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The phylogenetic pattern of macroalgal community function

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Summary

Understanding the link between organisms' genetics and functions is key to unraveling the mechanisms of community species assembly. Integration of a phylogenetic perspective with functional trait measurement has become popular in community ecology of vascular plants; however, the phylogenetic and functional relationships are not well understood in seaweed communities. Macroalgae present a challenge for examination of links between phylogeny and function, with several million years of convergent evolution between the red, brown, and green clades. I develop a novel dated phylogeny linking these clades. I used this phylogeny to investigate whether functional traits related to ecological processes such as photosynthesis and decomposition rate (e.g., specific thallus area, dry matter content) evolved at the rate expected based on the organism's evolutionary history, with measurement of their phylogenetic signal. I then applied a combined phylogenetic and functional traits approach to a case study of community-level data. Specifically, using community data from a 14-year macroalgal chronosequence, I examined how functional and phylogenetic alpha diversity metrics varied through community age and whether phylogenetic dispersion was as expected based on the community composition. Functional diversity increased with community age and species richness. Phylogenetic diversity metrics had different relationships with the community depending on the specific metric used. Functional and phylogenetic diversity provided complementary information on community assembly and biodiversity, while the community dispersion was phylogenetically even throughout the chronosequence. The results presented here illustrate that using a phylogenetic approach in macroalgae can inform understanding of their functional traits and patterns of community diversity observed in the field.

DECLARATION AND STATEMENTS

I, Olga Koppel, declare that this work has not previously been accepted in substance for any degree and is not being concurrently submitted in candidature for any other degree.

Signed .....[redacted].....

Date .....April 9, 2018.....

STATEMENT 1

I, Olga Koppel, declare that this thesis is the result of my own investigations. A full bibliography is appended, giving credit to any piece of work that is cited within the text.

Signed .....[redacted].....

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STATEMENT 2

I, Olga Koppel, hereby give consent for my thesis, if accepted, to be available for photocopying and for inter-library loan, and for the title and summary to be made available to outside organizations.

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Acknowledgements

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List of figures and tables

Introduction

Phylogenetics is the study of the evolutionary history of organisms. Phylogenetic trees are used to reconstruct evolutionary relationships between lineages and group species based on similarity of their molecular sequences (Claverie and Notredame 2011). Organisms that share a common ancestor tend to be more genetically similar and are placed more closely on a phylogenetic tree. Genetic variation within a community impacts how species establish themselves within the community, their interactions and even their contributions to ecosystem functions (Díaz et al. 2007, Swenson 2011, Cadotte et al. 2013, de Bello et al. 2017). For example, phylogenetic diversity has been demonstrated to explain productivity patterns over time in terrestrial plant communities (Maherali and Klironomos 2007, Cadotte et al. 2009, 2012). Furthermore, Cadotte et al. (2009) have found phylogenetic diversity to be the best predictor of biomass production in a long-term successional grassland savannah community. In community ecology, phylogenetics can be used to investigate how species evolution impacts coexistence and ecological patterns (Gerhold et al. 2015).

Functional traits are phenotypic expressions of an organism's evolutionary history that affect the fitness of an organism and its response to the environment (Wright 2004, Moor et al. 2017). Functional traits reflect measurable trade-offs between morphological, physiological, and biochemical capabilities of an organism in response to the environmental pressures an organism faces to arrive at a cost-benefit strategy (Wright 2004, Moor et al. 2017). Furthermore, functional traits can influence the contributions of organisms to ecosystem functioning, i.e., the culmination of the energy and material cycling within the system resulting from the activities of organisms and determine the degree of niche differences among organisms in a community (Srivastava and Vellend 2005, Violle et al. 2007, Laliberte and Legendre 2010).

It is often overlooked in community ecology that the display of functional traits in organisms, and consequently trait diversity, is linked to their phylogeny (Felsenstein, 1988; Revell, Harmon, & Collar, 2008; Díaz et al. 2013; Bässler et al. 2014a). An organism's genetic composition, which dictates the phenotypic possibilities for an individual, combines with the environmental pressures and ecological processes affecting

phenotypic expression to contribute to a cost-benefit strategy dictating functional traits (Figure 1).

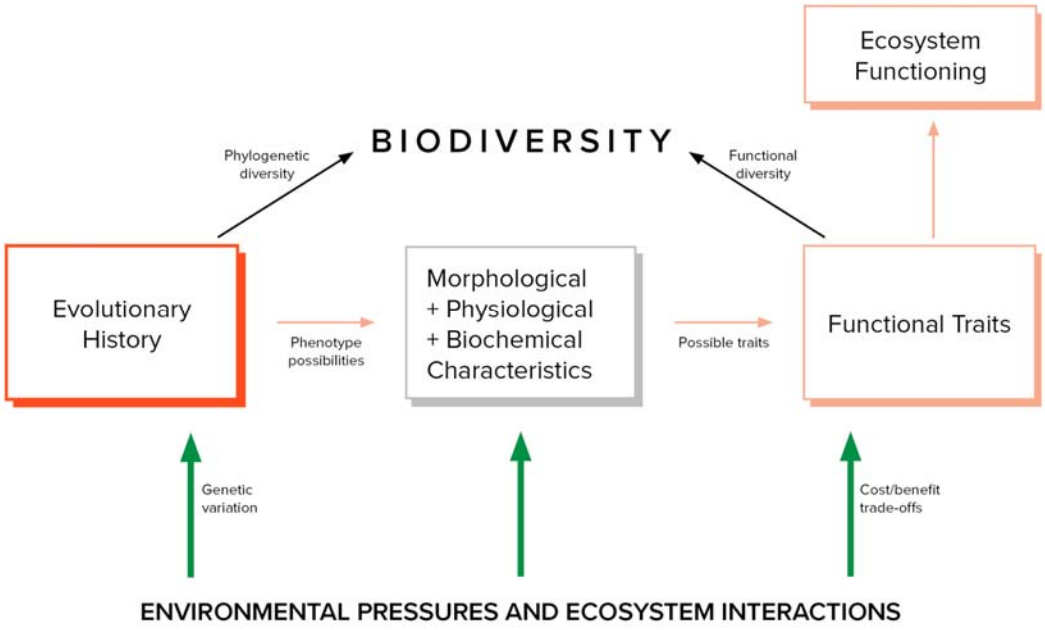


Figure 1. A schematic representation of the role of the previously missing link, evolutionary history, in the interaction with functional traits and contribution to biodiversity.

Analytical models for continuous trait evolution can harness knowledge of evolutionary relationships between lineages to infer functional trait values (Simpson, 1944; Díaz et al. 2007; Harmon et al. 2007; Beaulieu et al. 2012). The Brownian motion model is one such model popular for its widely applicable mechanism for trait inference (Helmus et al. 2007). Applied in a phylogenetic context by Joseph Felsenstein (1973), the model assumes that trait evolution is governed by random variance which increases linearly over time (Wiener 1923). Drawing from the expected character change over an evolutionary timeframe under Brownian motion, a phylogenetic signal can be assigned to traits to compare their observed evolution rate to that expected for the phylogenetic relationship (Blomberg et al. 2003). A higher than expected signal indicates species traits are more conserved than expected from the phylogeny, and may imply a strong role of niche conservatism in determining traits. A signal lower than expected indicates species trait values are more distantly related than expected, due, for example, to resource competition leading to diversification of resource uses (Munkemuller et al. 2012).

Functional traits of macroalgae

The functional diversity of rocky shore communities is determined mainly by the variety of functional traits of macroalgae, or seaweed, which dominate those ecosystems (Lobban & Harrison, 1997). As primary producers, the trait diversity of macroalgae has a direct role in influencing ecosystem functioning (Cardinale et al. 2011). Thallus dry matter content (TDMC), the surface area to volume ratio (SA:V), specific thallus area (STA) and thickness are four functional traits which capture important aspects of ecosystem productivity (Lavorel and Garnier 2002). These traits apply across photoautotrophic groups and are analogues of traits used to capture photosynthetic metabolic rates and variation in function in terrestrial plants (Duerte, Sand-Jensen, & Neison, 1995; Enríquez et al. 1996). TDMC is the ratio of dry to wet mass of the macroalgal thallus (Elger and Willby 2003). The thallus comprises the main body of a macroalgal individual in addition to its holdfast attachment to surface or substrate, sometimes connected to the thallus with a stem-like stipe (Lobban and Harrison 1997). The main role of TDMC is considered to be in mitigating desiccation; it has been observed to slow decomposition (Littler & Littler, 1980) and can be used to estimate productivity (Elger and Willby 2003). SA:V is linked with high nutrient uptake and high photosynthetic rates in algae (Rosenberg and Ramus 1984, Wallentinus 1984). Organisms can display a larger surface area in return for reduced volume capacity (Margalef, 1958; Littler & Littler, 1980; Nagatani et al. 2007). STA captures a similar aspect of variation, relating the surface area of a macroalgal thallus with its dry mass and providing information on the relationship of photosynthetic tissue with internal structural compounds. In plants, the analogous trait, surface leaf area, is known to increase with increased nutrient content (Vile et al. 2005). Reduced photosynthetic capability may be a trade-off in favour of higher mass per area of the photosynthetic structures (Onoda et al. 2017). In plant communities, specific leaf area is also found to co-vary with leaf thickness (Wilson et al. 1999). The thickness of leaves in marine mangroves has been found to play a role in resisting desiccation and positively correlate with water storage (Camilleri and Georg 1983). Yet despite the above-described links between traits and functions that are likely to occur in macroalgae, variation in these traits across species has not been systematically studied. The last major foray into explaining functional variation

in seaweed was by Littler and Littler through classification of species into functional-form groups (1980). These functional-form groups use qualitative categorization of morphology (e.g., foliose, sheet-like, and leathery) to group species. A more quantitative and measurement-based approach to seaweed functional diversity, analogous to that used in terrestrial vascular plants, is now overdue.

Integration of functional traits and phylogeny in macroalgal communities

The wide evolutionary history of macroalgae, in contrast to land plants, provides a unique opportunity to compare diverse evolutionary history with functioning of extant species. Chlorophyta, Rhodophyta and Phaeophyta, commonly known as the green, red, and brown seaweeds, are three macroalgal clades that are deeply polyphyletic, but strongly convergent. Divergence between these clades is estimated to coincide with the diversification of other eukaryotes, approximately 800±100 million years ago (Berney and Pawlowski 2006, Tomitani et al. 2006, Knoll 2011). Rhodophyta are considered to be primitive because of the presence of proteins and synthesis pathways which are characteristic of cyanobacteria (Graeve et al. 2002, Cunningham et al. 2007), but absent from other macroalgae. Indeed, the Rhodophyta is thought to contain some of the earliest photosynthetic eukaryotes (Kenrick & Crane, 1997). The first known fossil evidence of seaweed belongs to an extant Rhodophyte species, *Bangiomorpha pubescens*, which is considered the first sexually reproducing organism (Butterfield 2000, Yang et al. 2016), and dates to approximately 1 billion years ago. This fossil evidence predates the colonization of land by terrestrial plants, and also the evolution of their close relative, the Chlorophyte algae, by 600 million years (Kenrick and Crane 1997, Karol 2002, Fang et al. 2017). Phaeophyte crown radiation, the point from which the most recent common ancestor to extant Phaeophyte species diverged, occurred during the Mesozoic era, under 250 million years ago (Silberfeld et al. 2010).

A dated phylogeny unifying all three diverse groups has not been previously constructed. Understanding the relationship between the varied evolutionary history of Phaeophyta, Rhodophyta, and Chlorophyta and the present day functional traits expressed by the species in these groups can be instrumental in understanding community dynamics *today* and aid in predicting future impacts on community composition.

