorded the dissimilarity index from which each d-d relationship was calculated. After these had been recorded, we categorised them as abundance based (Bray-Curtis, Horn-Morisita, Euclidean, Hellinger, Theta), binary (Jaccard, Raup-Crick, Sørensen, Simpson, βsim), or phylogenetic (Unifrac, Rao, β-mean nearest taxon distance, β-mean pairwise distance).  *Study taxon*  We categorised d-d relationships into broad taxonomic categories (Archaea, Bacteria, Eukarya, Fungi). If a d-d relationship was based on multiple taxa, then an appropriate category was added as necessary (I.e. bacteria + archaea).  *Scale*  We recorded scale as the maximum distance separating communities (in km). If this was not stated in text or provided in supplementary material (e.g. in a geographic distance matrix), it was calculated from given geographic coordinates, or estimated from the d-d graph itself or from scaled maps, if no coordinates were provided.  *Biome*  We categorised d-d relationships based on their biome (agriculture, air, aquifer, indoor, coral, desert, dune, flower, forest, grassland, ice, lake, marsh, mine, ocean, paddy, river, sediment, sewer, sponge), reflecting the type of environment the communities occupied.  *Environmental material*  This variable represents the type of material that the sampled communities occupied. We categorised d-d relationships as air, host, sediment, soil, or water.  *P value*  As an additional comparison, we also recorded *P -*values for d-d relationships where possible. We recorded unadjusted *P*-values, and here use a global alpha value of 0.05 for simplicity, regardless of multiple tests conducted by each study. |

