Full title: Multivariate methods for testing hypotheses of temporal community dynamics

Short title: Testing hypotheses of temporal community dynamics

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ABSTRACT

For biological research to make important contributions towards understanding and managing temporally-variable global change processes, such as responses to land-use and climatic change, we must have effective and comparable ways to quantify and analyse compositional change over time in biological communities. These changes are the sum of local colonisation and extinction events, or changes in the biomass and relative abundance of taxa, within and among samples. We conducted a quantitative review of currently available methods for the analysis of multivariate datasets collected at temporal intervals. This review identified the need for the application of hypothesis-based approaches to understand temporal change in community composition. To address this gap, we: (1) summarise possible temporal patterns in community dynamics and appropriate methods for their analysis; (2) present analytical multivariate approaches to test hypotheses relating to the underlying temporal mechanisms community dynamics; and (3) demonstrate their use and implementation through three fully-worked case-studies, complete with R code for data analysis and visualisation.

KEYWORDS

Beta diversity, biodiversity, community variation, compositional change, multivariate analysis, species turnover, temporal change, temporal variability, time series, zeta diversity

INTRODUCTION

A hallmark of the Anthropocene Epoch is the increasing pace and variance of environmental change[1]. A key task for ecologists is to monitor, quantify, analyse, and predict temporal changes in the structure of biological communities as a function of ongoing changes in climate, land use, and other environmental drivers. Ecological communities comprise populations of multiple taxa. Community structure—the number of taxa (‘richness’) and their relative abundance (‘composition’)—changes through time because of fluctuations of individual populations, and local colonisations and extinctions of taxa [2,3]. These temporal

dynamics are governed by both intrinsic factors, including intraspecific and interspecific interaction, colonisation, and extinction, and extrinsic factors, which are processes that relate to periodic disturbances and directionally changing environmental conditions [4,5]. Understanding the temporal dynamics of community structure can illuminate fundamental ecological processes, including effects of individual life histories on rates of ecosystem change; the relative importance of biotic and abiotic factors in determining community structure; or how species, and the networks in which they are embedded, respond to environmental change [6–8]. Temporal changes in species richness and composition also can provide both simple summaries of ecosystem change, and information that can be translated rapidly into action, either to protect and restore declining species, or to staunch unsustainable growth of unwanted organisms.

Representations of community structure as multivariate datasets that include information on multiple taxa can be more sensitive to changes in explanatory or predictor variables (e.g., pH, temperature, soil type), than univariate summary statistics, such as species richness, diversity, or biomass [2,9], and hence more informative about causal processes. However, quantitative methods for investigating and testing hypotheses about temporal dynamics of multivariate measures of community structure (henceforth ‘community dynamics’) also are more complex than methods used for univariate data [10,11]. Methods for the analysis of spatial multivariate community structure data (e.g., [11,12]) have received far more attention than methods for analysis of temporal multivariate data. Well-known concepts used to describe spatial changes in community structure—grain, extent, nestedness, beta diversity, species turnover—have been transferred to the temporal domain [3,13,14], and some well-developed multivariate methods for large spatial datasets have been used to detect temporal changes in community structure [3]. Although temporal studies of compositional change are commonly undertaken, it is not always clear which analytical methods are

appropriate or available for any given research question, data type, or community structure; indeed, there may not be one ideal method for all situations and a range of methods may be required to answer a given question [12]. Comparisons through time of multivariate ecological data pose unique, non-trivial challenges because these analyses also must account for spatial variation in the data, [2,10,15,16].

In this paper, we summarise recent trends in the use of specific methods to analyse compositional community dynamics, describe the possible hypotheses of compositional dynamics for biological communities, and show which methods are most appropriate for testing particular hypotheses. Further, we describe the key considerations that should be made when testing hypotheses of community dynamics and presenting the results. We illustrate these ideas through three worked case-studies in which we test for: (1) significant temporal community dynamics in a grassland grazing experiment; (2) neutral community dynamics across spatial scales using the eight censuses of the 50-ha forest census plot on Barro Colorado Island, Panama, taken over a 35-year period [17,18]; and (3) seasonal community dynamics of marine bacteria. Code for the software programme 'R' [19] is provided to allow readers to implement our recommendations more easily (S1 to S3 Code – R code files: one file for each case study). Our goal is to contribute to the use of more consistent and quantitative methods across data types and study systems and promote the appropriate use of hypothesis testing in community dynamics research.

LITERATURE REVIEW

DATA COLLECTION AND ANALYSIS

We reviewed and described the multivariate analyses of community dynamics in studies published up until September 2017 to provide an overview of available methods, their benefits and drawbacks, and what they can reveal about community dynamics. Specifically, we asked: (1) are data from different taxonomic groups analysed in particular (or different)

ways; and (2) have combinations of organisms, habitats, and time-scales been investigated more or less comprehensively over the past three decades?

We searched the literature for studies investigating changes in ecological communities over time. We searched the ISI Web of Science Core Collection on 5 September 2017 for articles using the following search statement: [“temporal” AND “composition*” AND “communit*” AND ("community dynamic*" OR "temporal dynamic*" OR "temporal community varia*" OR "community change" OR "compositional change")]. We refined this search to include only document types listed as ‘articles’ and published in ‘English’. This search yielded 692 articles for review. Of these, we excluded 241 papers that lacked community data (i.e. those that included fewer than three taxa), had no temporal replication or pooled all temporal data, used only artificial communities or simulated datasets, or used space-for-time substitution (e.g., using chronosequences) to infer community ages. Although we did not constrain the search window, our search terms only returned papers after 1990, indicating that the specific terms we used were not part of the compositional change literature prior to the 1990s.

We extracted and collated key attributes relating to each study in the remaining 451 papers from the ISI database (e.g., publication year, number of citations). Specific study attributes were determined from the body text of each manuscript, including information on (i) habitat type (e.g., estuarine); (ii) habitat location (e.g., U.S.A.); (iii) taxonomic identity (e.g., vertebrate; fish); (iv) manipulation (e.g., natural variation, experimental resource manipulation); (v) spatial extent (e.g., local, global); (vi) temporal grain and extent (e.g., minutes, months); (vii) how composition was measured (e.g., presence-absence, relative abundances of species); (viii) how time was included in each analysis (i.e., explicitly as a predictor variable, or where other variables represent time, e.g., climatic factors recorded at different times); and (ix) the key perceived research question (e.g., exploration of yearly

dynamics) (S1 Table). We categorised organisms being studied into the following taxonomic groups: plants (Kingdom Plantae); vertebrates (Phylum Chordata); invertebrates (Phyla Annelida, Arthropoda, Cnidaria, Ctenophora, Echinodermata, Mollusca, Platyhelminthes, or Porifera); microeukaryotes (algae and Kingdoms Fungi and Protista); prokaryotes (Domains Bacteria and Archaea); and viruses (non-cellular pathogenic organisms). The data types collated and included papers are provided in the supplementary material (S1 Data).

LITERATURE REVIEW RESULTS

Our search identified 451 research papers investigating multivariate community dynamics. Publications in this field have increased following a near monotonic relationship to the present day, from less than five publications per year in the early 1990s to more than 30 per year in the last five years (S1 Fig). Few publications (20 papers; 4%) contained more than one independent dataset; those instances were treated as multiple studies that were analysed as separate data points (increasing our total sample size *n* to 475). The majority of studies (70%) were conducted within North America and Europe (Fig 1). Prokaryotes and invertebrates were studied most frequently (Fig 1); research on plants and vertebrate fauna accounted for less than one-third of all studies of community dynamics. Studies in the southern hemisphere were more likely to be focussed on the analysis of plant or invertebrate data, whilst prokaryote community data were more common in the northern hemisphere.

Studies spanned a broad range of habitat types, taxa, spatial, and temporal scales (S2 Fig). There were two-to-three times more studies at small spatial extents (at micro, point sample, and local scales) across the 14 habitat categories identified from the articles, and the most frequently-sampled (73%) habitats, common to both scales, were freshwater (119 studies), marine (93 studies), grassland (64 studies), and forest habitats (54 studies) (S2 Fig A). At small spatial extents, there also were more studies in artificial, estuarine, and intertidal habitats.

Across all communities, the most common temporal extent (i.e., the time between the collection of the first and last samples) of individual studies was years, and only a minority of studies on plant and vertebrate communities extended over a period of one decade or longer (S2 Fig B). Smaller organisms were often measured at finer temporal resolutions (grain) than larger organisms, and many studies on short-lived prokaryote and viral communities lasted more than one year. The temporal grain of sampling generally was smaller for communities of microorganisms than for communities of vertebrate animals and plants, for which the most common grain for repeated sample collection was years rather than months or seasons (S2 Fig B). Across research aims and for most methods, most time series had fewer than 15 observations (mean = 14.9, median = 7, mode = 4); the few studies that used network analyses and machine-learning methods used 50 or more observations taken through time.

Across all studies, 1–8 methods were used to analyse community dynamics (Fig 2A); about two-thirds of the studies applied ≤ 3 methods to analyse their datasets; most used two. Detailed descriptions of the types of methods used by reviewed studies can be found in the 'Visualising Temporal Patterns' section, or the supplementary S1 Text (Recommended methods) and S2 Text (methods encountered in the literature review that are not recommended).

Descriptive methods (i.e., visual, non-statistical comparisons) were used most widely (33% of all applied methods), with ordination (22%), raw dissimilarity (18%), time-lag analysis (9%), and cluster analysis (7%) comprising the next most common types of methods; the remaining 12 methods were used infrequently (Fig 2B). Several methods captured by this review were not multivariate, including univariate linear methods, temporal stability (*a.k.a.* coefficient of variation), frequency change, and multiplicative change. Although approaches to analysis of community dynamics have begun to incorporate more computationally complex methods, descriptive and ordination methods have been used most consistently since

1990, along with clustering and time-lag analyses (S3 Fig A). Since 2005, the use of raw dissimilarity methods has increased considerably. In the same decade, a steady stream of alternative methods that incorporate time explicitly have been applied, albeit with poor uptake across the wider literature in this area. These methods often are 'one-off' analyses that have been applied only a handful of times; we refer to these as 'minor' methods (S3 Fig A). Preferred ordination methods changed from detrended correspondence analysis (DCA) and principal components analysis (PCA) in the early 1990s, to canonical correspondence analysis (CCA) in the late 1990s, and, since the early 2000s, to non-metric multidimensional scaling (NMDS) (S3 Fig B).

There was a wide range of motivations for analysing community dynamics; most studies aimed to characterise temporal changes in community composition rather than exploring environmental effects or studying the effects of biotic processes or interactions (S4 Fig). For the vast majority of research aims and analysis types, community dynamics analyses were based on fewer than 25 temporal samples, with many based on 10 samples or less (Fig 3).

CONCLUDING REMARKS FROM LITERATURE REVIEW

Although the number of studies addressing community dynamics have increased dramatically since the 1990s, descriptive methods and ordination have remained the most commonly-used analyses, regardless of the research aim. Three-quarters of all analyses compiled were for observational studies aimed at describing patterns of community change or related to monitoring communities. Researchers frequently used a range of different analytical methods, even within one study, to enable them to understand the complexities of observed temporal changes. Nonetheless, most community dynamics datasets are of short duration and included fewer than 15 temporal observations. The relative rarity of long-term community datasets could be limiting the wider use of more data-intensive methods.

ANALYSING TEMPORAL COMMUNITY DYNAMICS

The two most common sets of methods seen in our review of the temporal community dynamics literature were descriptive and ordination-based methods. Although these techniques are useful for detecting temporal trends and qualitatively exploring patterns in the data, they are used less often for testing hypotheses of patterns in community dynamics. We recommend that researchers combine or replace these analyses with methods that quantify temporal dynamics in species composition in more powerful ways, thus allowing (1) more quantitative and potentially comparable descriptions of spatiotemporal patterns; and (2) explicit hypothesis-testing of mechanisms underlying observed patterns in community dynamics.

Here, we present a sequence of steps that researchers can use to approach an analysis of temporal dynamics (Fig 4). First, before analysis is undertaken, features of the dataset and the study design that will influence the choice of analysis and how results will be presented should be considered. Second, an exploratory data analysis (EDA) should be performed that can give a deeper understanding of the dataset and provide information on the most appropriate analyses. Third, knowledge of the dataset should be combined with testable hypotheses and predictions for patterns of community change to select the appropriate methods of analysis. Finally, aspects of the data analysis workflow should be reported in the paper. The selected analysis may be influenced by the desired results outputs (graphs, significance testing, etc.), so we describe some simple methods for the visualisation of temporal community data that may compliment outputs from the selected analyses.

PRE-ANALYSIS CONSIDERATIONS OF THE DATASET AND STUDY DESIGN

a. Response variable type

A community dataset may be an abundance matrix (number of individuals), relative abundance matrix (e.g., percent cover, sequence abundances), rank-abundance matrix

(ordinal abundance ranks), or a species-occurrence (presence-absence) matrix. The form of the response variables collected can influence the choice of analysis methods, such as which distance measure to use to calculate pairwise community dissimilarity values for samples (see below). It may be appropriate to use both presence-absence and relative abundance data to calculate multivariate comparisons of species composition in space and time (e.g., [20]). Comparing results based on presence-absence data with those generated using relative abundance data allows us to evaluate the role of taxon abundances in generating temporal patterns. Estimating abundances of each population within the community has its own important considerations, i.e., variation in species' detection probabilities, which have been addressed elsewhere (e.g., animals [21]; metagenomics data [22]).

b. Dataset structure

The degree of spatial and temporal replication is important in determining the method of analysis because few sites with many measurements over time would be analysed differently from many sites measured only a few times. The number of independent replicates in space and time should be identified and reported (see [23] for guidance on statistical independence).