In this study, I will first model the evolution of macroalgae over the vast evolutionary time scale that they span by constructing a phylogeny consisting of species from the three major clades: Phaeophyta, Rhodophyta, and Chlorophyta. Harnessing this newly developed insight into macroalgal evolution, I will use phylogeny as tool in determine the expected rate of evolution of individual traits, specifically testing for the presence of a phylogenetic signal in ecologically relevant traits. I predict that the phylogenetic signal will be apparent within, but is unlikely to hold across, the three seaweed clades, due to their vast evolutionary spread.

I will then apply the combined phylogenetic and functional perspective to a case study of intertidal rock pools, dominated by seaweeds and spanning a 14-year successional chronosequence, to integrate phylogenetic and functional perspective at the community level. A successional community offers a good opportunity to examine these interactions, where species interactions within the community may change over time and species functional strategies and traits are also likely to vary, reflecting commonly documented successional patterns at larger spatial scales (Odum 1969, Lichter 1998). Changes in community composition with succession will be quantified by various metrics of functional and phylogenetic diversity. These metrics emphasize different aspects of biodiversity and are influenced differently by changes in species richness. I therefore expect them to show a range of patterns across succession. I expect the functional diversity metric (Petchey & Gaston, 2002) to increase over time in the chronosequence, as higher competition leads to the persistence of functionally diverse species which mitigate competition through partitioning of ecosystem resources (Newton et al. 2007). Meanwhile, I also expect Faith's phylogenetic diversity metric (Faith 1992) to increase over time, since this metric is sensitive to increase in species richness, which I also expect an increase. I expect two other phylogenetic diversity measures, mean neighbor taxon distance (Webb et al. 2002) and mean pairwise distance (Webb 2000), to decrease over succession, as these are distance-based metrics, and addition of new species to the pools should decrease the distances between close relatives.

The phylogenetic pattern will also be examined for over- or under- dispersion with regards to the species assembly over the successional stages in the community (Liu

et al. 2016). Phylogenetic over-dispersion, compared to the phylogenetic relationships expected based on the community species composition, may be the result of community assembly through mechanisms of biotic interactions. Meanwhile, phylogenetic under-dispersion may result from strong community influence of niche conservatism and environmental filtering. A community with a phylogenetic diversity that is as expected is considered 'phylogenetically even' (Brown et al. 2013, Bell 2015).

Aim and objectives

The aim of this study is to understand the links between phylogeny and functional traits in macroalgae. To meet this aim I will address the following objectives:

1. To present a dated phylogeny spanning the three macroalgal clades, Chlorophyta, Rhodophyta, and Phaeophyta.

2. To investigate whether the observed trait variation in 33 macroalgal species, spanning the three macroalgal clades, can be predicted by phylogeny, i.e., whether they show a strong phylogenetic signal.

3. To compare how overall phylogenetic and functional diversity in an assemblage vary over succession and relate to one another. This will be investigated using data from a macroalgal successional chronosequence.

Methods and Materials

Note on author's contribution to data collection

Seaweed functional traits are being collected as part of a wider project at Swansea University and the sampling for traits was ongoing during my MRes research period in spring and summer 2017. Between May and August, I took an active role in species sampling from the field and trait screening in the laboratory, and led a field campaign to sample in Cornwall. I performed all the phylogenetic analyses and analyses of the

chronosequence data independently; this is the first time such information has been incorporated into the broader project.

Trait collection

Field sampling was conducted between May and August in 2016 and 2017 at six sites across the United Kingdom: Bay of Skaill (59.05 N, 3.34 W) in Scotland; Oxwich Bay (51.56 N, 4.15 W), Overton Mere (51.55 N, 4.20 W), and Bracelet Bay (51.57 N, 3.99 W) in Wales; and Portlooe (50.34 N, 4.47 W) and Portwrinkle (50.36 N, 4.31 W) in England (Figure 1). 33 macroalgal species were collected in total: 3 from Scotland, 28 from Wales, and two from England. Six replicates per species were collected, each at minimum 2 metres apart to reduce the chance of sampling clonal individuals that share a rhizoidal, or creeping, holdfast. The sites in Wales, and specifically those close to Swansea on the Gower Peninsula, are core sites for the seaweed traits sampling program. The other sites in Scotland and England were sampled to be able to include additional British seaweeds that are not found in the Gower Peninsula region– taking advantage of geographic turnover in the composition of communities – and to begin to expand to other areas in order to increase the generality of findings.

Laboratory analysis

Samples were cleaned of debris and epiphytic material and rinsed with distilled water to remove excess salt. Large samples were subsampled by half lengthwise prior to laboratory analysis. Volumes of each individual's frond, stipe, and holdfast were measured separately in a graduated cylinder. Ten measurements of frond thickness were taken with a digital thickness gauge at arbitrarily selected points along the frond. Samples were patted dry and fresh mass was measured. A lightbox with base illumination was used to capture photographs of samples that were cut to lay flat against the surface, to facilitate accurate surface area measurements in image post-processing. Sample dry mass was recorded after one week in a 70^{o}C drying oven. Thallus Dry Matter Content (TDMC) was calculated as the ratio of sample dry mass to fresh mass. Specific Thallus Area (STA) was calculated as the ratio of surface area to sample dry mass (cm^2/g). Image

analysis was performed using ImageJ software to measure individual sample surface area (Schneider et al. 2012).

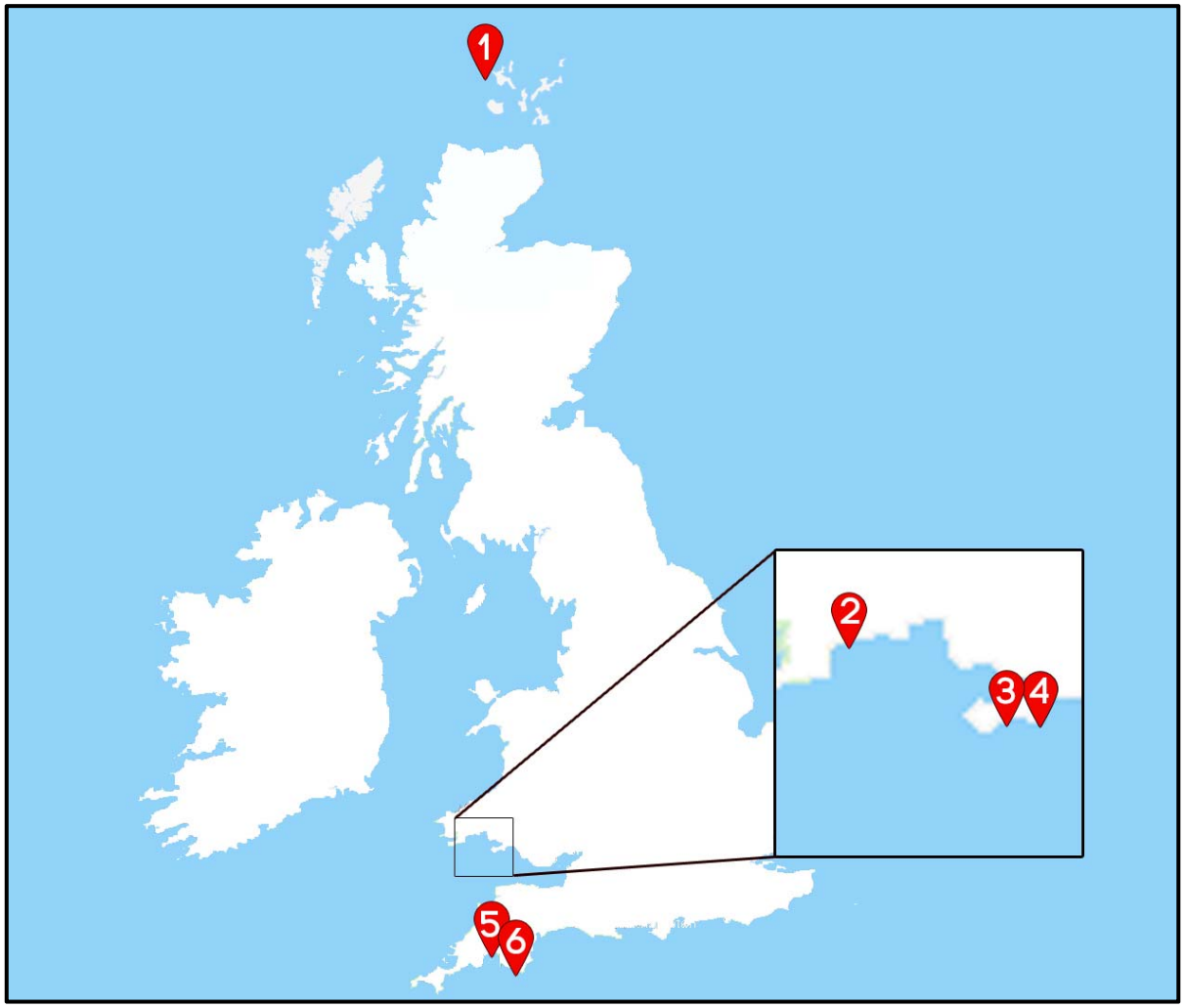


Figure 2. Sampling sites across the United Kingdom. 1: Skaill, Scotland; 2: Oxwich Bay, Wales; 3: Overton Mere, Wales; 4: Bracelet Bay, Wales; 5: Portlooe, England; 6: Portwrinkle, England.

Phylogeny construction

A phylogenetic tree was constructed for the 33 macroalgal species collected. All sequence analyses and Maximum Likelihood (ML) phylogeny estimation were performed in MEGA7 (Kumar et al. 2016). Bayesian inference of phylogeny was performed in

BEAST (BEAST v.1.5.3, (Drummond and Rambaut 2007). The ML and Bayesian methods were used in complement to ensure they produce congruent results (Hedges et al. 2004, Zhang et al. 2012, Liu et al. 2015).

Molecular sequences for chloroplast loci rbcL and psbA, and nuclear locus 26S, were obtained by data mining from GenBank. The combination of chloroplast and nuclear loci with different evolutionary rates were included for a more robust multi-loci phylogeny; low-copy genes such as the nuclear 26S are highly conserved and can provide resolution to the taxonomic level (Sang 2002). Gaps in the sequence matrix, with one or two missing sequences for some species, were allowed in favour of inclusion of more genes for improved tree resolution (Wiens 2006, Wiens and Morrill 2011); higher phylogenetic resolution will impact detection of a phylogenetic signal, and influence metrics of the phylogenetic diversity within a community (Swenson, 2009). For species that could only be identified to the genus level, a representative species from the genus was chosen (see Appendix 1 for sequences used and GenBank accession numbers). Sequences were manually trimmed and alignments were created for each loci using MAFFT, an alignment method chosen for its relative speed and accuracy (Katoh et al. 2002). Separate loci were combined with concatenation, a popular tree method for multiple loci (Tonini et al. 2015). The best-fit model of nucleotide substitution with the lowest Bayesian information criterion score was estimated using ModelTest (Posada and Crandall 1998) to be a General Time Reversible model with gamma distribution and invariant sites considered (GTR +G+I; BIC = 31378.39). General Time Reversible model accounts for unequal rates of variance in genes, important in this multi-locus alignment.

A Maximum Likelihood (ML) consensus tree was generated with bootstrapping (n=1000). Evolutionary distance calculations were based on the Tamura-Nei model (Tamura et al. 2011). The Bayesian inference method was also used to construct a dated molecular phylogeny with uncorrelated relaxed clock methods MCMC chain with 2 000 000 generations, and a burn-in of 200 000. Prior node constraints were set, estimated by published node dates from a combination of fossil data and published phylogenies implementing a Bayesian molecular relaxed clock method (Appendix 1).

Statistical analysis

Phylogenetic trees were read into R and manipulated using the *ape* package (Paradis et al. 2004). Data analyses were conducted in R 3.4.1 (R Core Team, 2013).

To test for pairwise correlations between traits, Kendall's τ (tau) coefficient was measured (Kendall 1938). τ is between 0 and 1.

