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Ecological impacts of the non-native Macrophyte *Crassula helmsii* on freshwater macroinvertebrate assemblages in Dartmoor National Park, UK

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Abstract

Invasive species are a major threat to biodiversity globally. In freshwater ecosystems, invasive macrophytes are one of the most significant hazards. This study evaluated the impacts of the non-native macrophyte *Crassula helmsii* on macroinvertebrate assemblages in ponds in Dartmoor National Park, UK. Sampled ponds differed in the extent of invasion, being uninvaded, partially invaded and dominated by *Crassula*. Samples were taken from macrophyte stands using a hand net and taxa identified to the lowest taxonomic level possible in the laboratory. Assemblages were compared using univariate and multivariate approaches. Taxonomic richness and Shannon-Wiener Index did not differ significantly between invasion categories. However, abundance and evenness were significantly different where *Crassula* was predominant. Despite considerable variation between assemblages in individual ponds, assemblage composition differed significantly between invasion categories. Ponds where *Crassula* dominated harboured greater average abundances of non-native macroinvertebrates, of which *Physella acuta* constituted a substantial proportion. Although *Crassula*-dominated sites appear to support as many invertebrate taxa as those dominated by native vegetation, the identity of many taxa differs. In particular, *Crassula* appears to facilitate the spread of some scrapers and detrital shredders. Allelopathy, effects on water circulation and increased periphytic growth are likely the main causes of the impacts observed.

Keywords: Invasive macrophyte, *Crassula helmsii*, Freshwater ponds, Macroinvertebrates, Allelopathy

Introduction

Despite constituting only a considerably small proportion of all Earth's water, surface freshwater bodies are among the most biodiverse environments, harbouring approximately 10% of all described species (Mittermeier et al., 2010; Dijkstra et al., 2014). Freshwater itself is essential to the survival of human populations, and the exceptional biodiversity of freshwater ecosystems underpins multiple fundamental aspects of society by providing numerous ecosystem services (Dudgeon et al., 2006; Reid et al., 2019). However, due to ongoing global change, mediated or directly driven by anthropogenic activities, freshwater ecosystems are undergoing unparalleled biodiversity declines globally (WWF, 2020).

Non-native invasive (NNI) species have been identified as one of the greatest threats to freshwater biodiversity (Dudgeon et al., 2006). The detrimental effects of biological invasions on freshwater ecosystems include change of physical structure (Emery-Butcher et al., 2020), disruption of fundamental processes (e.g., litter processing, MacNeil et al., 2011), habitat degradation (Hermoso et al., 2011), changes in community structure (Schultz & Dibble, 2012) and, in the worst instances, extinction of native species (Ricciardi et al., 1998; but see Gurevitch & Padilla, 2004). Moreover, the impacts of NNI species can also have serious implications for humans: by influencing the processes that underpin ecosystem services, NNI species can interrupt the provision of essential resources (e.g., drinking water, Connelly et al., 2007) or, also, serve as vectors for parasites and pathogens (Plummer, 2005).

Amongst NNI organisms, aquatic plants, or macrophytes, have gained increased recognition as a major concern in freshwater ecosystems (Evangelista et al., 2014; Tanner et al., 2017). Typically, native macrophyte stands are associated with higher faunal biomass than unvegetated areas, particularly macroinvertebrates (Humphries, 1996; Thorp et al., 1997; Khudhair et al., 2019). The architecture of macrophytes is considered as one of the main factors structuring freshwater communities (Warfe & Barmuta, 2006; Thomaz & Cunha, 2010), as it can provide protection against predation and increase the availability of microhabitats, thereby influencing the diversity and abundance of macroinvertebrates (Clemente et al., 2019 and references therein). Further, native macrophytes are a source of food for a number of herbivorous and omnivorous taxa, and, upon death, plant matter can constitute an important food source for detritivores (Thomaz & Cunha, 2010). Because NNI macrophytes can supplant native vegetation (e.g., Michelan et al., 2010), successful invasions can substantially alter waterscapes and, consequently, disrupt biotic interactions. Thus, understanding if and how NNI macrophytes impact biological communities is critical to preventing biodiversity loss and the impairment of ecosystem function.