If the data have a nested structure, e.g., samples are nested within sites, this will need to be accounted for in the analysis, particularly if one wants to report significance values. For example, if we recorded plant species composition in quadrats that were nested within transects that were nested within sites, then quadrats are not fully independent replicates. This can be accounted for in, for example, a constrained ordination analysis of plant quadrat composition by including 'site' as an explanatory variable in the constrained analysis [24,25]. Similarly, information from technical replicates (such as PCR products) could be kept separate at this stage because they provide useful information about variability at different

scales in the dataset. It is inadvisable to average or pool data until the significant sources of variation are understood.

c. Missing data

Whilst long-term data and monitoring are highly valued (e.g., [6]), it is unfortunately common for long-term datasets to contain missing values; these values should be denoted as missing (i.e., 'NA' in R) and not given zero or other numerical values. The importance of missing values and how to deal with them during data analysis will depend on the number of data points that are missing and how they are distributed across the dataset. Many distance measures cannot be computed if there are empty samples or missing values. Deleting entire rows or estimating missing data so that the dataset can be analysed may be an option in some cases [26]. However, other measures, such as the Gower coefficient, can be calculated while ignoring comparisons among samples with missing data [11; coefficient S15: 278].

EXPLORATORY DATA ANALYSIS (EDA)

As with any data analysis, the first step in the analysis of temporal community dynamics is an exploratory data analysis (EDA) phase [27]. This generates basic information that can be used to both error-check the data and to gain an understanding of key aspects of the dataset (Table 1). R code is provided in the three worked examples for the relevant EDA steps (S1 to S3 Code).

a. Count the number of taxa, the number of samples and the number of empty samples

The ratio of the number of taxa and samples is an important consideration for some multivariate analyses because some multivariate methods are more susceptible than others to the influence of rare taxa [28] and these may be of more or less interest, depending on the research aim. Some multivariate analyses, e.g., some ordination methods, cannot be applied

to datasets containing samples where no taxa are present [26]. However, empty samples may be important for analyses where we are interested in understanding responses to disturbance and local extinctions. The ratio of the number of explanatory variables to the number of independent replicates is also important for temporal analyses because it affects the degrees of freedom available for statistical testing [29].

b. Examine taxon frequency distributions and response curves

Some multivariate analyses assume particular frequency distributions of taxa (a histogram of the number of individuals, or relative abundance, across samples for each taxon), e.g., CCA assumes that species have optima along those environmental gradients ('unimodal' responses). In this case, our focus is the response of species to time and any other explanatory variables. Examining the shapes of taxon frequency distributions is necessary for determining whether species are responding strongly to environmental gradients in the dataset and whether their distributions conform to analysis assumptions.

c. Determine the ratio of temporal to spatial variation in composition

Where samples were taken both at different times and in different places, we want to determine where in our dataset the variation in composition lies. If the interest is primarily in temporal dynamics, but there is a high degree of spatial variation in the dataset, this may alter the analysis or how results are presented and interpreted [30]. The amount of change in taxonomic composition can be estimated from the differences in sample scores on DCA ordination axes, e.g., the 'gradient length' statistic quantifies the turnover in species composition [28].

d. Determine the optimal dissimilarity measure

'Dissimilarity measures', also called 'distance measures' or 'beta-diversity', are the inverse of 'similarity measures'. Each quantifies the compositional distance between two samples

(pairwise dissimilarity). To ensure that abundance data from different taxa are comparable using dissimilarity, data should be standardised prior to analysis if they are measured on different scales. For example, taxon abundance values within each sample can be divided by the total abundance within the sample, or can be standardised across all samples so that each taxon has a mean of zero and standard deviation of one [31]. Alternatively, a distance measure that incorporates a standardisation can be used, e.g., Hellinger distance [11,13,32]. The commonly used Euclidean distance measure is not usually appropriate for taxon abundance data, which are often characterised by non-linear relationships among taxa, abundance values measured on different scales, or by the 'double-zero problem', which is the idea that samples that are missing the same species are not necessarily more similar [31].

BASING THE ANALYSIS ON PREDICTED PATTERNS OF TEMPORAL DYNAMICS

The results from the EDA are then combined with the research aims to determine appropriate analysis methods to address the research question(s). The predicted nature of the change in species composition is of vital importance for selecting an appropriate analytical method that suits both the structure of the dataset and the research aims. Compositional change may take a variety of forms such as no change, directional change, homogenisation, divergence, synchronicity, turnover in one subset of taxa but not another (e.g., Table 2). Hypotheses of biological processes causing dynamics may be very specific, such as a decrease in certain prey species in response to a predator, or more general, such as a shift towards a grassland community in response to fire disturbance of a woodland or an increase in annual plant species in spring compared to winter. More specific and directional (increases or decreases) hypotheses lead to more powerful tests of compositional change; we strongly encourage researchers to generate such hypotheses *a priori*.

The majority of papers in our review of the literature were focused on characterising the dynamics within their community of interest, often without specifying a clear prediction

for what they expected. In other cases, researchers were interested in the effect of environmental change, such as seasonality or climatic cycles over longer time periods. In many cases, researchers will have some notion of how the community of interest might be changing, and that has driven them to do temporal sampling in the first place [33]. However, many long-term datasets exist because previous researchers began data collection with a rationale and method that was different from the current research questions, and so we are often limited by past sampling designs. It is beneficial to enter the analysis pathway with a clear prediction of the compositional pattern(s) expected and the biological hypotheses that underpin those predictions. These different predictions of pattern will manifest differently in terms of the expected change in species composition and compositional dissimilarity (Table 2).

ANALYSIS CHOICES

To aid researchers in selecting appropriate analysis methods, all recommended methods are fully described in S1 Text. Further, each of the recommended methods has been cross-referenced with (1) which hypotheses it is best suited to test; (2) whether or not it allows the identification of individual taxon effects on, or responses to, temporal change; (3) whether or not explanatory and spatial variables can be explicitly incorporated; (4) the type of statistical testing normally used; and (5) any requirements for the length of the time series (number of temporal samples) (Table 3). R code for each of these recommended analyses is provided for each of the worked case study examples (S1 to S3 Code), except for Moran's Eigenvector Maps (MEMs), which is given in Legendre & Gauthier [3] and Multivariate Autoregressive State Space modelling (MARSS), which is presented in the documentation associated with the R package 'MARSS' [34].

Our literature review revealed that in numerous cases, researchers perform and compare separate analyses on their community matrices from each sample time, e.g., separate

nestedness analyses at each sample time [35]; we do not specifically recommend or address these applications here in any depth because they do not explicitly evaluate community dynamics through time. We also focus our recommendations on methods that are truly multivariate, rather than methods that are applied separately to individual taxon abundances; however, if less than ten taxa are of interest, it may be that hypothesis testing via univariate approaches (e.g., separate linear regressions of taxon abundances) will be adequate for drawing synthetic conclusions about community change. In the supplementary material (S2 Text), we summarises univariate methods that were commonly used but were excluded from our recommendations: univariate linear methods, temporal stability (*a.k.a.* coefficient of variation), frequency change, multiplicative change, and Markov chain modelling. Cluster analysis, network analysis, and machine learning and other data-mining methods were excluded because we believe the methods presented here provide better alternatives, but we nonetheless provide an explanation of these (S2 Text) because they appeared in our literature review.

The selection of analytical method also can be influenced by the kind of output desired (Table 4). For instance, some analyses result in outputs that require more effort to interpret than others, whilst others show differences in species' responses or generate outputs that can be added to a geographic map.

ASSESSING THE STATISTICAL SIGNIFICANCE OF COMPOSITIONAL CHANGE

Most ecologists interested in community dynamics seek a way to determine if the changes that they see over time are greater than, or less than, they would expect by chance alone, i.e., testing for statistical significance. Some of the recommended methods presented here have their own built-in significance tests, often based on randomisations of the community matrix (e.g., canonical correspondence analysis). Commonly-used tests include the Mantel test [11] and permutational multivariate analysis of variance (PERMANOVA) [11,36]. However, as

with most statistical ecology methods, there are important considerations that need to be accounted for when assessing significance. Preserving the Type I error rate (where we infer that an effect is statistically significant, when it really is not), which is usually set at 5%, requires that we avoid repeated testing using the same data. When compositional change is assessed with a series of univariate tests using the same data, such as the change in the abundance of a set of species with changing soil chemistry, it is often recommended that a *P*-value correction, such as the Bonferroni correction, should be applied [11]. Using multivariate statistics, as recommended here, avoids multiple testing caused by treating each taxon independently. This is a significant problem in datasets where the number of taxa is large, which is the norm in high throughput sequencing studies [29].

When distance-based methods are applied to multivariate data resulting in, for example, an ordination diagram, we want to know if the distribution of samples in 'species space' is significantly different from if taxa were randomly distributed among samples and times. Null models, whereby community matrices are randomised under sensible criteria [37–42], can be used to test the significance of any observed change in dissimilarity. The primary consideration in applying any randomisation test is what assumptions are being made about taxon niches and whether they are valid. If we are interested in testing the effects of interactions among taxa on their co-occurrence, but our randomisation procedure (null model) allows taxa to occur in samples where they are unable to occur for other reasons, such as habitat differences, we may falsely detect a difference among samples (an inflated Type I error rate). Thus, if we are to make strong inferences about compositional differences, we must be sure that the differences among the groups of sites that we are testing are the only difference. Similarly, we must be sure that our sample size is high enough to allow us to detect differences among sets of sites for the given level of compositional variability (Type II error rate).

Schaefer, et al. [10] introduced a novel method for detecting significant shifts in community composition using randomisations under null models of taxon mean abundances through time. They demonstrate that this approach, which uses both compositional distance and the coefficient of variation (CV), could detect significant dynamics in community composition, even where only small amounts of change had occurred. In this method, temporal changes in communities are assessed by using a compositional distance measure that is selected by the user. This quantifies the multivariate distance between the randomly generated communities (null model randomisations) and observed 'reference' samples (e.g., 'Time 0' samples, compared to randomisations of 'Time 1' samples). If the observed distance is greater than under randomisation, then we can infer statistically significant temporal change. Because this can be done in an ordination context, increases and decreases of particular taxa driving the change can be determined from the ordination plots. Limitations of this method are that it requires a relatively large degree of temporal change in the data compared to spatial variation (i.e., more temporal, than spatial, variation) and it is relatively computationally intensive; however, we provide the R code for a worked example in the S1 Code supplement.

REPORTING THE ANALYSIS WORKFLOW

There are several aspects of the data analysis workflow that should be reported in papers presenting such analyses (Table 5). By fully reporting these aspects, something that was not consistent for many of the papers we read in the literature review, this ensures that the results are robust, represent the variation deemed important for the research aim and are reproducible. The number of taxa, samples, and replication in space and time all should be clearly reported in the Methods or Results section of the paper. The dissimilarity measure selected, and the rationale and procedures used to arrive at that decision, should be reported in the Methods section of the paper. We encourage users to refer to a specific source, such as

Legendre & Legendre [11], or to give the formula applied to maximise the repeatability and transparency of their research; these indices can be inherent in particular methods or built-in as defaults in software, but nevertheless should still be reported. Different methods of dissimilarity or ordination (or other analysis types) that were compared, e.g., in the EDA phase, should be listed in the Methods section of the paper stating that their results were consistent with each other. The choice of dissimilarity or ordination method is subjective, but rules of thumb can be used for assessing different methods, so that the method selected optimises the reduction of dimensionality in the data and thus maximises the variation accounted for by the analysis [see ,26 for comprehensive advice].

VISUALISING TEMPORAL PATTERNS

Some of the analysis methods that we recommend do not produce simple visual outputs that clearly show temporal trends in community datasets and visualising temporal dynamics can be difficult for species-rich datasets or complex sampling designs. However, there are a variety of useful methods that can be used to complement a hypothesis-driven analysis (Table 6). Many descriptive methods work best where a small group of taxa is measured only a few times. However, there are examples of descriptive methods being used effectively for larger, more complex examples. Pereira et al. [43] used a heat map to show compositional changes of 808 bacterial genera, at a single location, sampled 24 times over two years. Certain descriptive methods are useful for only specific types of temporal patterns, e.g., ‘time cores’ is a method that identifies ‘core’ groups of taxa that remain present over time [44]. Others, such as Venn diagrams and measures of overlap in composition, are useful only for small numbers of temporal comparisons. We recommend and provide R code (S1 Code) for time cores and Venn diagrams because these methods are suitable for assessing hypotheses of taxon replacement while specifically identifying the taxa driving compositional shifts (Tables 3 and 4). Relative abundance distributions (RADs, *a.k.a.* ‘rank abundance curves’), can

provide relatively sophisticated visualisations of community dynamics. RADs are plots of the relative abundances (on the *y*-axis) of all species in a community ranked (on the *x*-axis) from highest to lowest. They are closely related to *k*-dominance curves, in which species ranks are shown on the *x*-axis, but cumulative proportional abundance (dominance) is plotted on the *y*-axis [45]. If samples taken at different times are plotted separately, the set of RADs can illustrate changes in community structure over time. RADs also can be used to explore community dynamics if a dissimilarity measure, such as Bray-Curtis, is calculated for the species' ranks at each time point. The resulting temporal dissimilarity of the RADs can be explored by plotting rank dissimilarity against time lag or by ordinating rank dissimilarities in the same way that spatial compositional dissimilarities are examined. For example, temporal changes in RAD dissimilarity can illustrate changes in species evenness over time as might be expected if compositional homogenisation and a decline in evenness occurs because of invasion by an increasingly dominant species [46].

WORKED CASE STUDY EXAMPLES

Here we provide three worked examples to demonstrate the hypothesis-testing approach we advocate for different types of community dynamics. We used open-access datasets to test for: (1) significant differences in temporal community dynamics among disturbance treatments imposed upon plant communities in experimental grasslands, (2) neutral community dynamics for a fully-censused forest plot, and (3) seasonal (cyclical) temporal dynamics in marine aquatic bacterial communities. We present a brief description of each dataset and describe the results and interpretation for the relevant EDA and hypothesis-testing analyses. More detailed descriptions, explanations and R code for each case study are provided as supporting information (S1 to S3 Code).