Blomberg's *K* (Blomberg et al. 2003) was used to measure phylogenetic signal of traits using R package *phytools* (Revell 2012). The *K* statistic tests against the null hypothesis that more closely related species do not have more similar trait values than expected by chance. If Blomberg's K = 1, traits are evolving as expected according to the Brownian motion model (Blomberg et al. 2003). A K value greater than 1 suggests that species trait values are more conserved than expected based on the model, while K less than 1 indicates species trait values are more divergent than expected. Blomberg's K statistic tests whether species relatedness is a strong driver in trait values.

To correct for potential autocorrelation between traits and determine which traits were driving most variation, a principal component analysis (PCA) was performed. Trait values were standardized with z-transformation prior to PCA analysis in order to normalize trait distribution (Legendre and Legendre 1998, Buttigieg and Ramette 2014).

To visualize trait evolution over time, trait values were estimated for ancestral nodes of the dated phylogeny based on trait value, Brownian motion evolution rate, and branch lengths which represent evolutionary time (Felsenstein 1988) with R package *picante* (Kembel et al. 2010).

Testing the application of a phylogenetic approach to macroalgal communities

To explore the utility of using a phylogenetic and trait perspective in macroalgal communities, phylogenetic and functional diversity measures were applied to a pre-existing dataset. The dataset captured changes in rockpool communities across 14 years (1992-2005) in a space-for-time substitution, or chronosequence, on Plymouth Breakwater, in Cornwall, England (50.33 N, 4.15 W). The chronosequence consisted of 12 concrete blocks with 2 pools on each. TDMC, SA:V, STA, and thickness trait values

for 11 of the 23 species in the chronosequence were available from sampling across the U.K. Note that the chronosequence dataset was originally reported in a PhD thesis (Griffin 2008) and analyzed with respect to changes in the species composition of the communities but not functional or phylogenetic diversities.

A molecular phylogeny was constructed for all 23 species in the assembly using previously described methods. Since phylogenetic trait estimation is based on ability to predict traits from phylogeny, a strong phylogenetic signal (Blomberg's K value close to 1) is necessary before proceeding. The *phyEstimate* function in the *picante* package was used to predict functional trait values using ancestral state estimation based on shared phylogenetic nodes between unmeasured and known traits (Garland and Ives 2000). A principal component analysis was performed on the 23 species in the chronosequence with estimated and measured trait values and the component explaining the majority of variation was extracted.

Diversity metrics

Phylogenetic and functional alpha diversity metrics were chosen to determine the amount of phylogenetic and functional diversity at the community level. There are many redundant diversity metrics used in conservation and community ecology, and the appropriate metric needs to be carefully chosen based on the aim of the analysis (Vellend et al. 2011; Pavoine et al. 2013; Tucker et al. 2017). Metrics were selected to represent different aspects of phylogenetic diversity to provide a fuller understanding on phylogenetic relationship with community assemblage.

Tucker et al. (2017) identify Faith's phylogenetic diversity (PD, Faith, 1992) and mean pairwise distance (MPD, Webb, 2000) as key metrics of phylogenetic diversity. Faith's phylogenetic diversity (PD) described the breadth of phylogenetic diversity in the assemblage by calculating minimum total branch lengths between all taxa, providing understanding of the 'richness' of genetic differences (Tucker et al. 2017). Distance-based phylogenetic alpha diversity measures examine range of pairwise relationships to determine if overall species composition is broadly or closely phylogenetically spaced within a community. MPD is a distance-based metric that considers pairwise distances among taxa across the assemblage. It examines the overall dissimilarity and can provide

insight into the divergence within the assemblage. It supplements PD by demonstrating how closely related species are within a community, not only the overall diversity of species. Mean neighbour taxon distance (MNTD; Webb et al. 2002), another distance-based metric, is a measure of the shortest distance between a species to any other in the community. Unlike MPD, this approach retains detailed information of interactions between closely related species and provides insight into whether species are more broadly or closely spaced in a community. Abundance-weighted versions of these metrics were used to incorporate additional information on the relative representation of traits distributed along the phylogeny (PD_{AB}, Barker, 2002; MPD, Warwick & Clarke, 1995; $MNTD_{AB}$, Cadotte et al. 2010). Functional diversity (FD) was calculated as the total branch length in a dendrogram built from a species trait dissimilarity matrix following Swenson (2014) in R package *picante*.

A generalized linear mixed effects model (GLMM; Bolker et al. 2009) was used to determine how diversity metrics relate to assembly age and species richness. Species richness was used as a covariate to investigate its interaction with age and contribution to explaining variation in the diversity metrics. Autocorrelation with block identity across the 12 blocks of different age in the chronosequence, each containing two species pools, was controlled for by adding block as a random effect.

Standard effect size of diversity metrics

To determine if communities are more phylogenetically over- or under- dispersed than expected based on the species assembly, diversity metrics were compared to those expected by chance (Cadotte et al. 2013, Purschke et al. 2013, Liu et al. 2016).

Taxa on the phylogeny tree were shuffled with 999 permutations following Swenson (2014) to produce a random distribution of phylogenetic relationships expected based on the species community assembly. Phylogeny was randomized in favour of randomizing community data to maintain species richness patterns (Swenson 2014). This null distribution was compared to the observed value for each diversity metric to determine if phylogenetic distribution of chronosequence species observed at each of the 24 pools is nonrandom with regards to phylogeny. Standard effect size of the measured diversity metrics were compared to null models using functions *ses.pd*, *ses.mpd,* and

ses.mntd in R package *picante*. If the observed standard effect size is positive, the observed value of the metric is greater than the average random expectation, and the quantile p-value will be greater than or equal to 0.975, and the metric is greater than expected, implying phylogenetic over-dispersion compared to the null expectation. If p-value is less than or equal to 0.025, the metric will be lower than expected based on community species assembly, implying phylogenetic under-dispersion.

To visually compare the phylogenetic species pairwise dissimilarities to the functional trait dissimilarities, a functional dendrogram was constructed on a dissimilarity matrix combining all four traits using Gower distance in R package *phytools.* Similarities between the phylogeny and the functional dendrogram were drawn using *cophylo* in *phytools*.

Results

Functional trait patterns

A multiple regression test on functional traits values of all thirty-three sampled species in Scotland, Wales, and England detected no spatial autocorrelation of functional traits across sampling country ($p = 0.825$).

Trait variation was captured mainly by a single principal component, PC1 (95.9% of variation, Fig. 3). 4% of variance was explained by principal component 2, and no proportion of variance was explained by remaining principal components 3 and 4. Traits SA:V and STA have the highest loadings on the PC1 axis (0.846 and 0.532, respectively) and contribute the most to explaining variation in the data in this axis while TDMC does not contribute appreciably (2.61×10^{-6}).

Pairwise correlation tests between traits (Table 1) show a strong positive correlation between STA and SA:V, while both are negatively correlated with thickness. Meanwhile, no correlation was observed between TDMC and any other trait. The first principal component (PC1) derived from the traits was unrelated to TDMC while it was strongly and positively related to the other three traits (STA, SA;V, thickness).

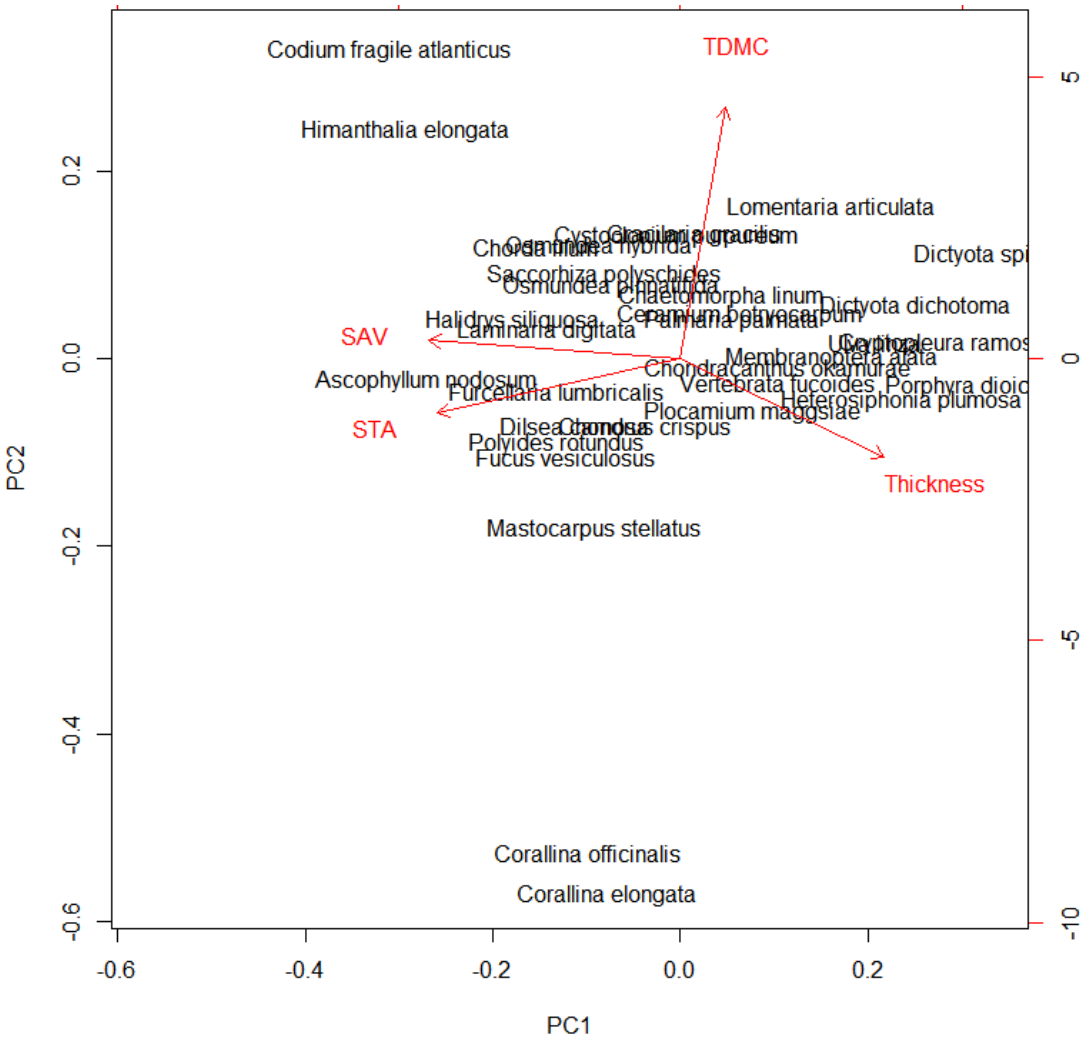


Figure 3. Principal component analysis showing the two main principal components for the four functional traits measured: TDMC, STA, SA:V and Thickness.