**Figures**

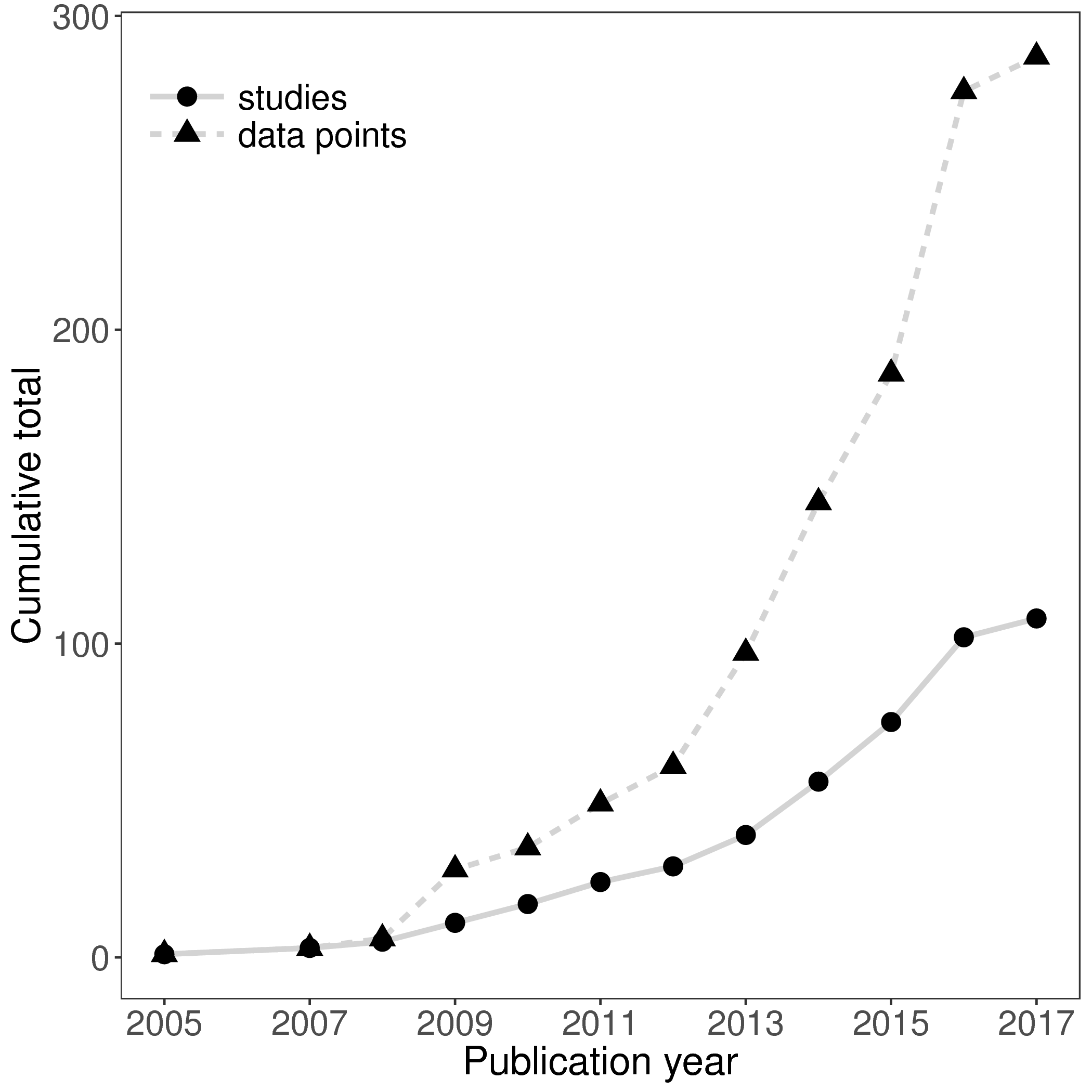
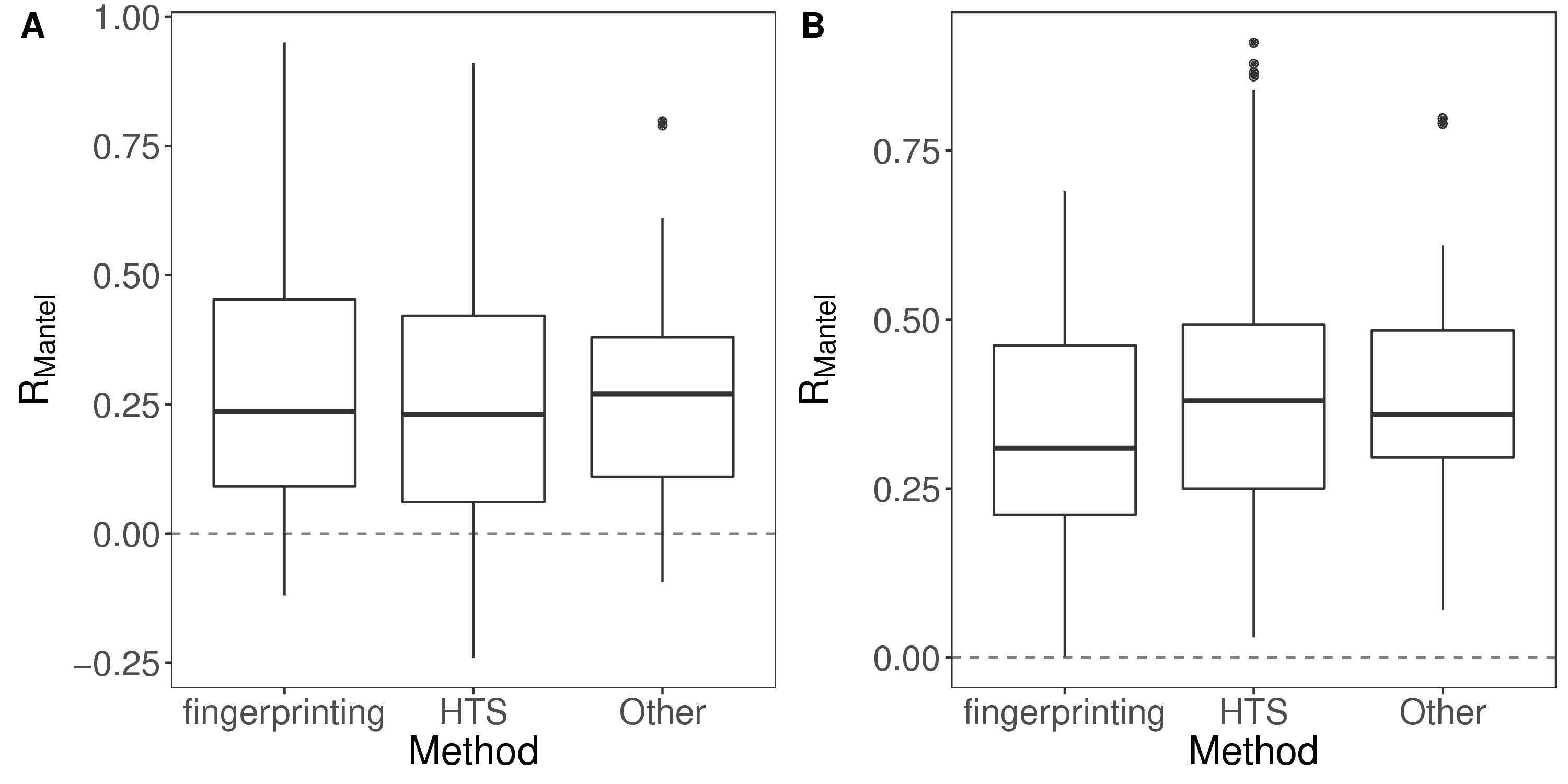


Figure 1. The cumulative number of distance-decay relationships and publications included in this study, through time.