TESTING FOR SIGNIFICANT TEMPORAL COMMUNITY DYNAMICS WITHIN A CONTROLLED EXPERIMENT

Porensky et al. [47,48] measured plant species composition annually from 2007 to 2014 in quadrats on 24 transects to which six grazing treatments were applied since 1982 (four transects in each treatment): none (exclosure), light, moderate, heavy, heavy-to-light (lightly grazed since 2007) and heavy-to-none (new exclosure since 2007). They calculated the mean cover in 25 quadrats for each species on transects. We tested the hypothesis that increasing grazing intensity causes increasing divergence (directional change) in species composition relative to the control (no-grazing) treatment.

EDA showed that the 24 transects were dominated across all measurements by ten common species out of a total of 92 species (S1 Code: row and column summaries, relative abundance plots and rank abundance curves). Species turnover through time was high; mostly 30% or greater for time period-treatment combinations (Fig 5). Principal co-ordinates analysis (PCoA) suggested that the main compositional differences corresponded to the grazing treatments, and that transect composition changed over time, but not markedly (S1 Code: PCoA analysis and figure). PERMANOVA showed that transects were significantly compositionally distinct and changed significantly over time in different ways (S1 Code: PERMANOVA outputs - all three coefficients for a two-way PERMANOVA with transect, year and their interaction were significant $P < 0.05$). Partitioning spatial and temporal beta diversity showed that two of the control (exclosure) treatments differed significantly (Fig 6). Principal Response curves showed that the grazed treatments differed from the control and that the relative abundances of four species in particular were influential in driving these differences among treatments, but did not show increasing divergence over time (Fig 7). This result is consistent with the analysis of temporal coherence (icc = -0.04; see S1 Code for detailed description of this statistic), which showed that transects were not synchronous in

their temporal changes (S1 Code: temporal coherence analysis). Thus, we conclude that treatment differences in turnover and shifts in composition were not consistent or directional and so we reject our hypothesis that increasingly grazed transects diverged more over time relative to the control.

TESTING FOR NEUTRAL COMMUNITY DYNAMICS ACROSS SPATIAL SCALES

The 50-ha forest census plot on Barro Colorado Island, Panama, has been censused eight times between 1982 and 2015 [17,18,49, http://ctfs.si.edu/webatlas/datasets/bci/]. This plot was established in part to test for neutral community dynamics [50] by fully censusing all woody stems greater than 1 cm diameter at breast height every five years. Under this hypothesis, we expect to see no directional or other patterns in temporal community dynamics. EDA showed that the dataset consisted of 324 species generating a highly right-skewed abundance distribution (most species were rare) (S2 Code: EDA analyses). A line graph plot of species' abundance values over time showed very little change in total abundance over time (S2 Code: line graph of abundance). Two analyses (S2 Code: zeta diversity and total turnover analyses) that allowed us to test for stability in community composition in time, i.e., change in species composition is lower than expected if individual trees were randomly distributed in time (keeping their spatial positions fixed), showed that change in species composition was lower than expected: (1) Zeta diversity analysis showed that the presence of species across census times was more consistent than expected (Fig 8) and (2) total turnover in species abundances at each of the seven time periods was lower than expected (Fig 9), particularly when the plot data were at larger spatial scales. Thus, we conclude that the observed lack of pattern is consistent with our hypothesis of neutral community dynamics.

TESTING FOR SEASONAL COMMUNITY DYNAMICS

Gilbert et al. [51] measured bacterial taxonomic composition over six years using pyrosequencing of the 16S gene for monthly water samples from a single site off the coast of Plymouth, United Kingdom (source data can be obtained from the journal's online supplementary file). We tested the hypothesis that subsets of the community would show cyclical patterns of temporal change due to seasonality. EDA showed that the dataset consisted of 74 operational taxonomic units (OTUs) across 62 temporal samples. Most taxa were common through time. Proportional turnover between temporal samples varied from 0.04 to 0.48 (S3 Code: turnover calculation), which is relatively low, and was consistent with the detrended correspondence analysis (DCA) result showing a short gradient length of 1.8 for axis 1 (S3 Code: DCA). Similarly, PCoA graphs showed little consistent change within years (Fig 10). However, 13 of the OTUs were deemed to be highly variable in that they changed by more than 500 reads across the sampling period (S3 Code: row and column summaries, relative abundance distributions and abundance plots).

Time-lag analysis showed that although there was a significant increase in dissimilarity within increasing time lag, variability was high, showing that temporal changes did not occur in a simple divergence over time pattern (Fig 11). Temporal synchrony analysis [sensu ,52] showed that some subsets of OTUs in the community were significantly changing in similar ways (Synchrony = 0.27; $P < 0.05$; S3 Code: temporal synchrony analysis). When patterns in these variable taxa were examined through time using zeta diversity analysis [53] (S3 Code: zeta diversity analysis), which is based on the presence of species in samples, winter samples shared significantly more species across sets of temporal samples than autumn, spring and summer (Fig 12; ANOVA, $P < 0.01$). Further analysis of the most variable taxa using the raw dissimilarity values based on abundance data calculated for temporal samples within seasons showed clear cyclical patterns (Fig 13; S3 Code). Thus, we

conclude that the patterns are consistent with our hypothesis of seasonal dynamics in community composition, largely driven by a small subset of OTUs that varied in their abundance, rather than presence, over time.

DISCUSSION

There is an expanding set of analyses that use a wide variety of multivariate and univariate methods to quantitatively address temporal questions about communities that continue to be developed. One of the key reasons appears to be the length of the time series, e.g., if the time series is long enough, methods such as temporal MEMs could be used; however, most datasets captured by our literature had relatively few temporal measurements (mean = 14.9, median = 7, mode = 4), consistent with other reviews (e.g., [54]). In other cases, the ability to implement some methods in temporal community ecology still requires development, such as the application of time-ordered and time-aggregated network analysis for spatiotemporal community datasets [55].

Some analysis types are well established for spatial data, but can be applied easily to temporal data, potentially yielding useful and comparable results for explicit temporal analyses. Further research is required to generate standard extensions and approaches using such methods for temporal data. For example, nestedness (*sensu* [56]) is usually applied to spatial data and the analysis is repeated for different time points; however, there is no reason that we cannot apply this method across times to look at whether sites are becoming more or less nested over time. If younger sites were nested within older sites, this may indicate that sites were becoming more homogeneous over time.

Our understanding of temporal dynamics in biological communities, and the methods we use to investigate them, have improved greatly over the past 25 years, probably because of increases in computational power and in dataset size. We can continue to improve our understanding by moving away from merely describing community changes, to linking these

changes to understanding and testing the processes driving community change and biodiversity; that is, moving from hypothesis-generating methods towards hypothesis-testing methods enabled by well-defined research questions with testable hypotheses that result in clear predictions for observed temporal patterns (e.g., Table 3). Even for exploratory studies of compositional variation through time, we emphasise the benefits of making *a priori* predictions for how we think composition should change through time.

Interactions between community composition and environment or other factors across space and time are complicated. For example, Brown [57] showed that temporal variability in species composition of stream invertebrates was reduced by high spatial heterogeneity, which may be because there are increased niches or refugia in changing environmental conditions. Manipulative experiments are useful, but not always possible, especially for long-lived taxa. Therefore, combining current knowledge of intrinsic and extrinsic factors controlling community change into null models and simulations may be the way forward [4]. Carefully considering sample sizes and appropriate replication of particular factors are also important if we are to properly test hypotheses of species coexistence over time [15]. There is a need for us to make useful predictions about how communities may continue to respond to ecosystem disturbances, such as climate change, invasive species, and habitat fragmentation [6,7]. Many methods commonly used to analyse temporal community dynamics are not quantitative, meaning conclusions can only be narrative descriptions, rather than clear tests of hypotheses. The methods recommended here push us to quantitatively assess dynamics, which naturally leads to clearer hypothesis testing.

There is a wide variety of methods available for analysing temporal dynamics of biological communities; however, many studies using these methods are descriptive and not focussed on testing hypotheses of change. We strongly recommend that researchers generate *a priori* hypotheses of compositional change. These predictions should be used to select

appropriate quantitative data analysis methods, as recommended here. The comprehensive description and hypothesis testing of temporal community patterns requires the use of multiple data analysis methods, but by using standard exploratory data analysis methods followed by testing *a priori* hypotheses, the methods selected can be explicitly focussed on the most appropriate tests for the situation. We hope that this synthesis will allow researchers to make informed decisions about their choice of data analysis for their research question and pave the way for more uniformity in reporting of these analyses and/or innovation in comparability. Our understanding of patterns and drivers of changes in species communities would benefit by having more longer-term and fine-scale studies.

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SUPPORTING INFORMATION

S1 Code. Example R code for plant community dataset

S2 Code. Example R code for BCI dataset

S3 Code. Example R code for marine bacterial community dataset (from high throughput sequencing)

S1 Table. Data attributes collated from the 451 reviewed papers.

S1 Data. Dataset of attributes extracted from papers in the literature review.

S1 Text. Descriptions of the recommended methods.

S2 Text. Descriptions of methods encountered in the literature review that are not included in the main text.

S1 Figure. Number of studies from the literature review published per year.

S2 Figure. Number of studies from the literature review by habitat types, taxa, and spatial and temporal scales.

S3 Figure. The proportion of different methods used through time from the literature review.

S4 Figure. The distribution of different studies from the literature review across different research aims.

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TABLES

Table 1: Recommended steps for exploratory data analysis (EDA) of community data prior to selecting analysis methods for temporal data. R code for the methods is provided in the supplementary materials S1-S3.

Step	Description	Rationale	Methods
1	Count the number of taxa, the number of samples and the number of empty samples	Some ordination methods are influenced by rare taxa; identify empty samples; determine replication for significance testing against explanatory variables	Row and column totals of the sample × taxon matrix. Plot the relative abundance distributions.
2	Examine taxon frequency distributions and response curves	Some ordination methods assume either unimodal or linear pattern of frequency distribution	Plot the abundance of species across time or other explanatory variables.
3	Determine the ratio of temporal to spatial variation in composition	If temporal change is to be detected within different spatial sampling locations, the analysis must adequately tease apart the variation in time and space. Where little spatial variation exists, spatial samples could be averaged at each time.	Mean spatial and temporal turnover should be calculated following Collins et al. [16] to allow a 'global' comparison of the amount of compositional variability among samples from each time and among times [see ,58 for details]; it can be calculated for different subsets of samples or times for which different predictions about temporal dynamics have been made, e.g., different experimental treatments. Ordination approaches, such as DCA [59], can be used to quantify spatiotemporal variation and to identify 'outliers', i.e., samples that are very different to other spatial or temporal replicates.
4	Determine the optimal dissimilarity measure and whether data should be standardised	The dissimilarity measure selected depends on the response variable type (presence data, relative abundance data, etc.) This choice of appropriate distance measure and/ or standardisation determines the degree of emphasis of sample richness,	Possible dissimilarity measures to compare samples should be selected using criteria listed in sources such as Anderson et al. [13], Legendre & Legendre [11] and Legendre & Cáceres [60]. Principal coordinates analysis or non-metric multidimensional scaling (NMDS) of the different

	composition or relative abundances in the resulting distance matrix, and thus may also relate to the research question [13].	dissimilarity matrices can be compared. The distance measure that gives the greatest spread of points in a few dimensions and, therefore better discrimination among the samples, should be selected. If NMDS is used, the stress values associated with the different distance measures can be compared, because lower stress values show that the ecological distances between samples are being better represented in ordination spaces.

Table 2: Testable predictions of patterns in temporal dynamics. These predictions are not all mutually exclusive.

Pattern of change	Prediction of change in community composition over time	Prediction of change in compositional dissimilarity over time
No change or persistence	No change in taxon presence or abundance.	No significant change.
Directional	Certain species are predicted to increase or decrease.	Dissimilarity increases.
Instability	Significant change, but it is not predictable in terms of which taxa increase, decrease or stay the same.	Dissimilarity increases.
Divergence	Taxonomic composition of two or more sites becomes less similar in a predictable way, i.e., a specific hypothesis about directional change.	Dissimilarity increases.
Synchronicity	Different groups of taxa change in similar ways at the same time. These groups may be functional or taxonomic.	Among-taxon-group dissimilarity increases. Within-taxon-group dissimilarity does not change.
Succession	Individual or groups of taxa are replaced in a predictable sequence.	Dissimilarity increases. The magnitude of dissimilarity is high, reflecting species replacement.
Resilience	Significant change, reverting back to the original state.	Dissimilarity initially increases at short temporal scales, but decreases at larger temporal scales.
Perturbation	Significant change that is initially high, but lessens over time.	Dissimilarity initially increases rapidly at short temporal scales, with the increase reducing in magnitude at larger temporal scales.
Cyclical or periodicity	Taxonomic composition diverges and then reconverges on the original composition.	Dissimilarity increases at short temporal scales, but decreases at larger temporal scales, and may alternate between these at increasing temporal scales.

Stablisation	Significant change that gets smaller over time.	Dissimilarity at fine temporal scales becomes lower over time.
Pivot point or threshold	Significant change across one relatively small unit of time.	Dissimilarity is low before and after the ‘pivot point’, but high across it.
Invasion	Increase in the dominance of one taxon, or functional group.	Dissimilarity decreases.
Degradation	Loss of rare species.	Dissimilarity decreases. The change in dissimilarity is large, reflecting species loss.
Homogenisation	Taxonomic composition of a set of samples becomes more similar, often with a concurrent decrease in diversity.	Dissimilarity decreases. The change in dissimilarity is large, reflecting species loss.
Convergence	Taxonomic composition of two or more sites becomes more similar in a predictable way.	Dissimilarity decreases.