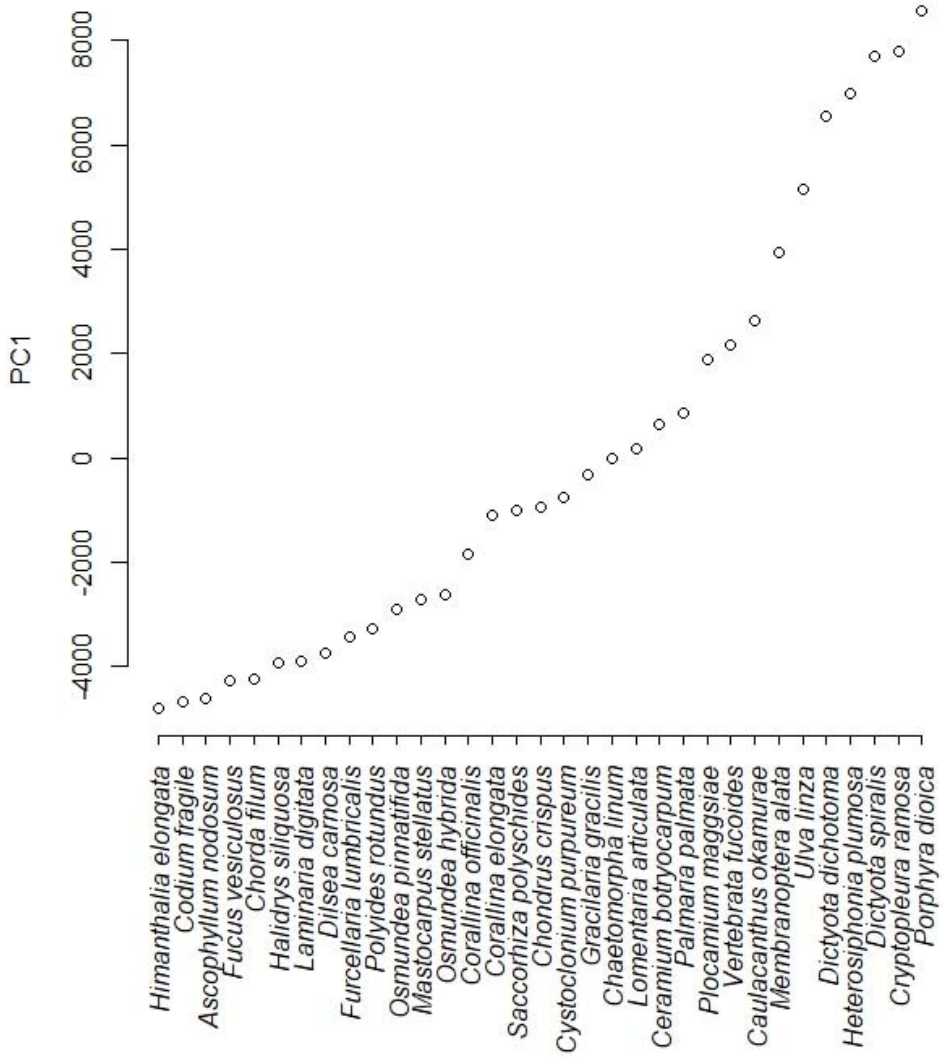


Figure 4. Principal component 1 (PC1) values for all 33 macroalgal species. Species positions on other axes are not shown because they explain <5% of overall variance in trait values.

Table 1. Pairwise trait correlation tests between all functional traits (TDMC, SA:V, Thickness, STA) and principal component 1 (PC1) measured by Kendall's τ (tau) coefficient with confidence level = 0.95.

	STA	**SA:V**	**Thickness**	**PC1**
TDMC	$\tau = -0.189$ $p = 0.126$	$\tau = 0.0379$ $p = 0.770$	$\tau = -0.098$ $p = 0.432$	$\tau = -0.049$ $p = 0.701$
STA		$\tau = 0.735$ $p = 1.113 \times 10^{-11}$	$\tau = -0.568$ $p = 8.619 \times 10^{-7}$	$\tau = 0.814$ $p = 3.553 \times 10^{-15}$
SA:V			$\tau = -0.742$ $p = 5.652 \times 10^{-12}$	$\tau = 0.837$ $p = 4.44 \times 10^{-16}$
Thickness				$\tau = -0.746$ $p = 3.998 \times 10^{-12}$

Evolutionary patterns

A dated phylogeny spanning the Rhodophyta, Phaeophyta, and Chlorophyta was developed. Bootstrap values for the ML phylogeny strongly support nodes leading to divergence between Rhodophyte, Chlorophyte, and Phaeophyte clades (Fig. 4). The Rhodophyte clade appears to have diversified earliest of the three macroalgal clades, while extant species of Phaeophyta are the most recently evolved with a crown node estimate of 205.24 million years ago (Fig. 5).

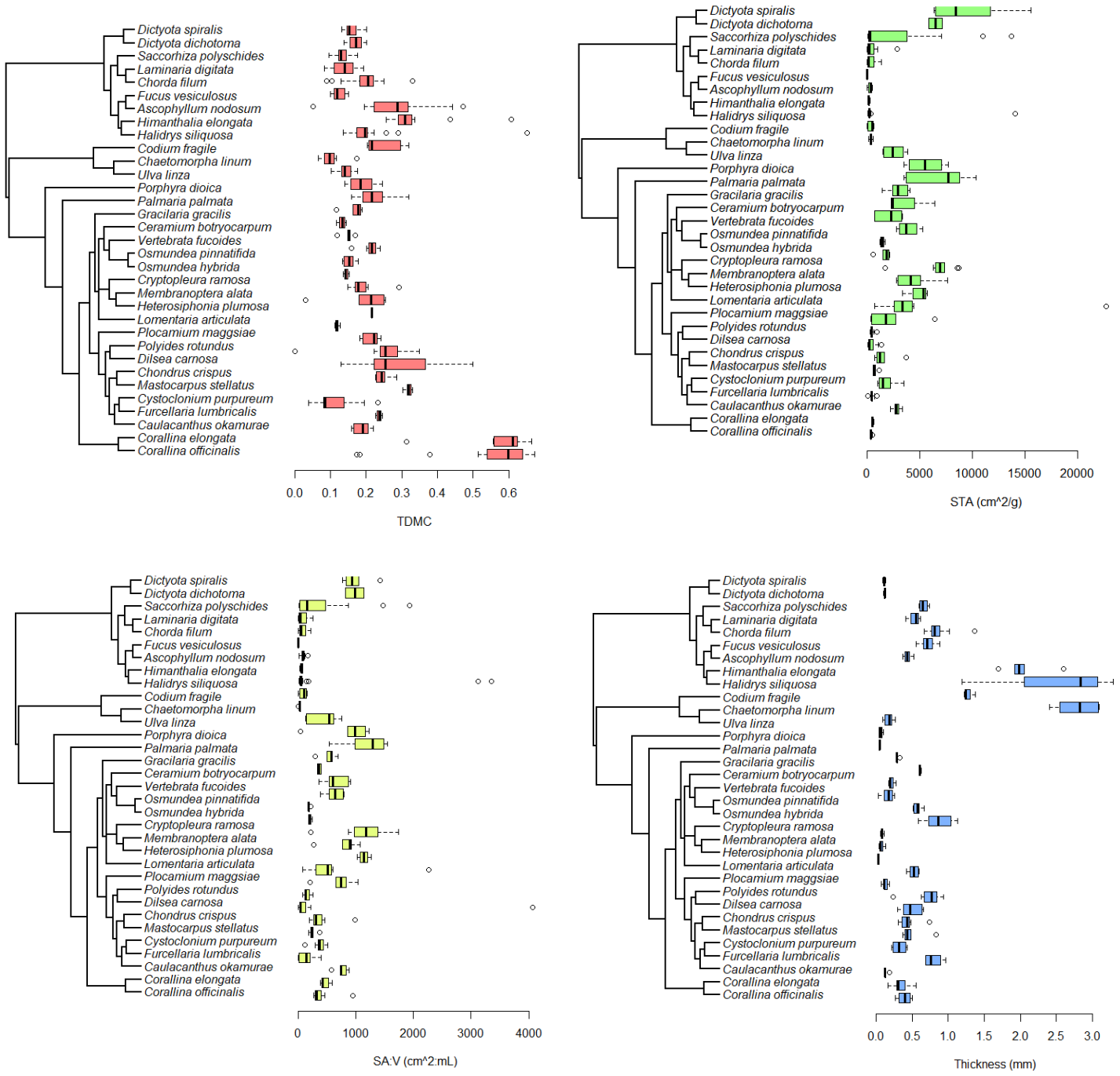


Figure 5. Four functional traits (TDMC, STA, SA:V, STA, Thickness) mapped onto Maximum Likelihood phylogeny of 33 species. Nodes display bootstrap support values (n=1000).

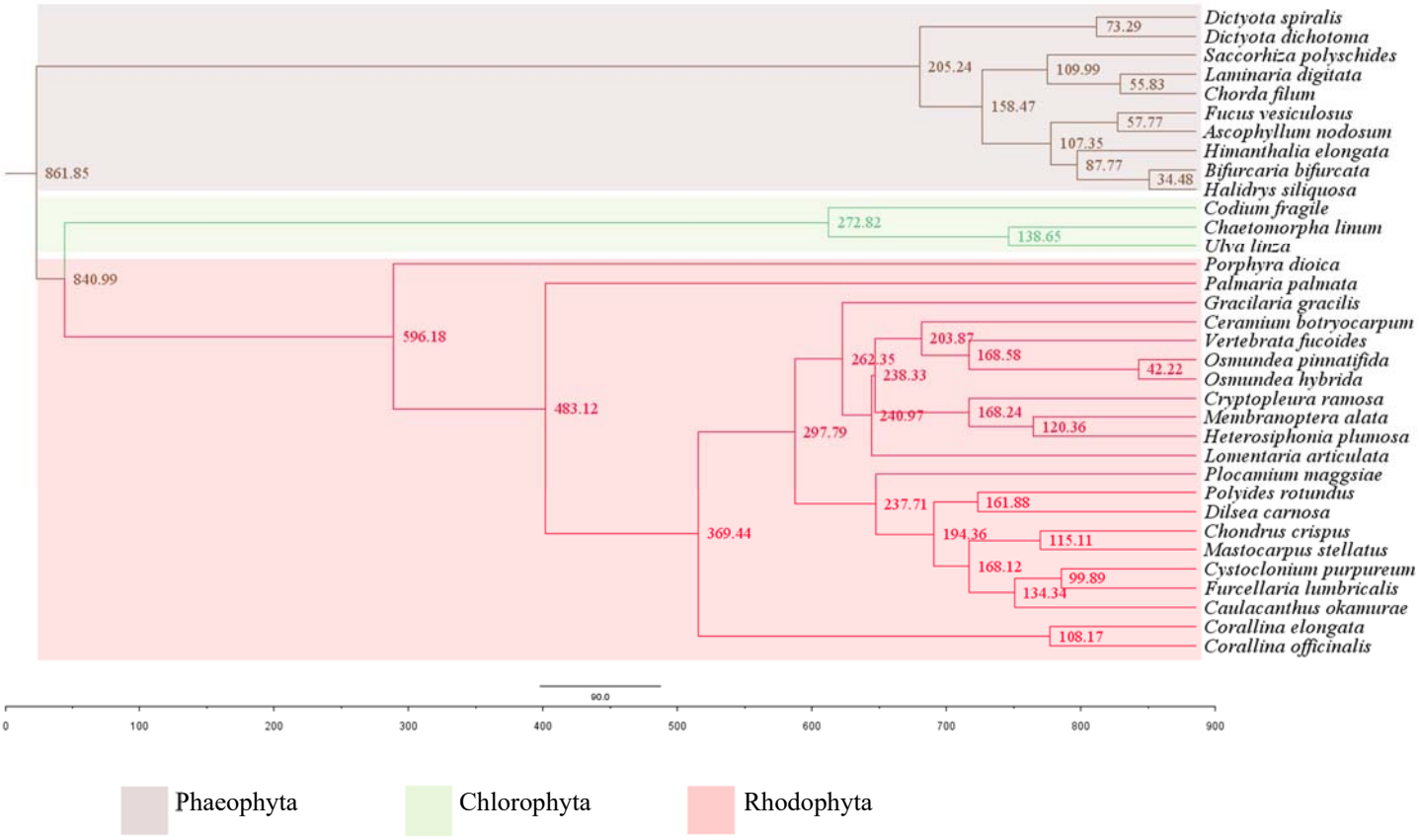


Figure6. Phylogenetic tree with Phaeophyte, Chlorophyte, and Rhodophyte clades distinguished by colour. Dated node divergence dates are presented in millions of years from root (MY).

Phylogenetic pattern in traits

STA, SA:V, TDMC and PC1 consistently show statistically significant phylogenetic signal both within Rhodophyta clades, and across these clades combined with Chlorophyta (All, Table 2). A strong phylogenetic signal was also displayed for TDMC within Rhodophyta clade ($K \geq 1$; Table 2, Fig. 6). These significant results suggest these traits have evolved as expected according to Brownian motion, and variation in these traits is dictated by the evolutionary distances between them. Values for SA:V, STA, and PC1 are significantly greater than K=1 within the Phaeophyta and suggest niche conservatism among closely related species. There is a low value of K (<0.6 for all traits) when measured across all three clades combined (Table 2) which may indicate that variance in trait values is partitioned within individual clades, as expected. Principal

component 1 on the PCA, which accounted for most of the variation in trait values among species, showed a significant phylogenetic signal (Table 2, Fig. 6). The phylogenetic signal was not calculated for Chlorophyta as the group is too small and the signal will have implicit variability (Blomberg et al. 2003).