Figure 2. (A) All, and (B) only statistically significant, Mantel correlation coefficients (RMantel) from studies based on high-throughput sequencing (HTS), community fingerprinting approaches (such as DGGE or TRFLP), or other low resolution/throughput methods (morphological identification, Sanger sequencing). Larger Mantel coefficients indicate stronger correlation between community dissimilarity and geographic distance.

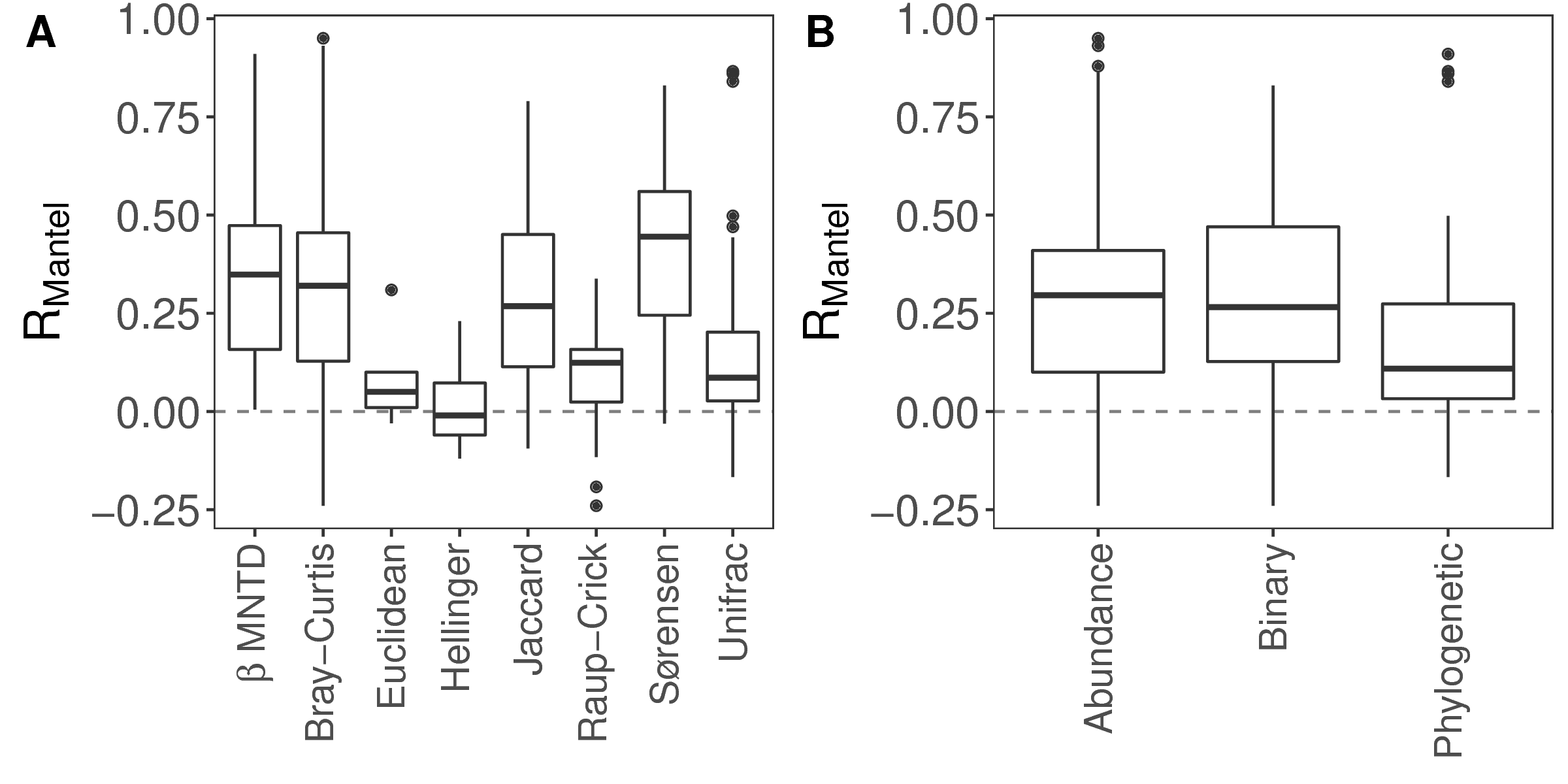
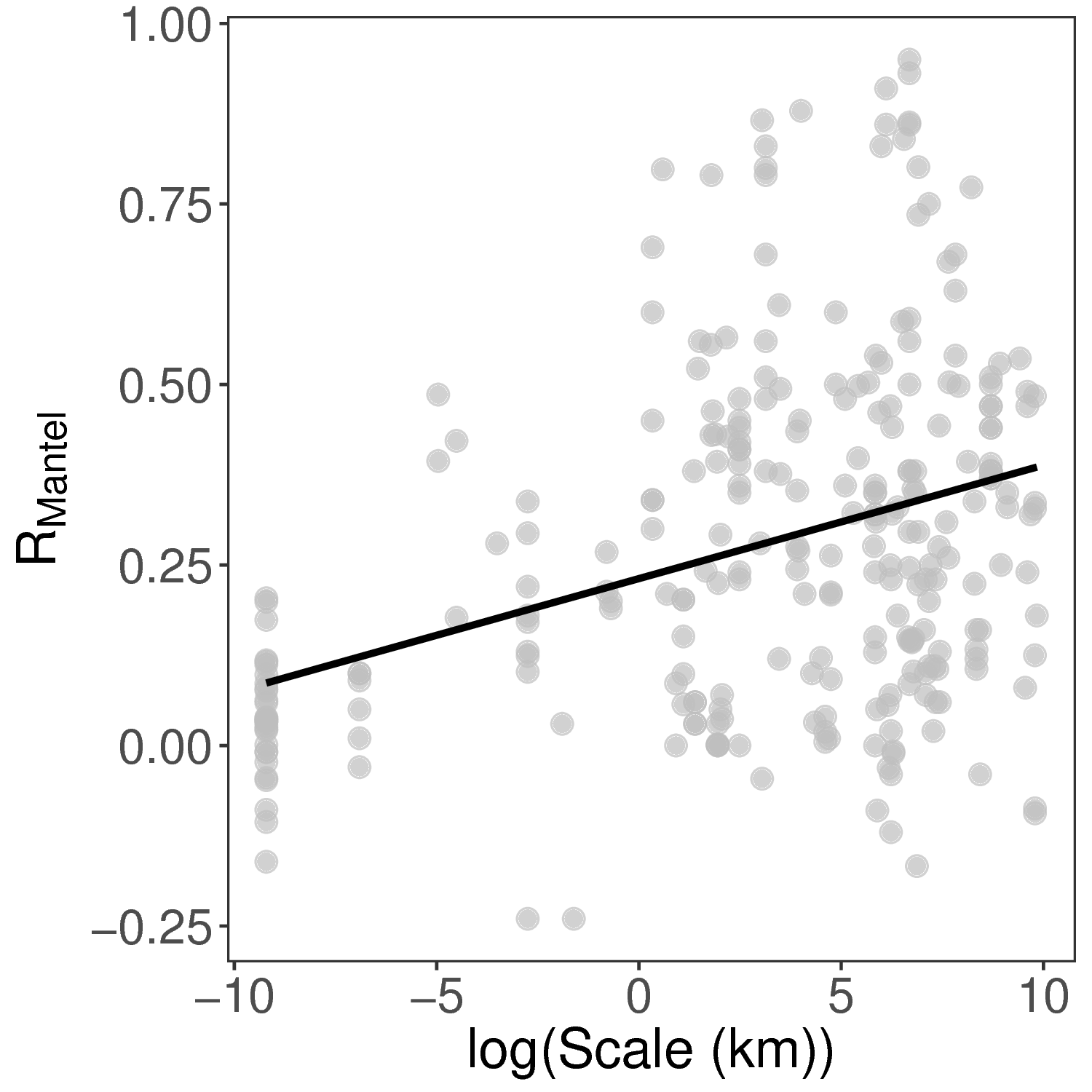


Figure 3. Mantel correlation coefficients from distance-decay relationships based on (A) different dissimilarity indices and, (B) different types of dissimilarity index. Index types reflect the different data requirements and type of distance (e.g. community composition or phylogenetic relatedness). Larger Mantel coefficients indicate stronger correlation between community dissimilarity and geographic distance.

Figure 4. The relationship between Mantel correlation coefficients and the geographic extent over which the distance-decay relationship was measured. The solid line shows the fit of a linear model (slope = 0.016, *P* < 0.001, adj-R2 = 0.12). The positive relationship indicates that larger scale studies tend to record stronger distance-decay relationships.