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Table 3: Guide to best-suited methods for the different predictions of temporal pattern in community dynamics, based on whether: environmental varibles can be included in the analysis (Env.); the analysis can be made explicitly spatial to account for temporal samples being clustered in different sites by including spatial variables (Spatial); or the role of individual taxa are identified in the analysis (Taxa), and the type(s) of significance testing (Sig.) that are used for the analysis (Rand. = randomisations, Mantel = Mantel test, Reg. = regression statistics, None = significance testing is not normally applied). Note that the null hypothesis for each of these predictions would be that there is no change over time in either taxon presence or abundance. Cell shading indicates the number of temporal samples in the time series for which each analysis method is appropriate (note that some hypotheses require more temporal samples than others): No shading—method applies well to time series of any length; cross-hatched pattern—method only applies well for few measures in a time series (< 5 temporal samples); Light grey—only applies well for ≥ 5 temporal samples; Mid-grey—only applies well for ≥ 10 samples; Darkest grey—only applies for ≥ 20 samples, but many more than 20 spatial and temporal replicates may be required, depending on the number of explanatory variables in the regression model; note that a time series of at least 20 temporal samples are required for calculating Moran's eigenvector maps (MEMs). + Requires a strong gradient in composition that is well represented by the ordination axis scores for samples. ++ Weak inference from species' positions on biplot. # Zeta diversity modelling can be combined with generalised dissimilarity modelling [61] to assess the effect of environmental variables. ## PRC requires a 'baseline' or a 'control' treatment level against which other samples are compared through time. LCBD refers to 'Local Contributions to Beta Diversity'. Refer to the supplementary materials for a detailed description of each method.

	Hypothesis of temporal dynamics								
Method	Directional Instability Divergence Invasion Convergence	Stabilisation Perturbation Resilience Cyclical	Synchronicity	Succession Replacement	Pivot point	Env.	Spatial	Taxa	Sig.
Mean dissimilarity	✓	✓	✓	✓	✓				Rand.
Beta diversity decomposition: LCBD, nestedness, turnover	✓	✓	✓	✓	✓			✓	Rand.
Zeta diversity	✓	✓	✓	✓	✓	#			Rand.
Synchrony			✓						Rand.
Temporal coherence	+		+						Rand.
Multivariate autoregressive state-space modelling	✓	✓	✓		✓	✓	✓	✓	Rand or reg.
Turnover rates	✓			✓	✓				Rand. or reg.

Venn diagrams, overlap and time cores				✓				✓	None
Beta diversity time lag graph or regression	✓	✓	✓	✓	✓				Rand.
Pivot days					✓				Rand.
Mean dissimilarity as response in regression	✓	✓	✓	✓	✓	✓	✓		Reg.
MEMs with db-RDA	✓	✓	✓	✓	✓	✓	✓	✓	Rand.
Constrained ordination	+	+		+		✓	✓	++	Rand.
Ordination site scores in glm or non-linear regression	+	+	+	+		✓	✓	++	Reg.
Principal Response Curves (PRC)##	+	+	+	+		✓	✓	✓	Rand.

Table 4: Recommended analysis methods for temporal change in community composition and a description of their output. R code for all of these methods is given in Appendix S4 with an example dataset apart from the Moran's eigenvector maps (Legendre and Gauthier 2014; Borcard et al. 2011) and MARSS (Holmes et al. 2012), where supplementary code is provided in the key references. LCBD refers to 'Local Contributions to Beta Diversity' and SCBD refers to 'Species Contributions to Beta Diversity'.

Method	Description of results	Key paper(s) using each method
Beta diversity decomposition (nestedness, turnover)	Beta diversity values for nestedness and turnover that can be used in subsequent graphs (e.g., time lag) or analyses of explanatory variables.	Baselga & Orme [62]
Beta diversity decomposition (SCBD, LCBD)	List of taxon relative contributions to the overall compositional variation. Matrix of LCBD values showing the relative contribution of each sample-time combination to the overall compositional variation. Values can be used in subsequent graphs or analyses of explanatory variables.	Legendre & De Cáceres [60]; Legendre & Gauthier [3]; Lamy et al. [63]
Beta or zeta diversity time-lag graph	Graph showing the relationship between temporal diversity and time lag (distance in time). In the case of zeta and multi-site dissimilarity, time-lag graphs are specific to each number of samples in the subset (e.g., zeta order).	Hui & McGeoch [53]; McGeoch et al. [64]
Beta or zeta time lag regression	Regression statistics indicating the significance of the relationship between temporal diversity and time lag (distance in time). In the case of zeta and multi-site dissimilarity, time-lag analyses are specific to each number of samples in the subset (e.g., zeta order).	McGeoch et al. [64]
MARSS	Auto-regressive models quantifying the relative effects of species (or species groups) interactions, environmental covariates, spatial structure, and observation error on relative abundances through time. Effects can also be output as estimated trajectory plots. Options exist to produce metrics of community stability.	Hampton et al. [65]
Mean dissimilarity	Mean dissimilarity values for data subsets. Values can be used in subsequent graphs, analyses or maps.	Collins [66]

Mean dissimilarity as response in regression	Regression statistics indicating the significance of the relationship between mean dissimilarity and explanatory variables, which includes time if dissimilarity is calculated for different spatial samples, or will only include change in non-time explanatory variables, if dissimilarity is calculated across time.	Collins [66]; Barros et al. [67]
Moran's eigenvector maps (MEMs) with db-RDA	MEMs results in a matrix of uncorrelated temporal variables that characterize scales of temporal variation in composition that can be used in the db-RDA. The RDA results in a table of ordination statistics, an RDA ordination diagram, and if paired with variance partitioning, a Venn diagram showing the amount of variance explained by each component.	Legendre & Gauthier [3]
Ordination (constrained)	Ordination diagram and table of statistics (see the text section 'Ordination' for more details and other possible outputs).	Anderson & Willis [68]; http://ecology.msu.montana.edu/labdsv/R/
Ordination site scores in glm or non-linear regression	Regression statistics indicating the significance of the relationship between species composition and explanatory variables.	Day & Buckley [69]
Pivot days	A list of time points are identified at which significant shifts in composition have occurred.	Lellouch et al. [70]
Principal response curves	Principal response curve diagram comparing trajectories in compositional space of different sites or samples over time relative to the baseline or control.	Auber et al. [71]
Synchrony	A single value representing the degree to which taxa are changing in a similar way over time, calculated for a given sample by species matrix across a set of times. Can be compared for different time windows or different subsets of samples (e.g., experimental treatments).	Loreau & Mazancourt [52]; Gouhier & Guichard [72]
Temporal coherence	A single value representing the degree of consistency in temporal change among samples for the dataset or subset considered (see the text section 'Ordination' for more details).	Rusak et al. [73]; Angeler & Johnson [20]

Turnover rates	Percent change in species composition between time points.	Diamond [74]
Venn diagrams, overlap and time cores	Venn diagram showing the number of taxa shared and unshared between two or more subsets of samples. Lists of shared and unshared taxa. Time cores results generate lists of taxa that occur consistently through time for a set of samples.	Vallès et al. [44]; O'Sullivan et al. [75]
Zeta diversity	Zeta diversity values for each zeta order (number of samples in subsets) that can be used in subsequent graphs (e.g., zeta diversity vs. order) or analyses of explanatory variables.	Hui & McGeoch [53]; McGeoch et al. [64]

Table 5: Recommendations for what users of analyses of temporal multivariate data should report regarding sampling design, data types, and the outcome of exploratory data analysis in the different paper sections.

Paper section	What to present
Field or lab methods	Number of spatial locations (or independent samples)
	Number of temporal replicates within each location (or sample)
	Total number of temporal replicates
Data analysis methods	Data type analysed, e.g., presence data, relative abundance, number of individuals
	Distance measures used for each multivariate analysis, preferably citing Legendre & Legendre [11] or similar, so that the exact measures used can be repeated.
	Groupings of taxa used in analyses should be clearly described, e.g., taxonomic families, functional groups.
	Number of taxa, if pre-defined.
Results	Number of taxa, if different from expected.
Supplementary material	R code (or other) and data so that readers can replicate the analyses.

Table 6: Data visualisation methods and considerations for their use in temporal community dynamics studies.

Methods	Description and example references	Considerations
Ordination axis samples scores	Symbols (e.g., [76]), envelopes (e.g., [77]), or trajectories (e.g., [78]) can be added to ordination diagrams to display the relative compositional change among temporal samples.	Useful for many samples and taxa. Ordination sample scores can also be used in other graphs or maps to display spatiotemporal compositional variation.
Relative abundance distributions	Plot the relative abundance of all taxa in the community [45,46,79].	Useful only for small numbers of comparisons.
Venn diagrams	Displays the overlap in species composition among samples (e.g., [75]).	Useful only for small numbers of temporal replicates. Suitable for visualising taxon replacement while specifically identifying the taxa driving compositional shifts.
Time cores	Identify ‘core’ groups of taxa that remain present over time [44].	Suitable for visualising taxon replacement while specifically identifying the taxa driving compositional shifts.
Heat maps	A grid representing spatial and/or temporal samples where cells are coloured by a gradient representing relative abundances, ordination sample scores, or compositional change (e.g., [43,80]).	Useful for representing change between time points across sample space.
Geographic maps	Grid cells or other geographic polygons displaying numeric values representing relative abundances,	Useful for representing change between time points across geographic space.

	ordination sample scores, or compositional change (e.g., [81]).	
Abundance graphs	Bar graphs, boxplots, scatterplots, or pie charts showing relative abundance of different taxa at different times (e.g., [82–84]).	Useful only for a small number of taxa and spatiotemporal samples.
Relative abundance tables	Relative abundance or percent dominance values across time (e.g., [85]).	Useful only for a small number of taxa and spatiotemporal samples.
Photographs of gels or electropherograms	Show the relative frequencies of characteristic taxon identifiers (e.g., [86]).	Useful only for a small number of taxa and spatiotemporal samples.

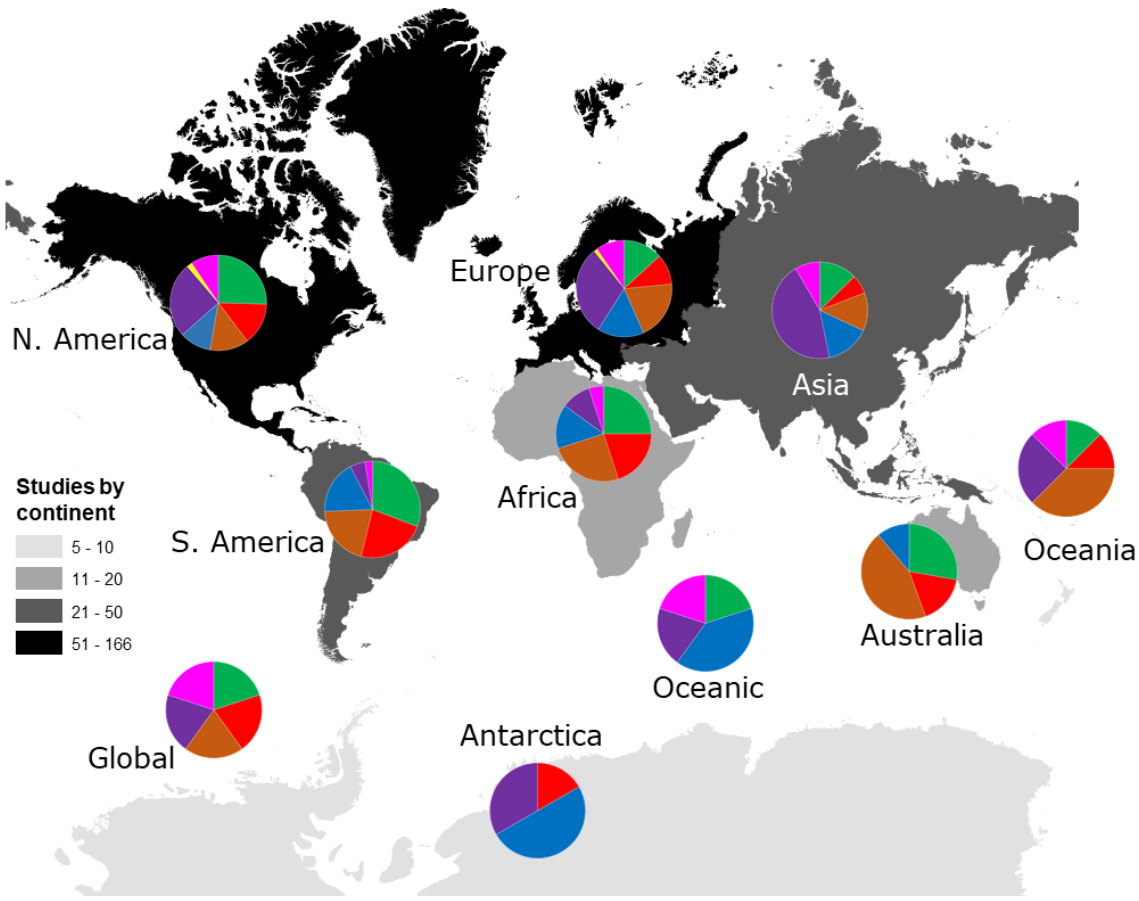
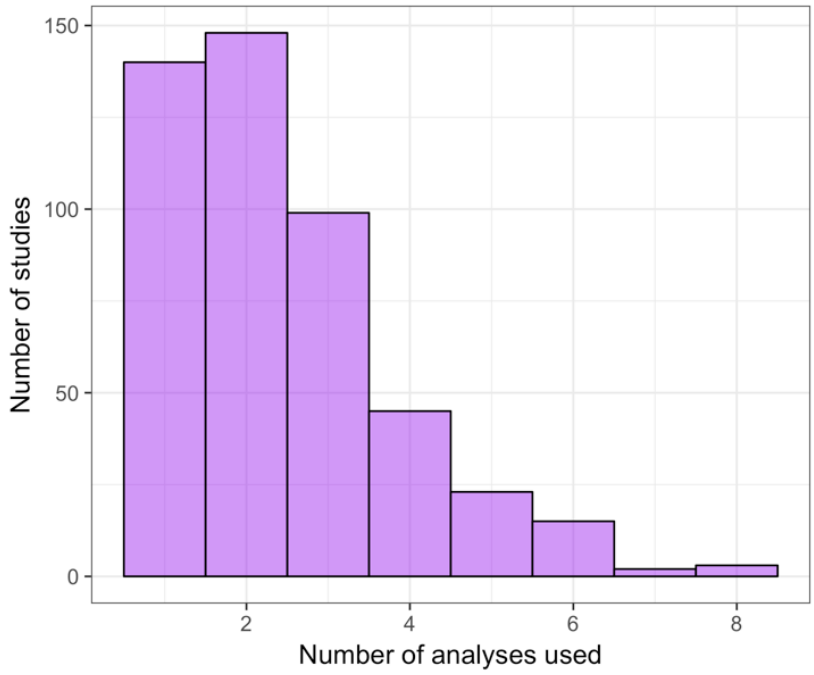


Fig 1. Number of studies identified by our search criteria from each continent. Numbers of studies were: Global (*n* = 5), Oceanic (*n* = 5), Africa (*n* = 20), Antarctica (*n* = 6), Asia (*n* = 47), Australia (*n* = 18), Europe (*n* = 166), North America (*n* = 161), Oceania (*n* = 8) and South America (*n* = 39). Darker colours indicate more studies have been conducted within those continents, including Oceania. Pie charts show the taxonomic focus of study data from each continent as being on (●) plants, (●) vertebrate animals, (●) invertebrate animals, (●) microeukaryotes, (●) prokaryotes, (●) viruses, or (●) mixed taxonomic groups.