Table 2. Phylogenetic signal (Blomberg's K) of the four functional traits and principal component 1 of the PCA combined across Phaeophyta, Chlorophyta, and Rhodophyta clades (All) and for the Phaeophyta and Rhodophyta separately.

	All	Phaeophyta	Rhodophyta
	K	K	K
TDMC	0.611**	0.786	1.162**
Thickness	0.320	0.786	0.577
STA	0.591**	2.30**	1.39**
SA:V	0.570**	2.53**	1.07**
PC1	0.588**	2.36**	1.18**

**$P<0.01$

Trait evolution

Trait evolution over time is demonstrated with trait state estimation at internal nodes of the phylogeny and visualised with a colour gradient (Fig. 7). As the dated Bayesian phylogeny is used for this plot, branch lengths represent evolutionary time in millions of years (MY). Convergent evolution is illustrated by emergence of similar colours towards terminal tips in different clades. The relative homogeneity of colour along branch lengths in thickness (Fig. 7 d) over evolutionary time and across clades is consistent with a lack of phylogenetic signal for this trait (Table 2). Outliers in trait values are immediately apparent (*C. elongata* and *C. officinalis*, Fig. 7 a; *D. spiralis* and *D. dichotoma*, Fig. 7 e). The presence of a calcite skeleton in *Corallina* may be the cause of outlying trait values.

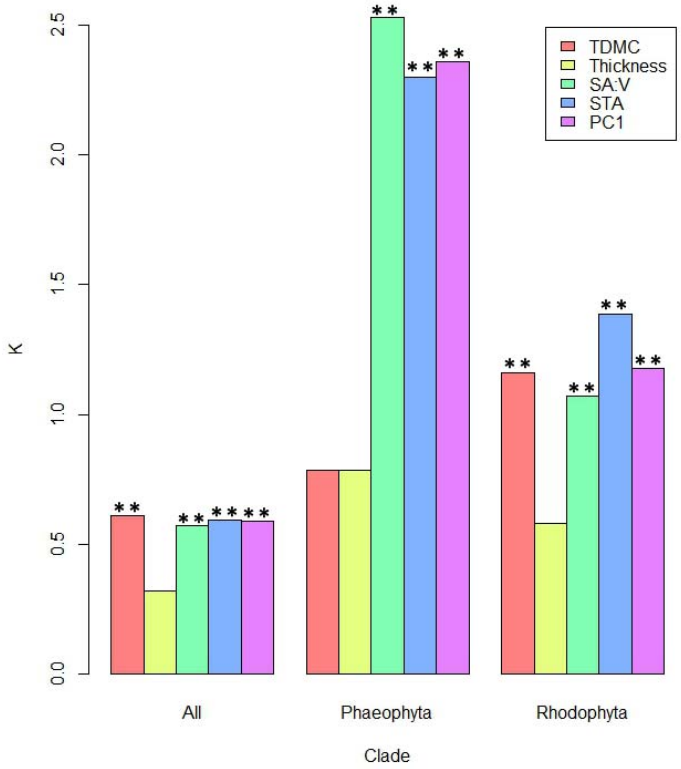


Figure 7. Phylogenetic signal (Blomberg's K) across the major phylogenetic clades represented in the dataset for individual functional traits and species scores for principal component 1. ***P*<0.01.

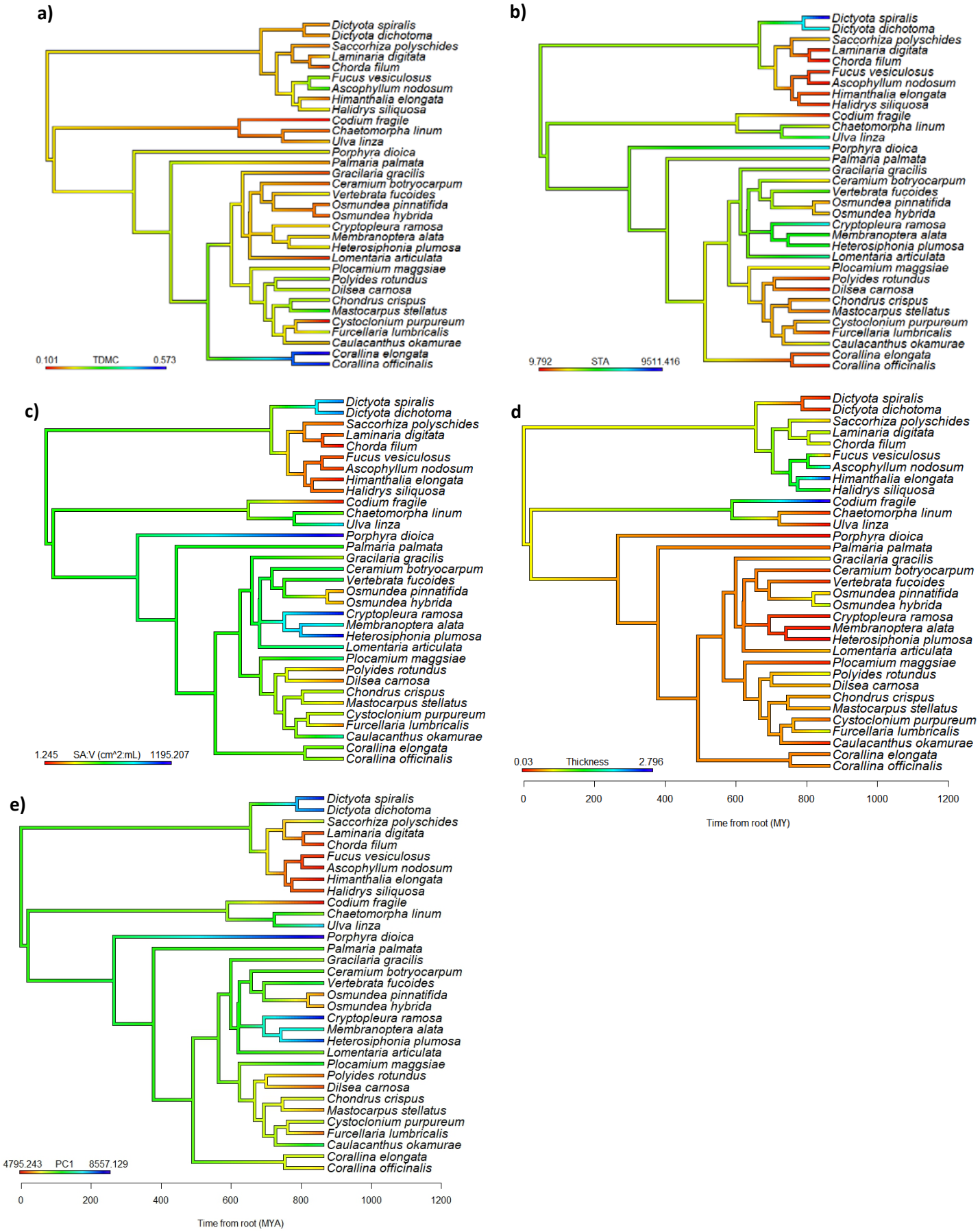


Figure 8. Ancestral functional trait state reconstruction for **a)** TDMC, **b)** SA:V, **c)** STA **d)** Thickness and **e)** PC1 across 33 macroalgal species. Branch lengths represent evolutionary time (MY).

Trait estimation

Trait evolution concordant with expected values based on phylogeny demonstrates a detectable pattern in macroalgal species trait values (Table 3), and is a requisite for phylogenetic estimation of traits.

Table 3. Estimated TDMC, thickness, SA:V and STA trait values and standard error (±SE) for the 3 Phaeophyte (*L. differmis*, *S. muticum, S. lomentaria*) and 8 Rhodophyte chronosequence species with unmeasured traits.

	TDMC		Thickness		SA:V		STA	
	estimate	SE	estimate	SE	estimate	SE	estimate	SE
Leathesia differmis	0.19	0.61	1.18	0.61	1378.41	0.61	739.93	0.61
Sargassum muticum	0.17	0.72	2.04	0.72	880.38	0.72	482.37	0.72
Scytosiphon lomentaria	0.19	0.61	1.18	0.61	1378.41	0.61	739.93	0.61
Apoglossum ruscifolium	0.27	0.72	0.50	0.72	3863.55	0.72	1776.89	0.72
Boergeseniella fruticulosa	0.27	0.72	0.50	0.72	3863.55	0.72	1776.89	0.72
Callophyllis laciniata	0.24	0.5	0.53	0.50	4122.56	0.50	2059.71	0.50
Cladophora sp.	0.18	0.51	0.23	0.51	8309.42	0.51	5260.45	0.51
Dumontia contorta	0.23	0.5	0.53	0.50	4042.37	0.50	2034.89	0.50
Gastroclonium ovatum	0.21	0.67	0.52	0.67	4106.18	0.67	2251.33	0.67
Gelidium crinale	0.31	0.72	0.48	0.72	3969.87	0.72	1595.73	0.72
Vertebrata fucoides	0.27	0.72	0.50	0.72	3863.55	0.72	1776.89	0.72

Principal component analysis

A principal component analysis including all chronosequence species with estimated and measured traits values returned PC1 as the main explanatory axis for trait variation (98%; Fig. 8). PC2 explains 0.02% of variation, and PC3 and 4 do not contribute.

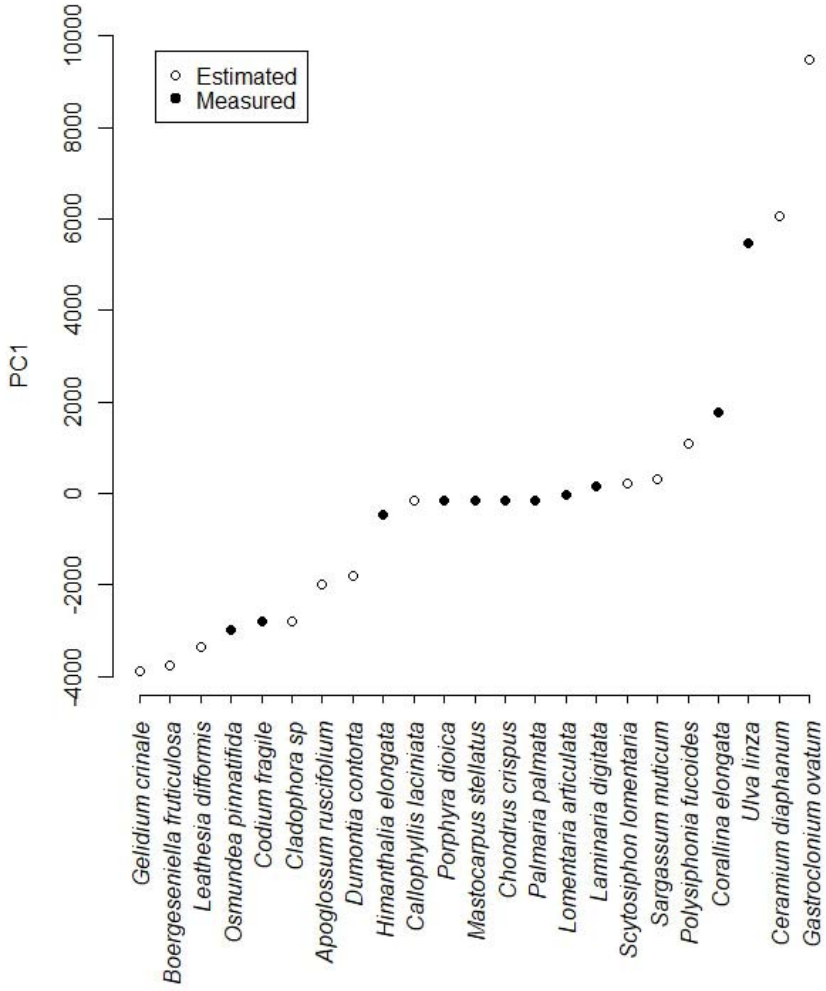


Figure 9. Principal component 1 (PC1) scores for the 23 species in the chronosequence with estimated and measured trait values.