Established NNI macrophytes can have severe impacts on invaded ecosystems. For example, the Brazilian waterweed (*Egeria densa*) has been shown to obstruct sediment resuspension and remove nutrients from the water column, influencing phytoplankton growth (Yarrow et al., 2009). Madsen et al. (1991) quantified the photosynthetic rates of six native macrophytes under dense *Myriophillum spicatum* canopies, documenting a suppression of carbon balances in the native species. Michelan et al. (2010) reported decreased native macrophyte species richness and beta diversity induced by *Urochloa subquadripara*. Moreover, NNI macrophytes can deter feeding and impair the growth of aquatic herbivorous species (e.g., Erhard et al., 2007), altering trophic interactions. However, as Schultz & Dibble (2012) noted, the response of biological communities to NNI macrophytes depends on the species-specific traits of invaders. Also, site-specific conditions of the recipient environment – e.g., physicochemical water parameters (Pulzatto et al., 2019) and abiotic disturbance (Thomaz et al., 2012) – can be significant in determining the success of NNI macrophytes. Furthermore, Grutters et al. (2015) showed that three NNI macrophytes were virtually equivalent to native flora in terms of refuge provisioning for prey species; in contrast, predator-prey interactions and prey-specific traits were primary determinants of prey survival. Thus, to better recognise the effects of biological invasions, these need to be studied with respect to their location.

The swamp stonecrop *Crassula helmsii* (Kirk) Cockayne is a NNI macrophyte widespread throughout continental Europe, Ireland, and Britain, where it is most abundant (Smith & Buckley, 2020). The plant was likely introduced from Oceania to Britain through the aquaria trade at the beginning of the 1900s, but it was first reported as naturalised in 1956 (Laundon, 1961). Despite an initial patchy distribution, *C. helmsii* rapidly became widespread in the south of Britain, thanks to its capacity to reproduce asexually from small fragments and its resistance to extended periods of drought and frost (Dawson, 1994). Various authors have highlighted a potential to suppress native flora (Dawson & Warman, 1987; Leach & Dawson, 1999; Watson, 2001; Sims & Sims, 2016). However, Langdon et al. (2004) reported no significant effects on floral diversity, despite providing evidence for inhibited germination of six plant species and delayed hatching of newt eggs. Circumstantial evidence indicates that *C. helmsii* is often associated with low macroinvertebrate diversity, but available studies report no significant differences between invaded and non-invaded sites (Ewald, 2014; Smith, 2015; Smith & Buckley, 2015). However, no research has been conducted to identify possible differences in faunal composition between invaded and non-invaded sites.

Given the widespread distribution of *C. helmsii* and its potential to affect freshwater ecosystems, several attempts have been made to control its spread (Smith & Buckley, 2020). However, none of the methods employed has been successful in eradicating the species effectively. Moreover, destructive solutions (e.g., seawater inundation, Dean et al., 2013) can impact other macrophytes and animals, possibly causing greater damage than if no actions are taken at all. Manual or mechanical eradication is likely to be an effective means of control (van der Loop et al., 2018), but it cannot be implemented as a large-scale solution due to practical and economic limitations. A clear understanding of the impacts of *C. helmsii* on freshwater ecosystems could incentivise control programmes with adequate resources, but such understanding is currently lacking.

The aim of this study was to measure the effects of *C. helmsii* invasion on macroinvertebrate communities in freshwater ponds in the Southwest of the United Kingdom (UK). Specifically, it explored whether *C. helmsii* has a measurable impact on macroinvertebrate assemblage structure, diversity, abundance and functional feeding group composition.