(A)



(B)

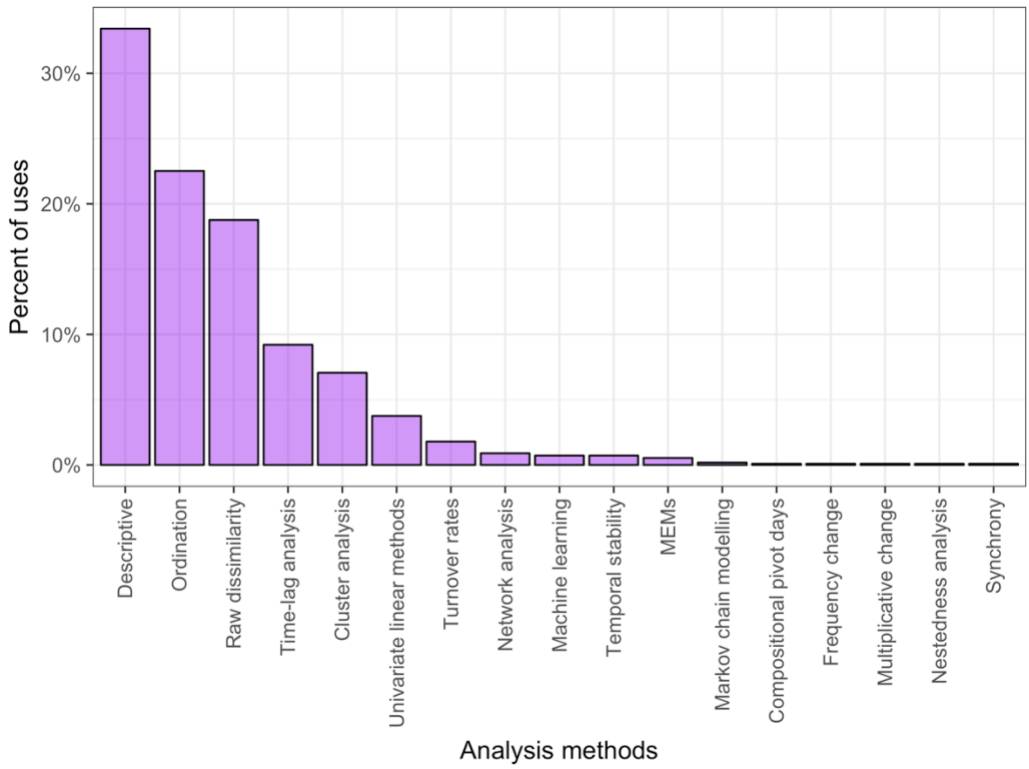


Fig. 2. The frequency of use of different temporal community dynamics analysis methods across all reviewed studies. Specifically: (A) the number of different analyses applied in each

study; (B) the percent of uses of each analysis type across all studies and times. Descriptions of analysis types are detailed in either S1 or S2 Text.

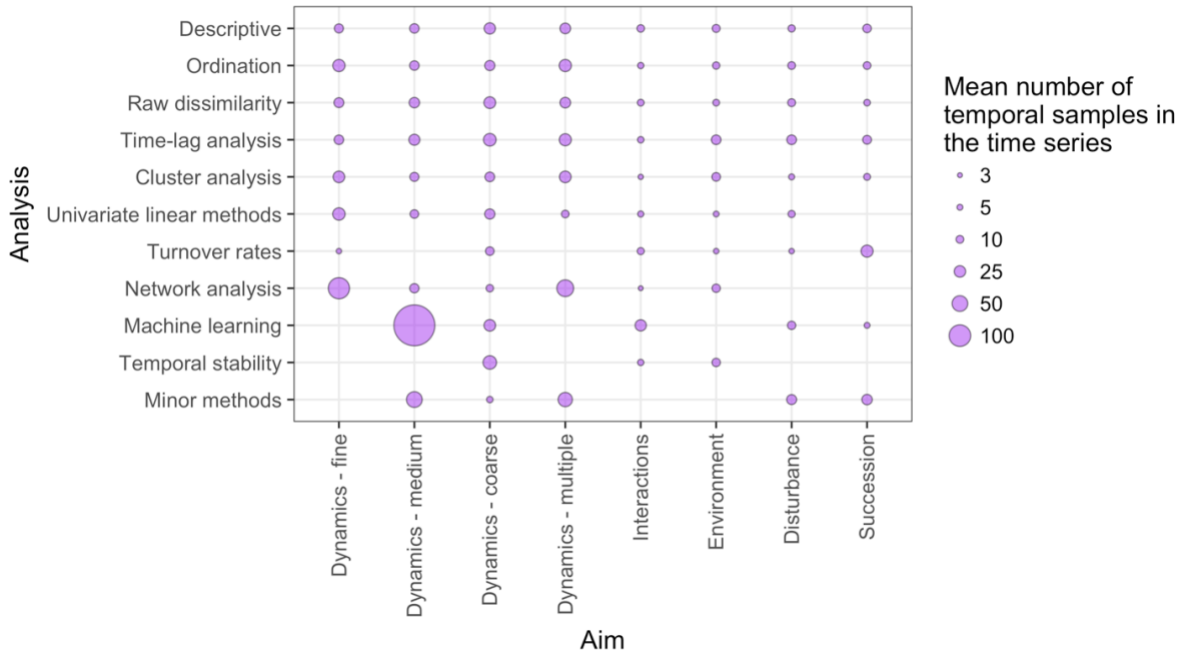


Fig 3: The relative distribution of the mean number of independent temporal samples across different research aims and temporal analysis methods. ‘Minor methods’ include compositional pivot days, frequency change, Markov chain modelling, multiplicative change, Moran's eigenvector maps (MEMs), nestedness analysis, and synchrony. Descriptions of analysis types are detailed in ‘Analysis Choices’ in the text or in S3 Text. Research aims vary from understanding compositional dynamics at fine, medium and coarse temporal scales, to investigating the effect on species composition over time of species interactions, environmental conditions, and ecological disturbance, to understanding successional change in communities.

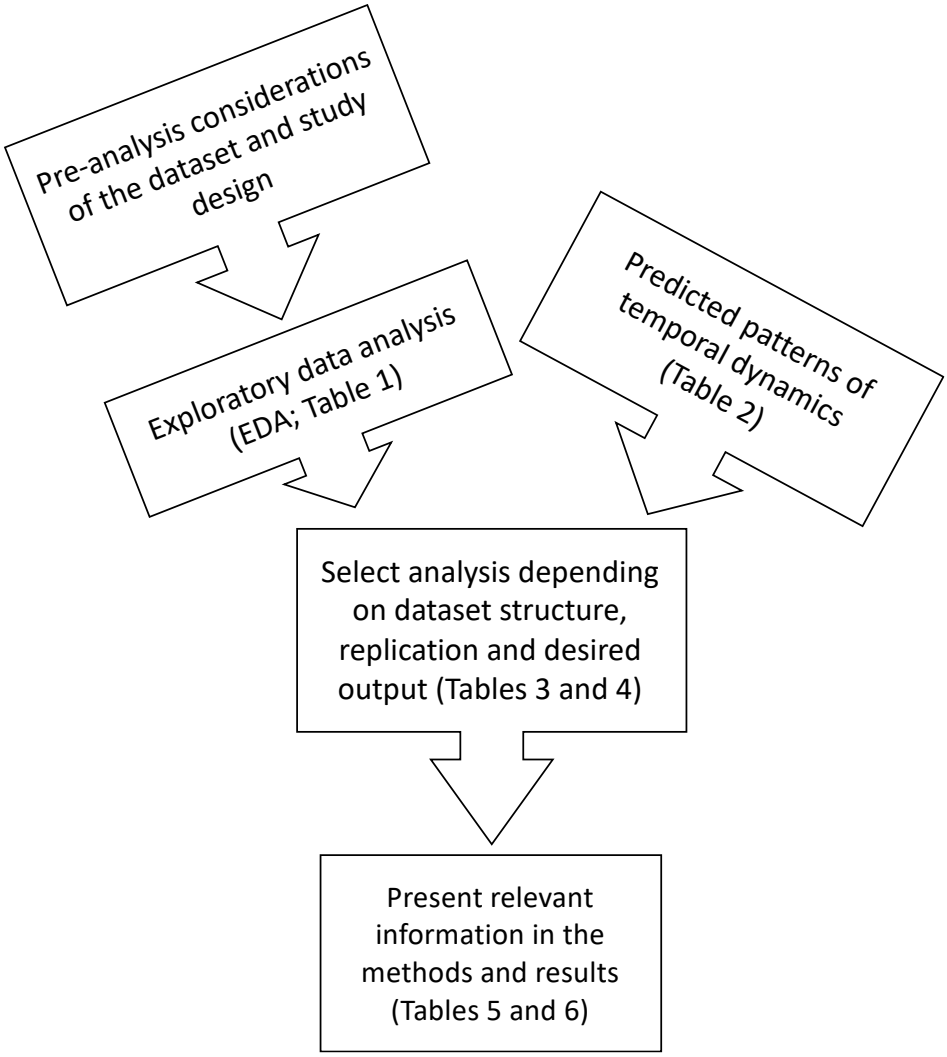


Fig 4. Workflow for the analysis of temporal community data, as recommended in this paper.

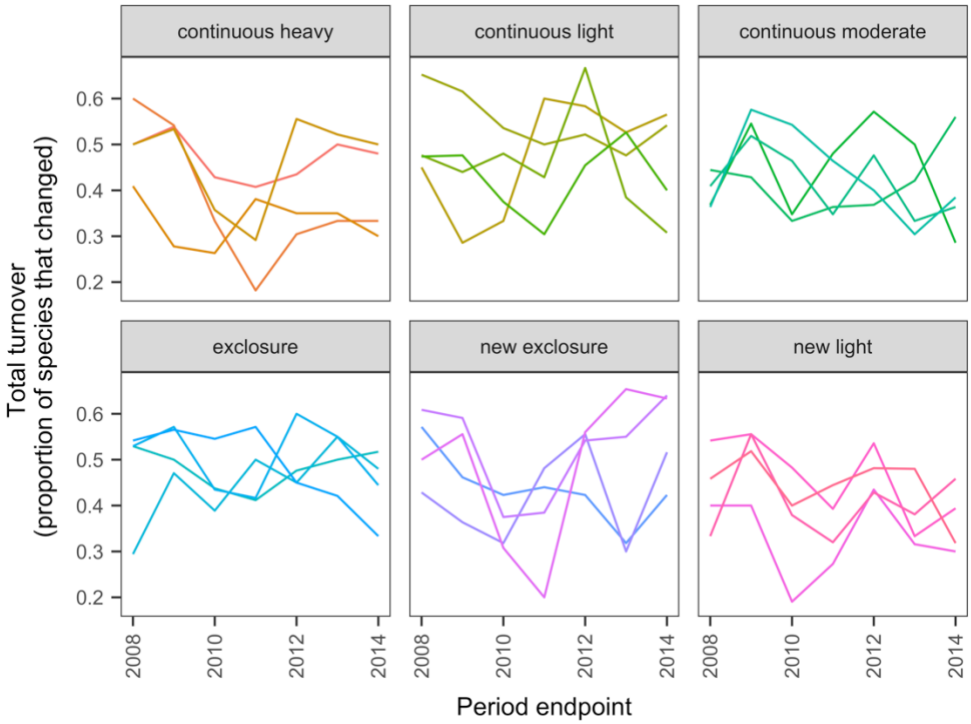


Fig 5: Changes in compositional turnover over time. Four transects were subjected to one of six grazing treatments and were sampled annually from 2007 to 2014. Note that "exclosure" treatment is the control (no grazing). The different lines represent different transects.