Functional and phylogenetic diversity within a community

Abundance-weighted and unweighted alpha phylogenetic diversity metrics explored the phylogenetic pattern of relatedness between macroalgal species in the chronosequence (see Methods). A generalized linear mixed effect model (GLMM) demonstrated phylogenetic and functional diversity variation with community succession,

considering species richness and assemblage age but controlling for non-independence with block number by including it as a random effect.

Two measures of phylogenetic diversity, PD and MPD, increased over succession in the community, while MNTD decreased (Table 4, Fig. 9 a, c, e). The significant relationship found between phylogenetic distance (PD) and mean pairwise distance (MPD) with species richness (Table 4, Fig. 9 a, c) is expected from the formula of PD, a tree-based metric which sums branch lengths across the topology, and MPD, a distance-based method that measures the mean pairwise distance between all species. Abundance-weighted phylogenetic diversity measures all showed a decrease in phylogenetic diversity with community succession.

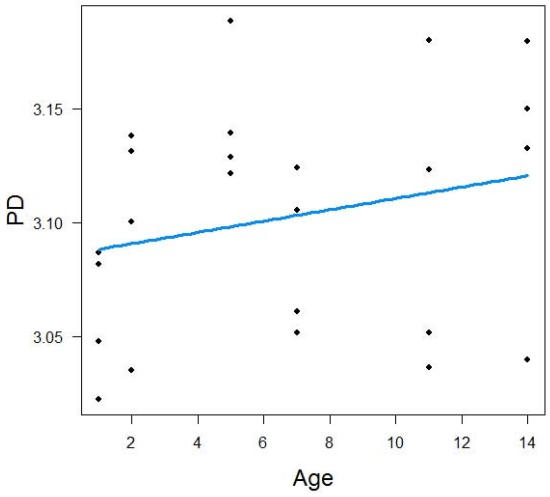
Abundance-weighted MPD has a significant negative trend. In contrast, there is a positive trend in unweighted MPD with community age, signifying that species are more closely spaced as species richness increases (Table 4, Fig. 9c-d). For PD and MPD, directionality of relationship is changed with unweighted and abundance-weighted versions of metrics (Fig. 9a-d). Significant negative relationships between abundance-weighted mean nearest taxon distance ($MNTD_{AB}$) (Fig. 9 f), and MPD (Fig. 9 c) are observed. Functional diversity increases over time (Fig. 9 g-h), and this relationship is explained by species richness rather than assembly age (Table 5). A positive relationship between functional diversity (FD) and phylogenetic diversity (PD) suggests that more distantly related species fill more functional roles.

Table 4. Generalized linear mixed effects model (GLMM) for indices of abundance-weighted ($_{AB}$) and unweighted phylogenetic (PD, MNTD, $MNTD_{AB}$, MPD, MPD_{AB}) and functional (FD) alpha diversity across differences in assemblage age and species richness (SR). Block identity was a random effect.

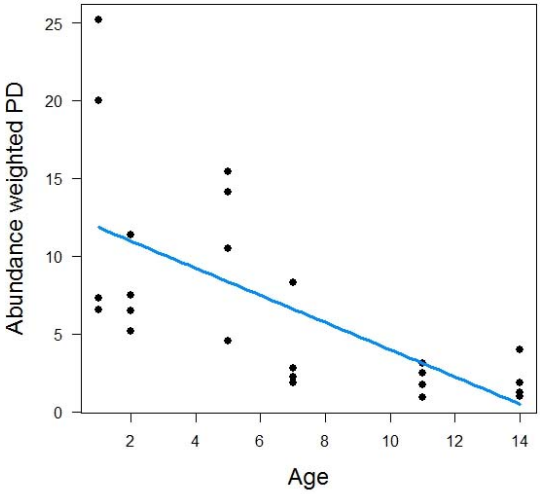
Metric	Fixed effect	Estimate	Standard Error	df	t value	Pr(>|t|)	Random effect Variance	Random effect SD
PD	Age	0.002	0.003	7.495	0.881	0.405	0.001	0.023
	SR	0.084	0.004	20.178	20.896	0.00*		
PD_{AB}	Age	-0.8732	0.2281	21.000	-3.828	0.001*	0	0
	SR	-0.2321	0.3459	21.000	-0.671	0.509*		
MPD	Age	0.010	0.006	21.000	1.635	0.117	0	0
	SR	-0.051	0.009	21.000	-5.400	0.000*		
MPD_{AB}	Age	-0.019	0.016	10.114	-1.248	0.240	0.037	0.192
	SR	0.024	0.019	20.465	1.258	0.223		
MNTD	Age	-0.010	0.004	21.000	-2.248	0.036*	0	0
	SR	-0.038	0.007	21.000	-5.622	0.000*		
$MNTD_{AB}$	Age	-0.085	0.024	7.604	-3.596	0.008*	0.125	0.354
	SR	-0.042	0.019	13.100	-2.212	0.045*		
FD	Age	0.005	0.003	21.000	2.067	0.051	0	0
	SR	0.045	0.004	21.000	11.291	0.000*		
FD_{AB}	Age	0.008082	0.023696	9.763	0.341	0.7403	0.05261	0.2294
	SR	0.070903	0.032437	20.89	2.186	0.040*		

*$P<0.05$

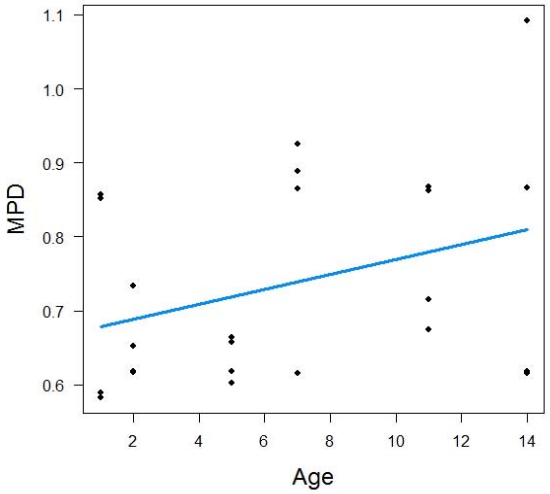
a)


PD
3.15
3.10
3.05
2
4
6
8
10
12
14
Age

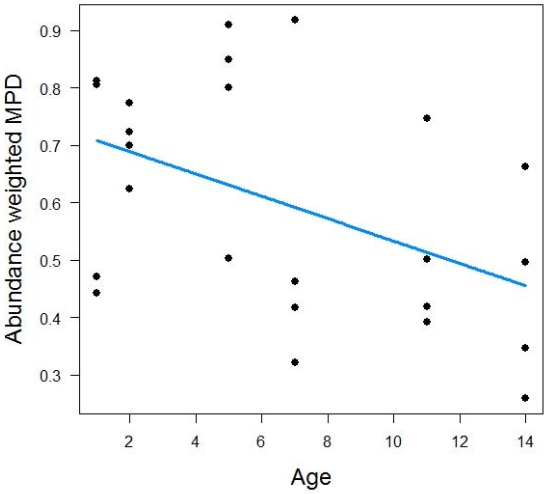
b)


Abundance weighted PD
25
20
15
10
5
0
2
4
6
8
10
12
14
Age

c)


MPD
1.1
1.0
0.9
0.8
0.7
0.6
2
4
6
8
10
12
14
Age

d)


Abundance weighted MPD
0.9
0.8
0.7
0.6
0.5
0.4
0.3
2
4
6
8
10
12
14
Age

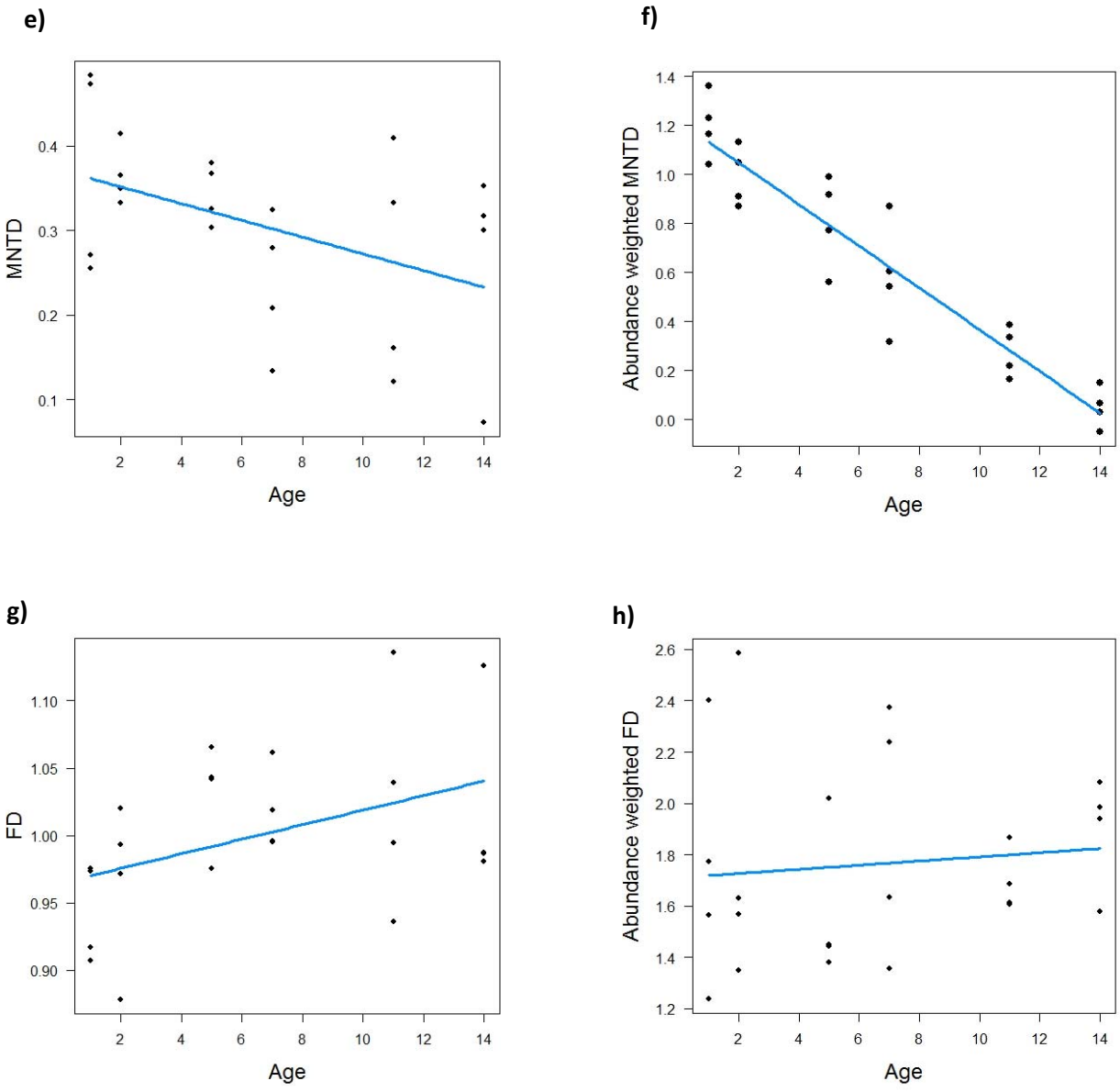


Figure 10. Regression plots for observed phylogenetic (**a-f**) and functional (**g-h**) abundance-weighted and unweighted alpha diversity metrics across community age for the 24 assemblages in the chronosequence. MNTD: mean nearest taxon distance; MPD: mean pairwise distance; PD: Faith's phylogenetic diversity; FD: functional diversity.