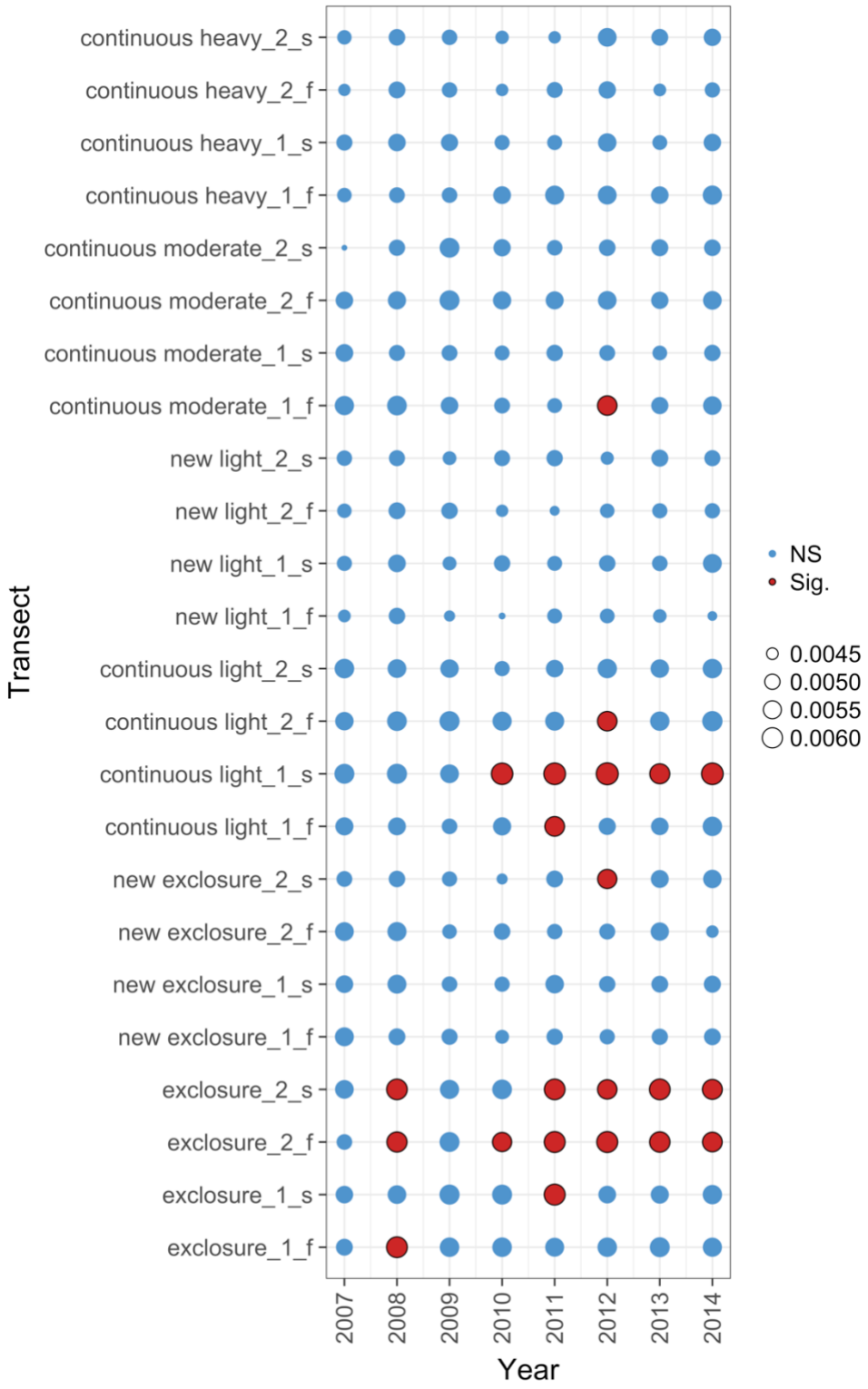


Fig 6: Local contributions to beta diversity (LCBD) for each transect at each sampling time. Plot shows the relative contributions of the different transects and times to the overall beta diversity. Symbol size is proportional to the LCBD value and significance is indicated by the red, outlined circles; blue circles are not significant. Transects had a grazing treatment and were established in one of two replicate pastures (1 or 2) and were either north-facing (f) or south-facing (s).

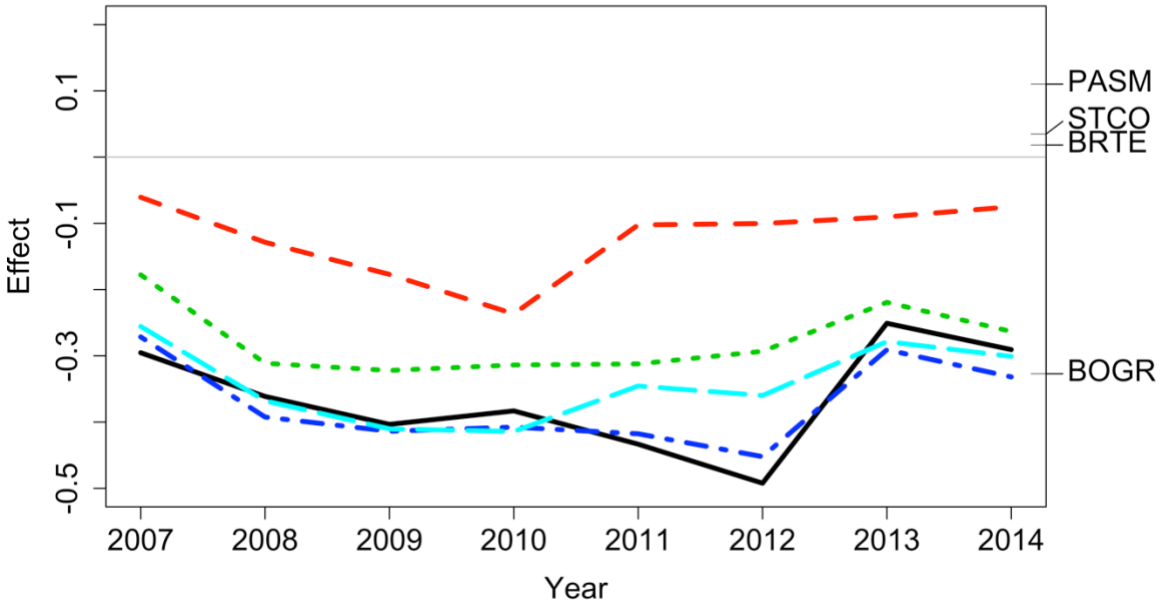
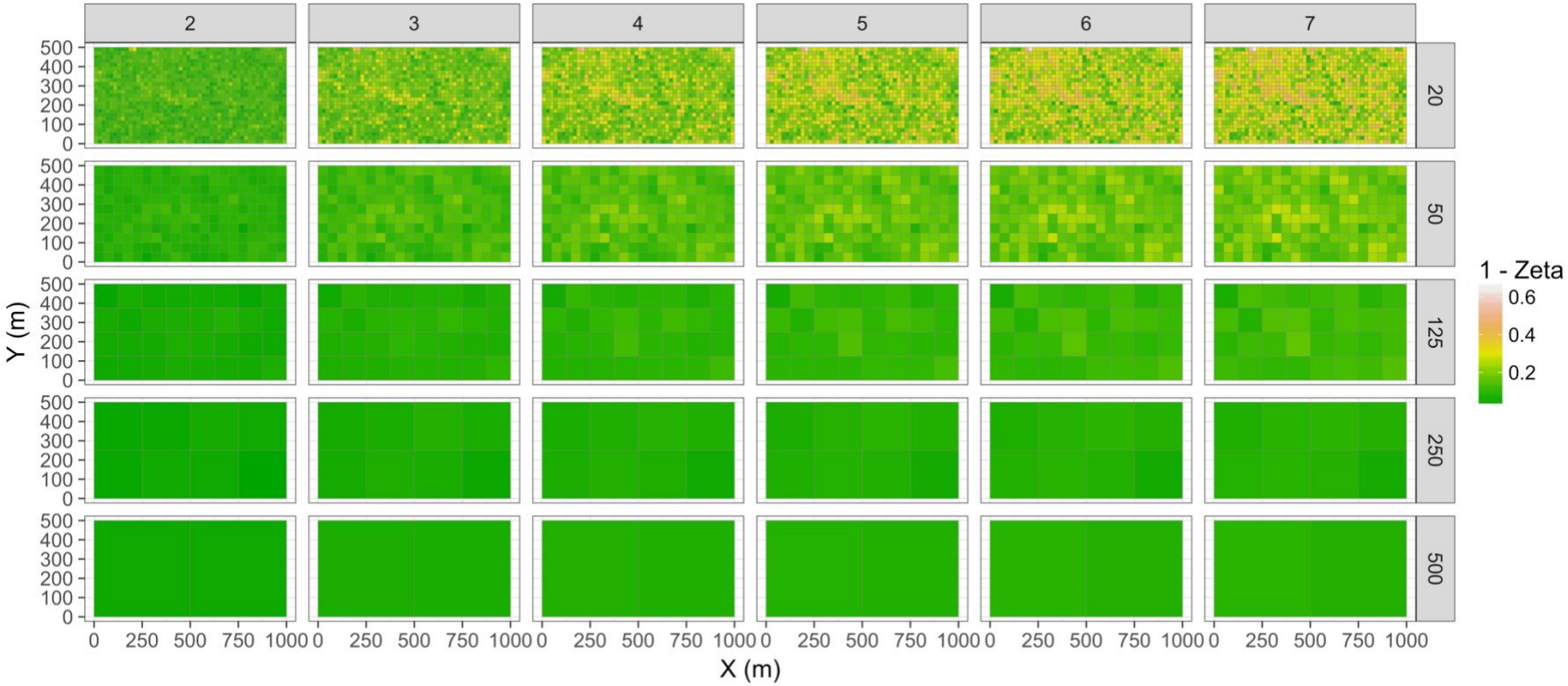
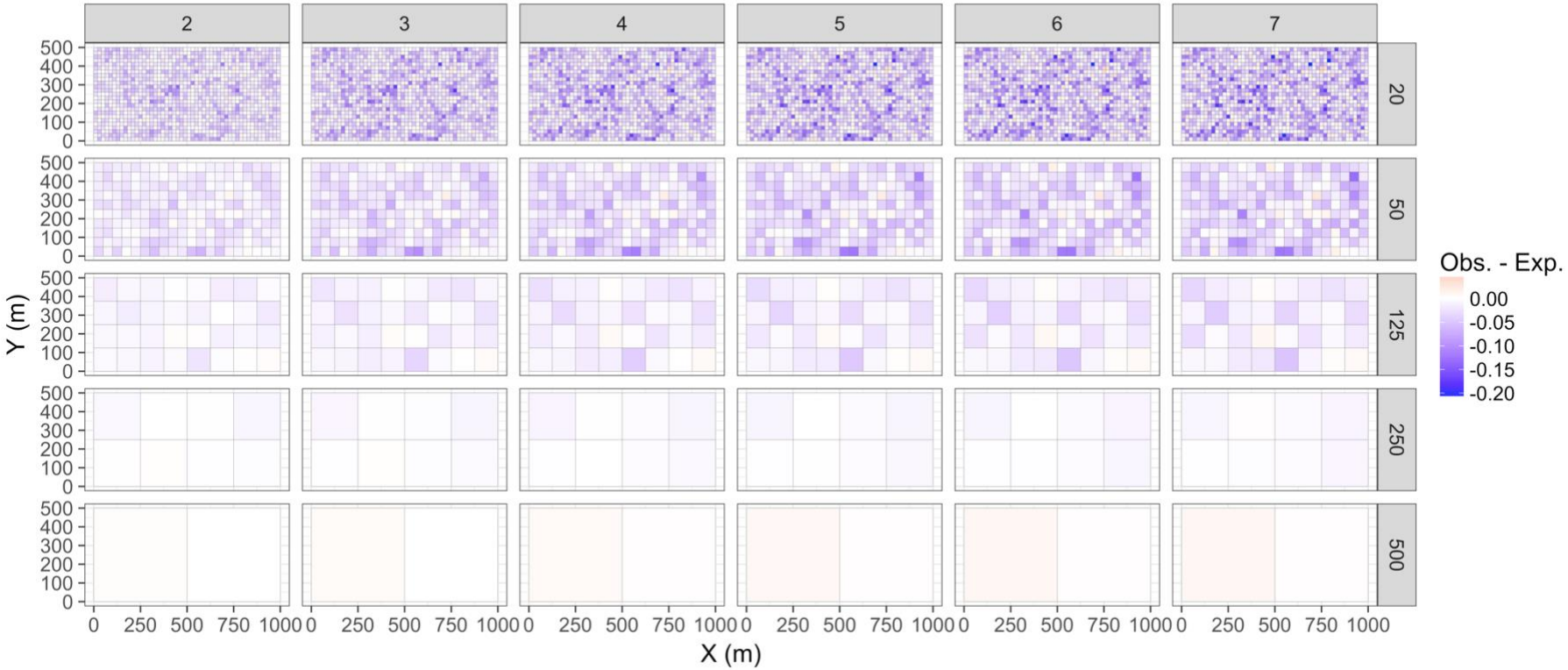


Fig 7: Principal response curve analysis. Results show that treatments differed in plant species composition from the control (exclosure; grey line) but the differences between treatments were inconsistent over time. Treatments were: new exclosure (since 2007; **dark blue**), continuous light (**red**), new light (since 2007; light blue), continuous moderate (**green**), and continuous heavy (**black**). Four particularly influential species where either lower (BOGR = *Bouteloua gracilis*) or higher (PASM = *Pascopyrum smithii*, STCO = *Hesperostipa comata*, and BRTE = *Bromus tectorum*) in abundance than in the control.