Dispersal of phylogenetic relationships

Alpha diversity metrics were used to determine whether assemblages in the chronosequence were over-dispersed (more distantly related) or under- dispersed (more clustered) than expected with regards to their phylogenetic distribution compared to a null model.

Expected values of MPD, MPD_{AB}, MNTD, $MNTD_{AB}$, and PD based on the assemblage were calculated by a randomization of phylogenetic taxa (n=999) for each assemblage. The null model is the species relatedness expected by chance from the assemblage. The rank of the observed metric value was compared to the distribution of randomized values.

Standard effect size plots show that the overall phylogenetic pattern of relatedness between species in all communities for each metric measured was not over- or under-dispersed when compared to expectation from the assemblage (Fig. 10). MPD values are statistically significantly over-dispersed with regards to expectation based on community assembly for three assemblages, indicated by red circles where quantile p-values ≥ 0.975 (Fig. 10).

The co-species associations between phylogeny and functional traits in the chronosequence species are visualized (Fig. 11). Dotted lines joining each individual species' location on the phylogeny to its location on the dendrogram serve to contrast the groupings of species that result from their phylogenetic and functional similarities.

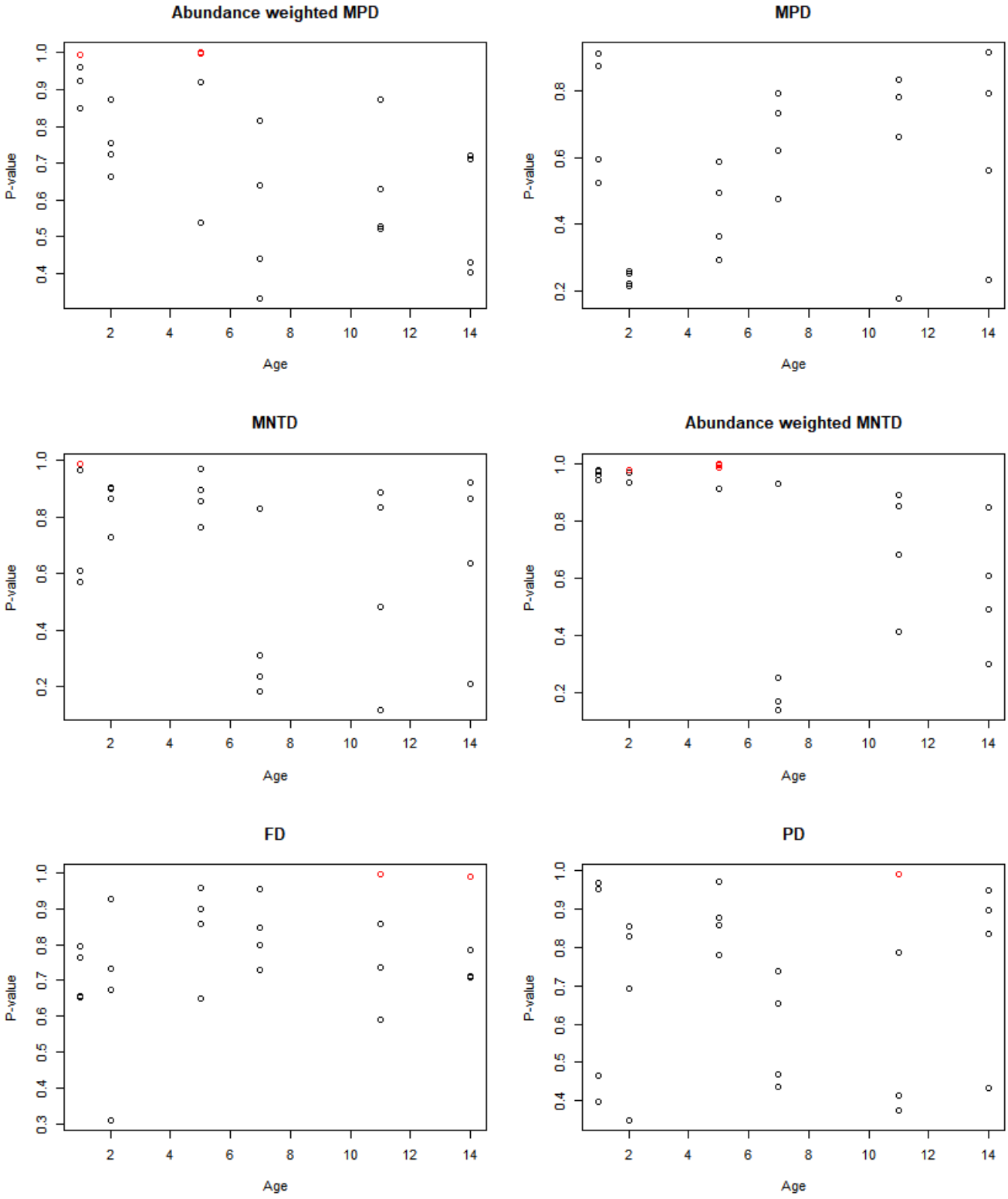


Figure 11. Quantile P-values of observed phylogenetic and functional alpha diversity metrics compared with null communities (n=999) over chronosequence age. Constrained randomization of phylogenetic taxa was performed with community and species richness held constant. Red points indicate statistically significant phylogenetic over-dispersion ($P \geq 0.975$). No under-dispersed communities ($P \leq 0.025$) were observed.

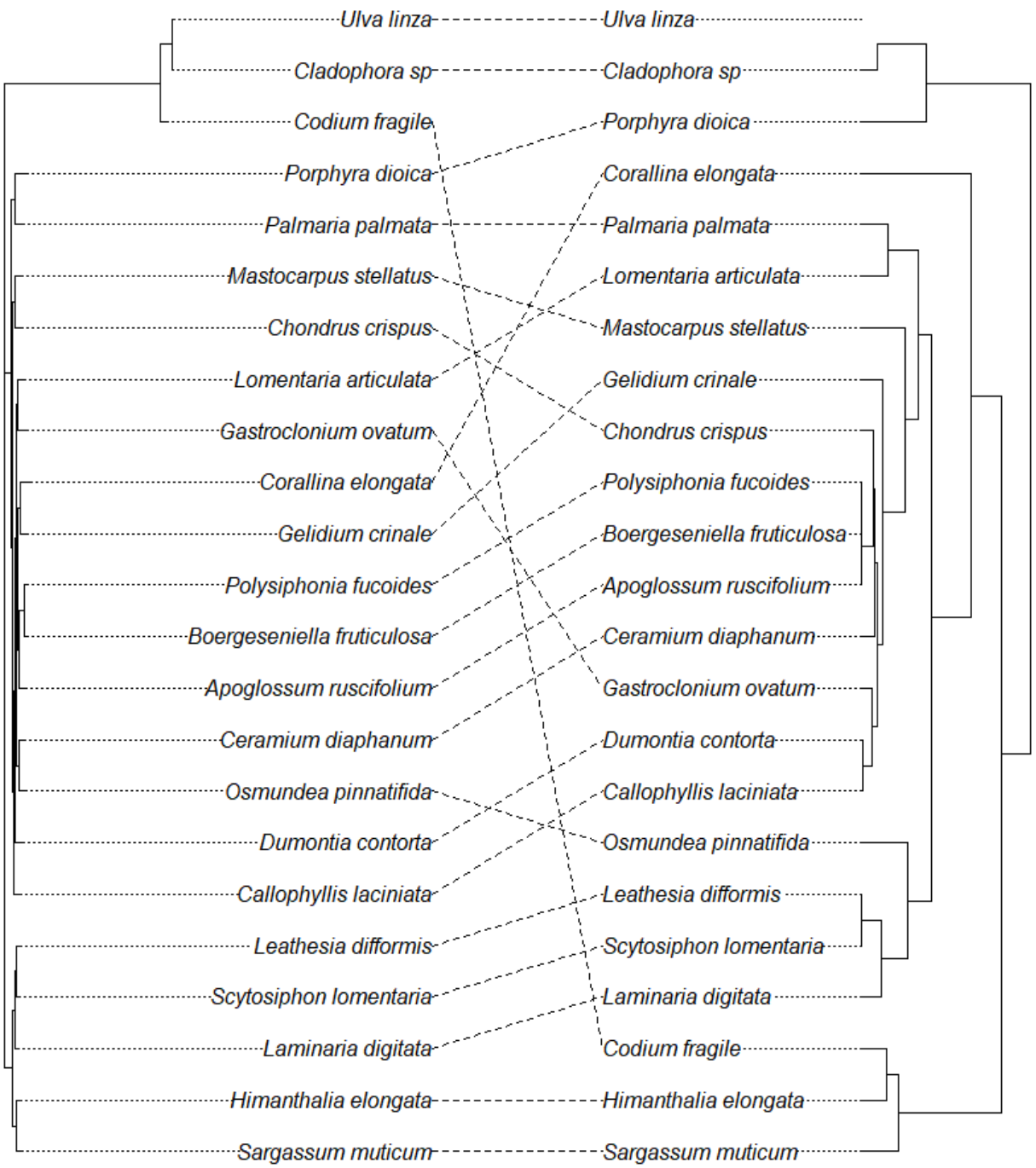


Figure 12. The relationship between phylogeny (left) and functional dendrogram based on phylogenetic and functional trait dissimilarity matrices for 23 macroalgal species in the Plymouth breakwater chronosequence community.

Discussion

A novel, dated phylogeny spanning the three macroalgal clades, Chlorophyta, Rhodophyta, and Phaeophyta was constructed. A strong phylogenetic signal for three of four traits, TDMC, STA, and SA:V, was observed within the two well-represented clades, Rhodophyta and Phaeophyta. These traits were demonstrated to be sensitive to phylogeny and evolve as expected according to a Brownian motion model of evolution. Thickness was not found to have a phylogenetic signal within the Phaeophytes or Rhodophytes. Functional diversity was demonstrated to increase with community succession. While phylogenetic alpha diversity metrics sensitive to species richness displayed an increase in phylogenetic diversity over community succession, all abundance-weighted metrics showed a decrease in phylogenetic diversity over community succession. The community was found to be phylogenetically even with regards to species assembly.

Topologies obtained in the Maximum likelihood (ML) and Bayesian inference phylogenetic analyses were congruent (Figures 4 and 5, respectively) and relationships match those published in phylogenies of individual clades (Karol 2002, Silberfeld et al. 2010, Verbruggen et al. 2010, Leliaert et al. 2012, Qiu et al. 2016). This suggests that it is possible to have some confidence in the phylogeny constructed here. Low bootstrap values for some nodes in the Rhodophyte clade may be the result of an incomplete concatenation matrix during sequence assembly, causing gaps that lead to lack of replacement for certain sequence regions. Including full genome data, rather than individual genes, would further increase accuracy of trait estimation (Cooper et al. 2001). Traits for every unobserved species were estimated using phylogenetic and trait data for the most recent common ancestor that is shared with that species. The traits are expected to vary directly proportionally to branch length between species (Garland and Ives 2000). The standard error of the *phyEstimate* calculations represents the error associated with the branch lengths used in the estimation of the true value and for this reason was identical in the species who had a recent common ancestor which was the same number of branch length units away. The use of fossil evidence as priors to specify some of the divergence nodes in the Bayesian inference phylogeny may also be a limitation to the accuracy of the

estimate; fossil evidence provides only a reliable minimum age constraint for the taxa identified, while an estimation of the maximum age, when the organism first appeared, cannot be known from a single piece of fossil evidence. As brown algal species have soft tissue and lack a calcareous skeleton, they are poorly represented on the fossil record (Silberfeld et al. 2010). To address this issue, this study uses published MRCA phylogenies to supplement fossil evidence to support these dates.