(A)


2
3
4
5
6
7
20
50
125
250
500
Y (m)
X (m)
0
100
200
300
400
500
0
250
500
750
1000
1 - Zeta
0.6
0.4
0.2

(B)


2
3
4
5
6
7
20
50
125
250
500
Y (m)
X (m)
0
100
200
300
400
500
0
250
500
750
1000
Obs. - Exp.
0.00
-0.05
-0.10
-0.15
-0.20

(C)

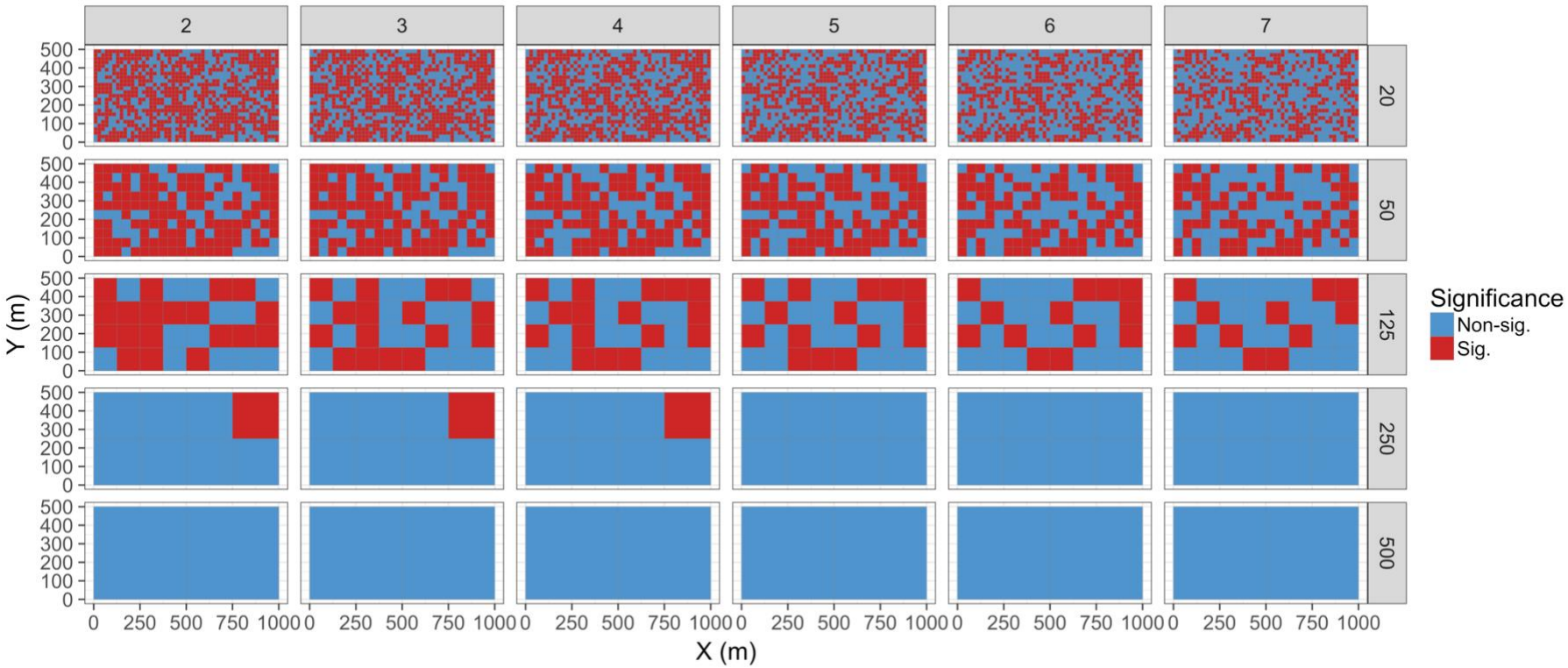
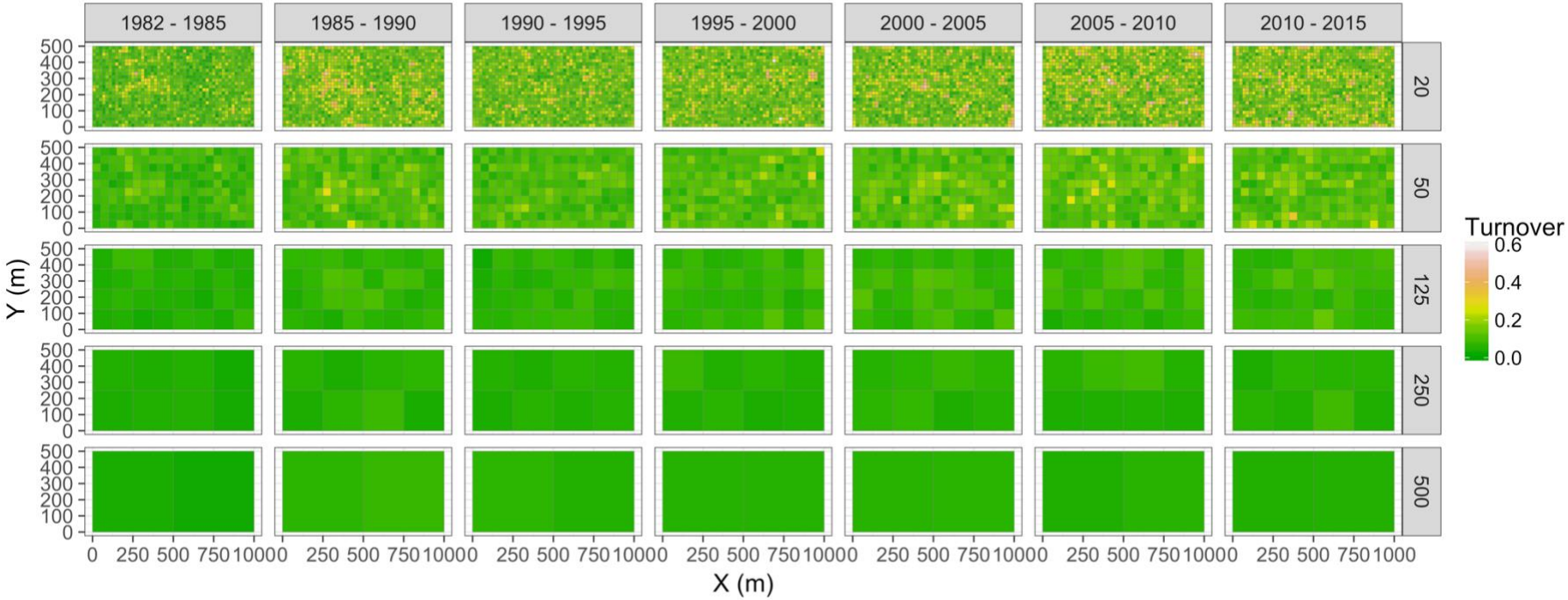
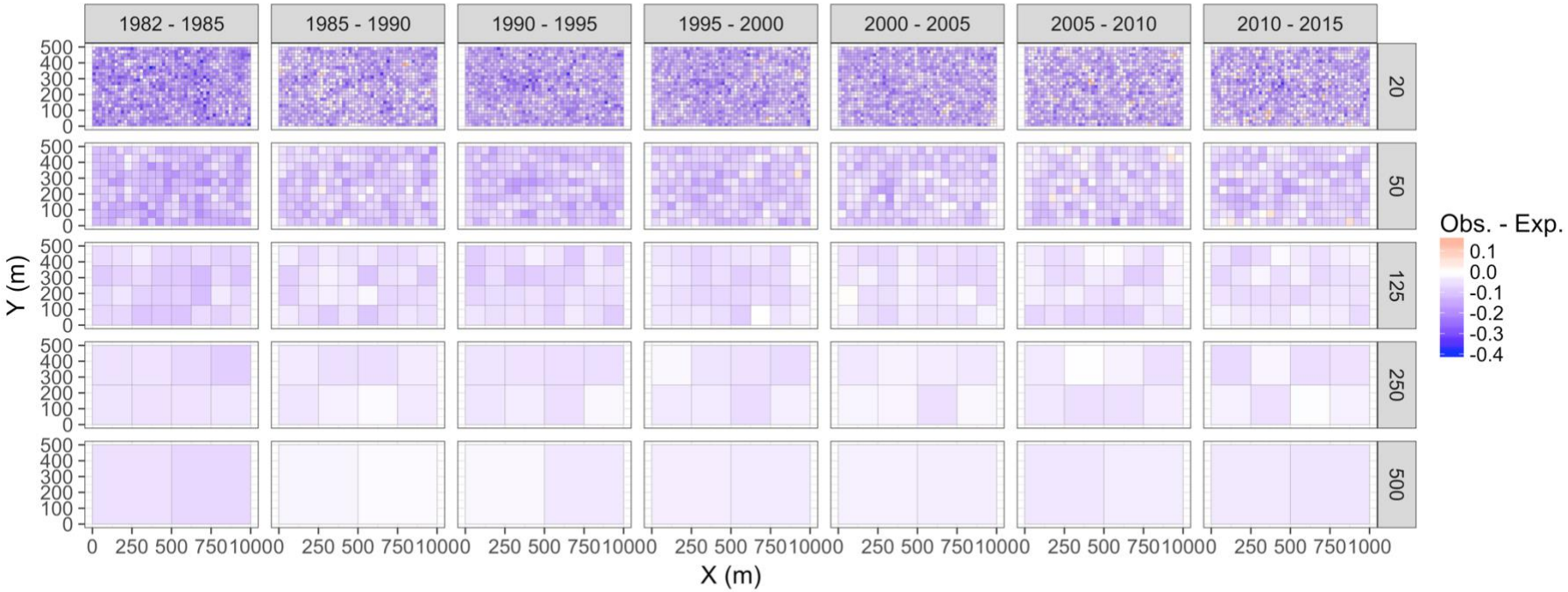


Fig 8. Zeta diversity analysis, based on the number of species shared by a set of temporal samples. Results show that the woody forest community in the 50-ha forest census plot on Barro Colorado Island has been significantly more stable over time than random expectation, particularly at large spatial scales where richness is higher. Maps show for 1 - zeta diversity calculated for quadrats of increasing sizes (20 × 20 m, 50 × 50 m, 125 × 125 m, 250 × 250 m and 500 × 500 m) and for between 2 and 7 orders of zeta (the number of temporal samples compared): (A) the observed values, (B) observed - expected values under a null model of random change in species composition in quadrats over time, and (C) significance of the observed values. Results are given as 1 - zeta so that they are comparable to the turnover results in that higher values represent higher dissimilarity in species composition.

(A)


1982 - 1985
1985 - 1990
1990 - 1995
1995 - 2000
2000 - 2005
2005 - 2010
2010 - 2015
20
50
125
250
500
Y (m)
X (m)
Turnover
0.6
0.4
0.2
0.0

(B)


1982 - 1985
1985 - 1990
1990 - 1995
1995 - 2000
2000 - 2005
2005 - 2010
2010 - 2015
20
50
125
250
500
Y (m)
X (m)
Obs. - Exp.
0.1
0.0
-0.1
-0.2
-0.3
-0.4

(C)

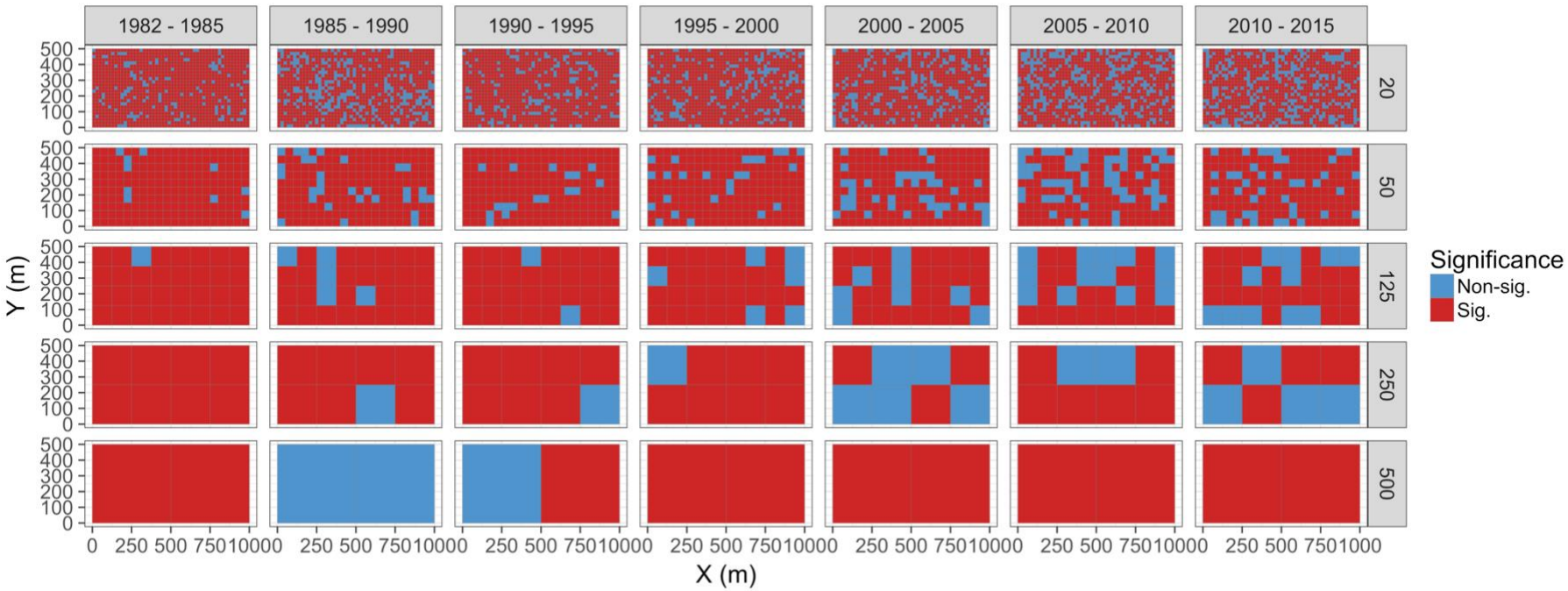


Fig 9. Turnover analysis results for the seven time intervals for the Barro Colorado Island forest community. Results show that the proportion of species turning over each measurement interval was significantly lower than under random expectation, particularly at large spatial scales where richness is higher and composition is more stable: Maps show proportion of species turnover calculated for quadrats of increasing sizes (20 × 20 m, 50 × 50 m, 125 × 125 m, 250 × 250 m and 500 × 500 m) across the seven time intervals: (A) the observed values, (B) observed - expected values under a null model of random change in species composition in quadrats over time, and (C) significance of the observed values.