The phylogenetic signal for STA, SA:V, TDMC, and PC1 was strong within Rhodophytes, indicating that variation in those traits can be predicted by phylogeny by this major macroalgal clade. STA and SA:V were the main contributors to PC1. The power of the phylogeny to predict PC1 suggests that it may be a good predictor of a function that both traits measure, which is mainly their contribution to primary productivity (Wallentinus 1984). Using multiple relevant traits for an ecological process of interest may allow phylogeny to predict not only a single trait, but shed light on an organism's contribution to an aspect of ecosystem functioning (Figure 1). In Phaeophytes, STA, SA:V and PC1 produced a statistically significant K value that was much higher than expected based on the Brownian motion model. This suggests that variation in these traits is more conserved in this group than expected by the model, and cannot be predicted by phylogeny alone. Conservatism in these traits also reflects the membership of all Phaeophytes expect two, of the *Dictyota* genus, to the same functional-form group of species with thick and leathery morphologies (Littler and Littler 1980). In contrast, the Rhodophytes measured span several of these groups. Another macroalgal study by Stepien et al. (2016) measured diversity in carbon concentrating mechanisms across clades, also an aspect of photosynthesis, similarly found a phylogenetic pattern within the Rhodophytes. This 2016 study examined whether evolution of carbon concentrating mechanism was conserved within red and brown clades. Similar to my research, this study postulated the presence of a phylogenetic pattern in traits that are related to photosynthetic capabilities that are measurable across marine macrophytes. Theseauthors report an absence of a pattern within the Phaeophytes across clades and postulate the difference in clade age may be responsible for the difference in phylogenetic signal level. This may limit the inferential ability from phylogeny across the

Phaeophytes as a group with this particular evolutionary model of phylogenetic signal estimation.

Variation in thickness was not found to be predicted by phylogeny (Table 2, Fig. 6). It was the only trait which did not display a statistically significant relationship at any organizational level in the phylogeny. The lack of a phylogenetic signal suggests that this trait evolution is directed mainly by a process that is external to its evolutionary history. The mapping of thickness evolution along the phylogeny visually represents the lack of any pattern in thickness evolution (Fig. 7). Based on the presence of a strong phylogenetic signal, trait values were estimated (Table 3). These estimations had low standard errors. However, direct measurement of trait values in these species, and comparison to those estimated based on the phylogeny, would be necessary to validate this claim and the use of phylogenetic information to impute trait values in macroalgae more generally.

The use of the Brownian model of evolution is one possible study limitation. The Brownian motion model of evolution used in phylogenetic signal detection has been criticized for being overly simplistic and limited in biological application to continuous traits: traits are biologically bounded by environment and so cannot have infinite random values, and linear evolution over geological time ignores biotic and abiotic interactions which drive speciation (Blomberg 2016). The former concern was addressed by development of Ornstein-Uhlenbeck model, which states trait values may diverge at random but the mean trait value is always the same (Beaulieu et al. 2012). The latter concern is addressed by the ‘Early burst’ model of adaptive radiation (Simpson 1944, Harmon and Glor 2010). Despite limitations, the Brownian motion model remains the most commonly used as the only model of the three to provide a widely applicable mechanism for trait inference (Helmus et al. 2007). Future studies could use Akaike Information Criteria to select the best model of evolution to measure the traits of interest (Posada and Crandall 1998)

This study has demonstrated various ways in which functional and phylogenetic diversity varied with community succession are related to one another. The generalized linear mixed model showed the increase of both abundance weighted and unweighted

functional diversity with succession. Both were significant with regards to the species richness covariate in the GLMM. This demonstrates that functional diversity increase in succession may be due to the increase in species richness. Functional richness, or the number of different functions performed in an ecosystem, has been demonstrated to increase with species richness (Mason et al. 2005). Ricklefs & O'Rourke (1975) report a correlation of functional diversity with species richness in different tropical moth assemblages, but hesitate to conclude that the relationship is not instead an indirect effect of competition, or environmental factors allowing a different diversity of species to co-exist in different communities. Because functional traits are rarely directly measured across species in macroalgal communities, and functional trait databases analogous to those for terrestrial plants are lacking, previous authors have not been able to apply a functional diversity approach to macro algal communities. My results represent an initial attempt, but further analysis to evaluate the potential non-linearity in the response of functional diversity to community age are warranted.

As expected, the response of phylogenetic diversity to community age, or successional stage, was found to depend on the diversity metric implemented. Faith's phylogenetic diversity metric increased with community succession. MNTD decreased over time while abundance weighted metrics displayed the opposite trend, which was predicted. Contrary to expectation, MPD was found to increase over time, showing a significant relationship with species richness. This finding is consistent with findings in a terrestrial plant community, which finds that presence of more distantly related organisms is related to an increase in this diversity metric (Valiente-Banuet and Verdú 2007).

Standard effect size plots show that the overall phylogenetic pattern of relatedness between species in all communities for each metric measured was not over- or under-dispersed when compared to expectation from the assemblage (Fig. 10). MPD values for three assemblages are statistically significantly over-dispersed (Fig. 10). However, significant values returned for one in twenty plots may be expected due to random chance (Cadotte and Davies 2016). There is likely little biological significance, and are too few over-dispersed values to support a general conclusion about a trend across the assemblage. Of the 24 pools examined, there is no single assemblage for which

phylogenetic over-dispersal is consistent across diversity metrics. This finding suggests that the communities are not being strongly structured on the bases of phylogeny. In other words, species in similar or distant parts of the phylogeny are not being preferentially assembled into particular communities; the communities are assembled randomly with respect to phylogeny, and are phylogenetically even. This suggests that perhaps phylogeny is not strongly related to the traits of species determining their abilities to colonize pools at a particular stage. The presence of distantly related Rhodophyte and Chlorophyte taxa (*Ceramium spp*. and *Ulva spp*., respectively) in early successional stages, rather than more closely related species, supports this suggestion.

Value of this study

A lack of standardized trait measurement and appropriate statistical analysis methods for robust functional trait measurement has severely limited understanding of macroalgal community composition in the past. Combining a phylogenetic hypothesis with functional trait measurement provides strong inferential power in explaining community biodiversity and functioning. The demonstrated functional-phylogenetic link provides a vital step towards understanding variability observed in species functioning and in macroalgal community composition.

Future directions

In this study, the longitudinal gradient through the United Kingdom was used out of necessity, to collect as many species as possible that do not co-exist in a single location, rather than to examine geographical effects on the results. In future, the study could be extended over larger geographical area. This would probe whether the relationship between traits and phylogenetic distance hold true for the species in different sites, at different latitudes, and with gamma and beta diversity.

Incorporating phylogenetic analysis in biodiversity studies represents a practical advantage. Rapid development of whole-genome sequencing methods and wide accessibility of sequences in global NCBI database, GenBank, has lead phylogenetic reconstruction or data-mining from growing online repositories to be pragmatic approach

to supplement trait data than collect functional information for all species of interest. Measurement of functional traits that directly capture an aspect of function, such as competitive effect and dispersal distance, often is often made difficult by practical time and resource limitations (Drenovsky et al. 2012). A phylogenetic perspective on macroalgal community ecology can make the most of trait measures and bioinformatics tools to understand community assembly and predict biological diversity impacts on ecosystem functioning.

The history of an organism, taken together with present-day function, could predict the future, such as species community composition in response to environmental changes, or the likelihood and consequences of invasions. This functional-phylogenetic link could be vital in understanding species response to global change (Cavender-Bares et al. 2009, Tucker et al. 2016).

Conclusion

A dated phylogeny spanning the three macroalgal clades, Chlorophyta, Rhodophyta, and Phaeophyta demonstrates the strong trait convergence in an evolutionarily diverse group. This study has demonstrated that the observed trait variation in 33 macroalgal species, spanning the three macroalgal clades can be predicted by phylogeny. This shows that the evolutionary history has a strong ability to determine trait values across these sampled macroalgae of Britain. The link of functional traits to evolutionary history can provide valuable context informing functional and phylogenetic diversity, both important aspects of biodiversity (Díaz et al. 2007, Swenson 2011, Cadotte et al. 2013, de Bello et al. 2017). Utilizing both functional traits and phylogenetic information in macroalgal communities provides a more complete picture of the variation in biodiversity observed between communities.

Appendices

Appendix 1. GenBank accession numbers sequences used in phylogenetic analyses. **Dilophus,* known synonym for *Dictoyta*. **Previously known as *Polysiphonia fucoides*, "-" sequence not available.

Species	Gene		
	rbcL	**psbA**	**26S**
Ascophyllum nodosum	AJ287853.1	AY528844.1	AF102971.1
Caulacanthus okamurae	AF321113.1	-	-
Ceramium botryocarpum	AF439288.1	-	-
Chaetomorpha linum	EU380531.1	-	-
Chondrus crispus	U02984.1	AY119746.1	XM_005719315.1
Chorda filum	AY372983.1	AY528848.1	-
Cladophora rupestris	-	-	-
Codium fragile var. atlanticus	KJ909148.1	-	-
Corallina elongata	KP834400.1	JQ422231.1	-
Corallina officinalis	KJ591676.1	KJ637655.1	-
Cryptopleura ramosa	AF254175.1	-	-
Cystoclonium purpureum	KC174804.1	-	-
Dictyota dichotoma	AY422654.1	KF270598.1	-
Dictyota spiralis	DQ472081.1*	EU395616.1	-
Dilsea carnosa	KT310705.1	-	-
Fucus serratus	-	-	-
Fucus vesiculosus	DQ307680.1	DQ307679.1	AF102932.1
Furcellaria lumbricalis	U04194.1	-	-
Gracilaria gracilis	AY049400.1	-	-
Halidrys siliquosa	EU681595.1	FM958294.1	-
Heterosiphonia plumosa	AF259494.1	-	-
Himanthalia elongata	EF990246.1	EU681644.1	-
Laminaria digitata	AY372984.1	AY528849.1	JX442505.1
Lomentaria articulata	KU726701.1	-	-
Mastocarpus stellatus	U02992.1	-	-
Membranoptera alata	JX110927.1	-	-
Osmundea hybrida	FJ785317.2	-	-
Osmundea pinnatifida	KU566560.1	-	-
Palmaria palmata	U28421.1	KT886248.1	-
Plocamium maggsiae	JX969788.1	KU501335.1	-
Polyides rotundus	KC130221.1	-	-
Porphyra dioica	JN787102.1	-	-
Saccorhiza polyschides	AB045256.1	EU681658.1	-
Ulva linza	DQ813497.2	-	EU888138.1
*Vertebrata fucoides***	EU492913.1	-	-
Apoglossum ruscifolium	AF312310.1	-	-

Species	rbcL	psbA	26S
Boergeseniella fruticulosa	JX828161.1	-	-
Callophyllis laciniata	KF280986.1	-	-
Dumontia contorta	JN403062.1	-	-
Gastroclonium ovatum	DQ307680.1	DQ307679.1	AF102932.1
Gelidium crinale	JX096521.1	-	-
Leathesia difformis	KU726701.1	-	-
Lomentaria articulata	KU726701.1	-	
Sargassum muticum	AB776776.1	-	-
Scytosiphon lomentaria	AB022238.1	-	-

Appendix 2. Estimated macroalgal evolutionary divergence dates in millions of years ago (MYA) from fossil data and Bayesian molecular relaxed clock analysis methods.

Group	Divergence date (MYA +/- SD)	Method	Literature
Phaeophyta	128.90 +/- 30	Molecular relaxed clock, fossil data	(Medlin et al. 1997, Sanders and Lee 2007, Silberfeld et al. 2010)
Rhodophyta	740.60 +/- 80	Molecular relaxed clock, fossil data	(Berney and Pawlowski 2006, Du et al. 2016)
Chlorophyta	337.37 +/- 150	Molecular relaxed clock	(Berney and Pawlowski 2006)
Rhodophyta and Chlorophyta split	930.21 +/- 110	Molecular relaxed clock	(Berney and Pawlowski 2006)

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