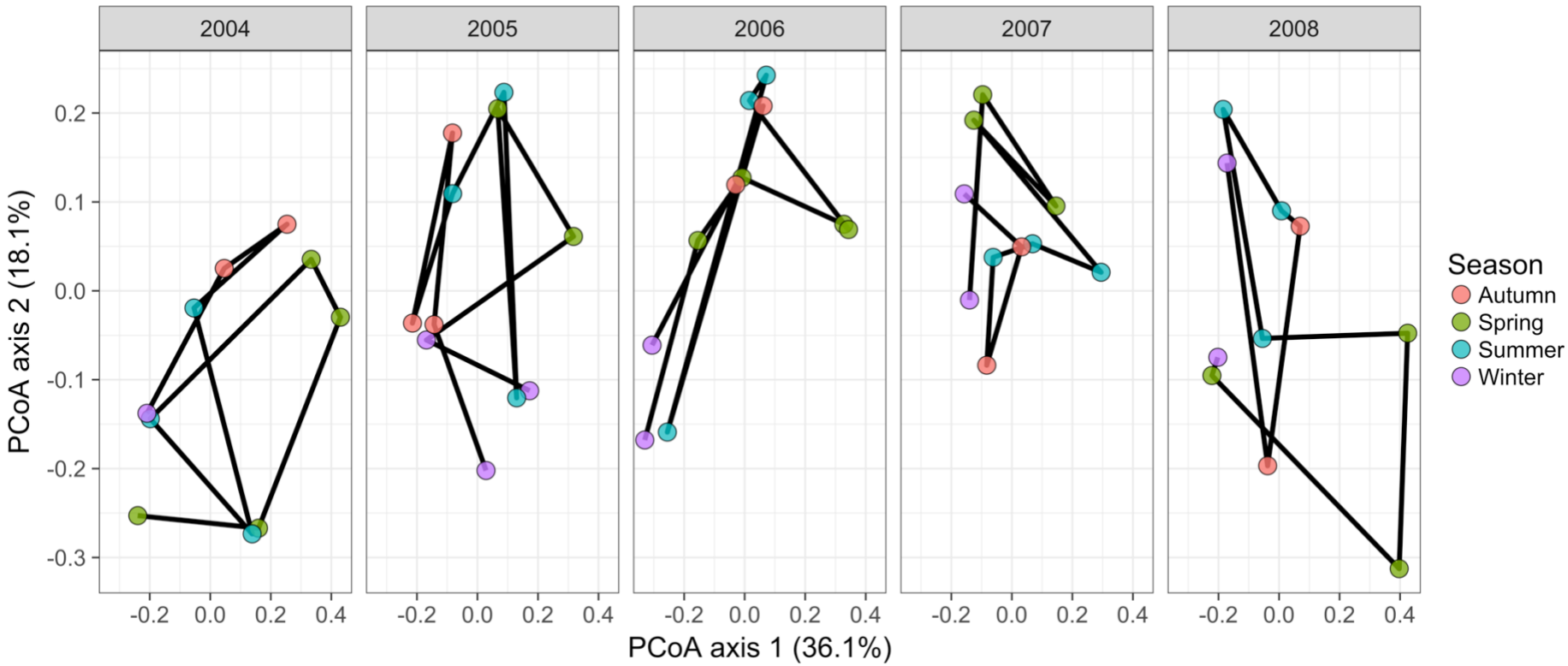


Fig 10. Principal coordinates analysis of 74 marine bacterial OTUs across 62 temporal samples. Results show no visible consistency in compositional change by season (coloured points) within or between years for the single site analysed. Black lines link samples taken consecutively within years.

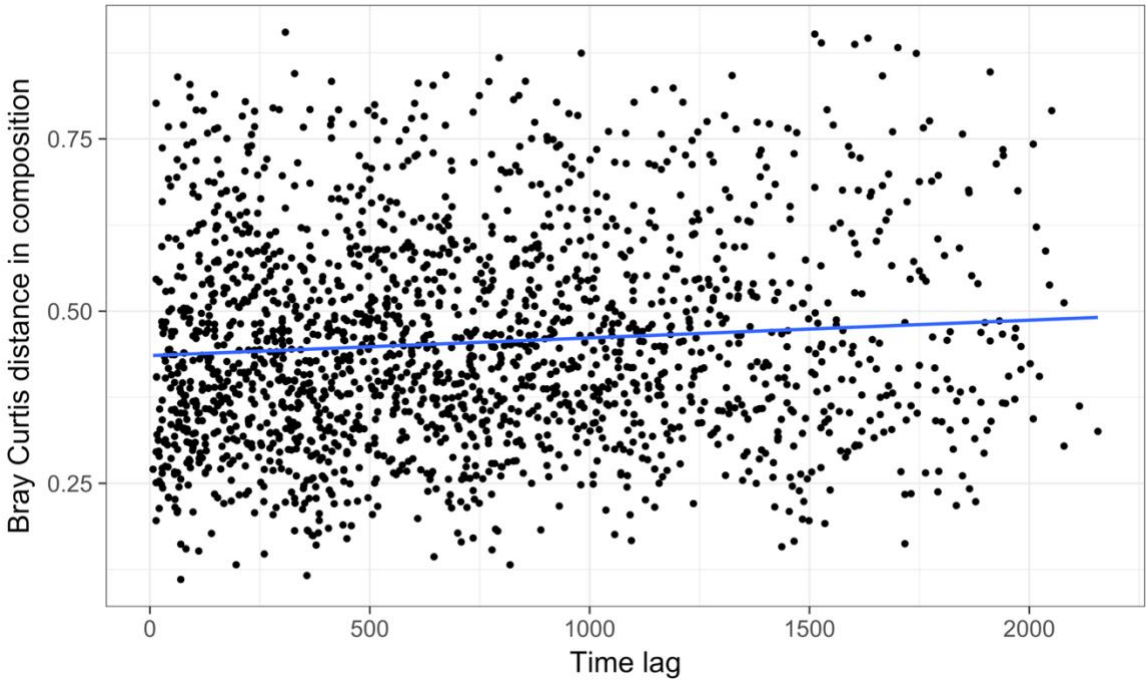


Fig 11. Time lag analysis of 74 marine bacterial OTUs across 62 temporal samples at one site off the coast of Plymouth, United Kingdom. Results show high variability in pairwise Bray-Curtis dissimilarity values with slight divergence occurring over time. The blue line is a linear regression line. The high variability suggests that there is not consistent, directional temporal change at this site.

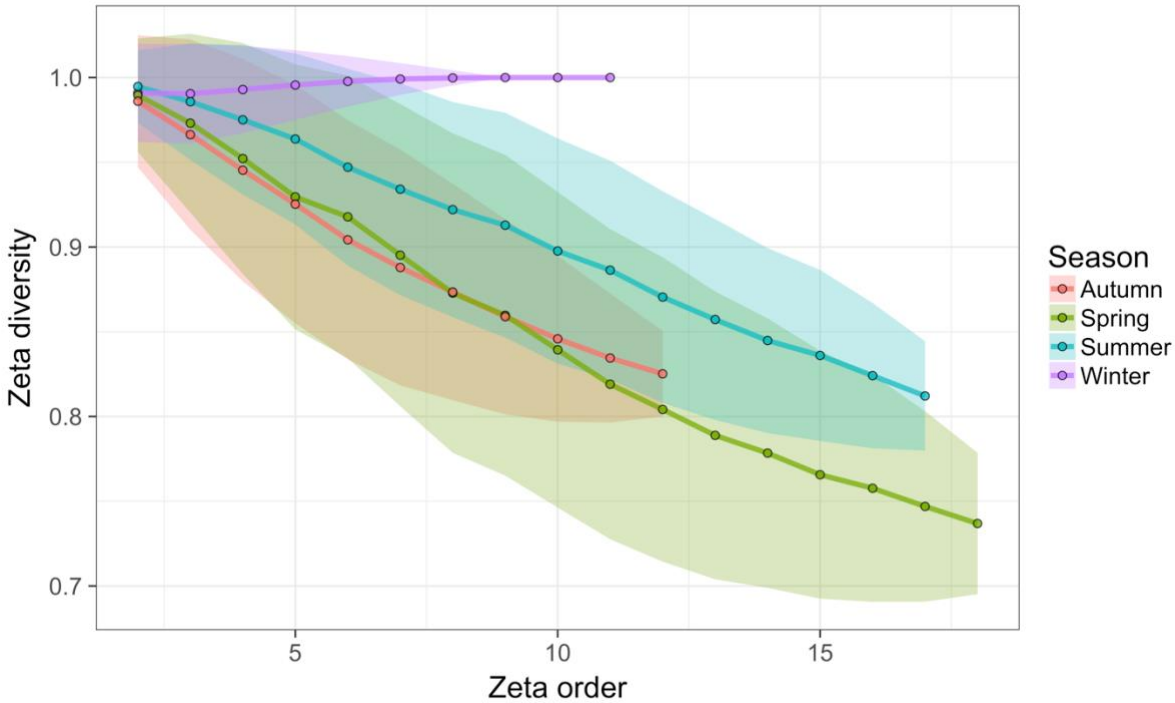


Fig 12. Zeta diversity analysis of the 13 marine bacterial OTUs that were most variable in abundance across 62 temporal samples. Results show that spring samples were more diverse than winter or summer samples. Zeta order represents the number of temporal samples compared and so each point on the graph shows the mean number of OTUs shared among these different numbers of temporal samples.

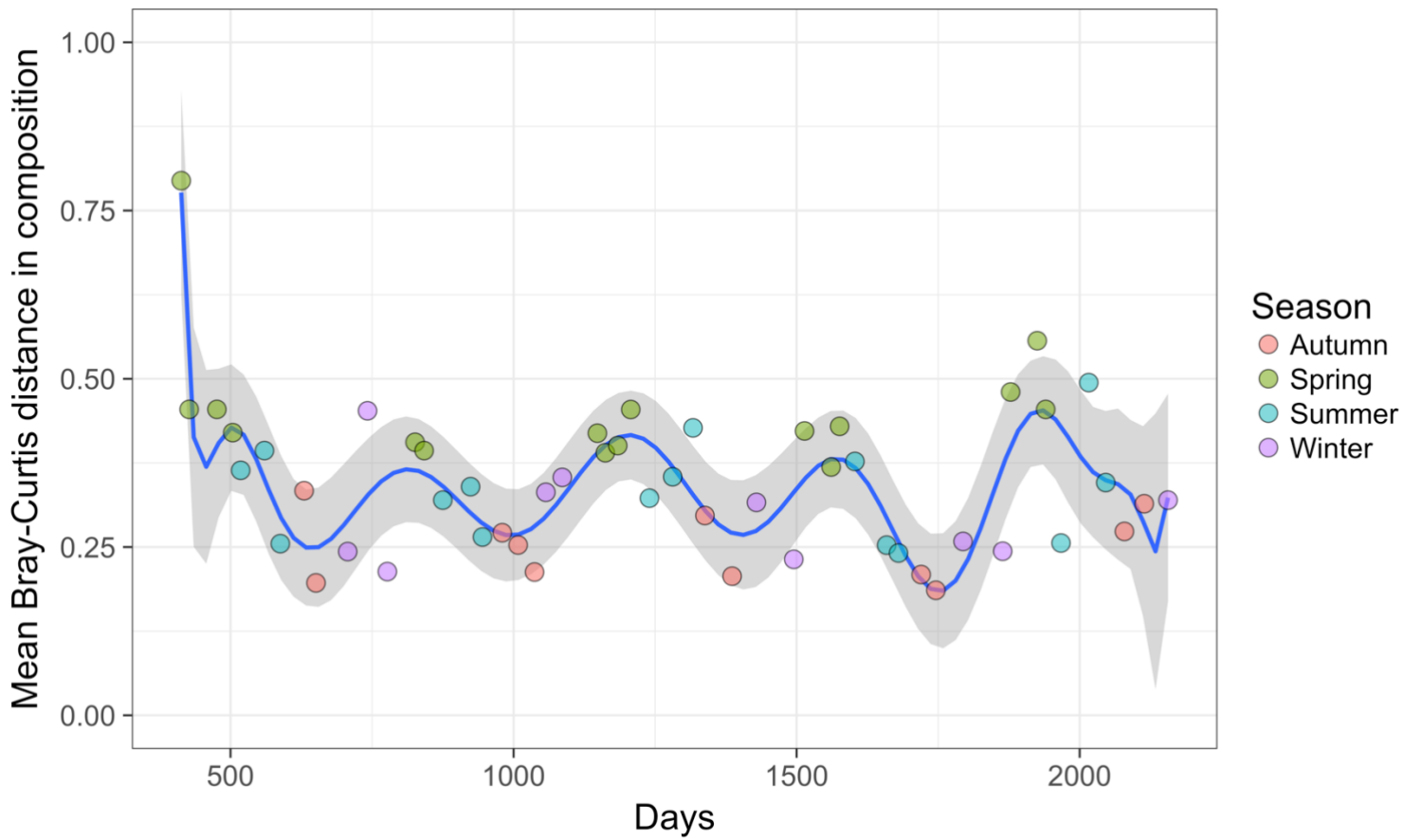


Fig 13. Change in the mean Bray-Curtis dissimilarity values calculated for temporal samples (n = 62) grouped by season. Results are for the 13 most variable marine bacterial OTUs at the one site analysed, showing cyclical community dynamics. The blue line is a polynomial regression line with 16 terms. Multiple comparisons from an analysis of variance showed that samples taken in spring were significantly less similar to other spring samples than were samples taken in summer, autumn, or winter ($P < 0.001$). Samples taken in summer were significantly less similar to other summer samples than were samples taken in autumn ($P < 0.05$).