Methodology

Study Area

Data were collected from Dartmoor National Park (Devon, UK) – an upland area underlain by fine and coarse granite, interspersed with igneous intrusions (Dearman & Butcher, 1959). The rock base layer is overlain with acidic soils exposed to frequent precipitation, which contributes to the formation of bogs, mires, and numerous seasonal ponds. Dry areas of Dartmoor are predominantly heathland, where acidophilic shrubs – mostly *Ulex gallii*, *Agrostis curtisii* and *Calluna vulgaris* – are widespread. Bogs and mires are dominated by species of perennial grasses, such as *Scirpus cespitosus*, *Eriophorum vaginatum*, *Molinia caerulea*, and several bog-mosses (*Sphagnum* spp.; JNCC, 2020). Due to the soil acidity, the area supports relatively extensive grazing (Meyles et al., 2006). Given its many small ponds, some of which were created for stock watering, the area is highly suitable for investigating the impacts of *C. helmsii*.

On Dartmoor, *C. helmsii* is found in terrestrial, emergent and submerged forms. Where it occurs, it can form dense stands which can obstruct the growth of other macrophytes. Reports of occurrence in Devon have risen considerably since the first record, and the number of reports reflects the trend of all British counties (see Smith & Buckley 2020).

Ponds

Sampling occurred over the course of one week between October and November 2020. Prior to fieldwork, Dartmoor was visited on several occasions and a list of past *C. helmsii* reports in Devon was obtained from the Devon Wildlife Trust to find invaded sites suitable for sampling. Of the waterbodies visited, 12 ponds were chosen on the basis of the extent of *C. helmsii* invasion (i.e., overall *Crassula* cover), with three invasion categories being distinguished: uninvaded ponds (“Absent ”, n = 6), invaded ponds where *C. helmsii* presence was limited to isolated patches within the waterbody (“Sparse”, n = 3), and ponds where dense *C. helmsii* stands were predominant (“Dominant”, n = 3; for examples of each category see Figure 1). Pond depth, pH, conductivity, and turbidity (Formazin Attenuation Units, FAU) were measured on site using portable probes (conductivity: YSI Pro2030; pH: YSI Model 63; turbidity: HACH DR900). Probes were recalibrated with deionised water after each measurement to avoid inaccuracy in subsequent records. Macrophyte species richness was estimated by counting the species present around the sampling area. Pond area was estimated from aerial images obtained from Google Earth Pro 7.3.3.7786 (Google, 2020) dated October-November for the years 2017-2019, in order to obtain an approximate measure of each waterbody’s area in the same season as sampling was conducted. Pond area and depth were used to calculate water volume. Table 1 gives a summary of the environmental parameters of sampled ponds.

Figure 1: Pictures showing examples of invasion categories at some of the ponds sampled. (a) CAD_B, a “Dominant” pond, where *Crassula helmsii* was the predominant submerged macrophyte as shown in (b) and (c). (d) CAD_C, a “Sparse” pond, where *C. helmsii* had a patchy distribution and was only present in some areas of the pond, as in (e); in (f) other macrophytes were present. (g) CAD_D, an “Absent” pond, where *C. helmsii* was absent and vegetation was only composed of other macrophyte taxa, as shown in (h)

Table 1: Environmental parameters and macrophyte richness (S_m) of the ponds sampled

Pond	Coordinates (degrees °)	IC	Depth (m)	Area (m^2)	Volume (m^3)	pH	Conductivity ($\mu S\ cm^{-1}$)	Turbidity (FAU)	S_m
CAD_A	50.46553889, 4.03593333	D	0.28	108.41	30.35	6.07	51.47	7	12
CAD_B	50.46493333, 4.03666389	D	0.17	67.35	11.45	5.88	62.8	13	8
CAD_C	50.46475556, 4.03391111	S	0.23	89.79	20.65	5.57	53.75	18	9
CAD_D	50.46237778, 4.02894722	A	0.36	171.54	61.75	5.36	39.85	3	6
CAD_E	50.46356111, 4.03540556	D	0.15	23.01	3.45	5.34	73.2	6	7
DART	50.55401667, 4.02712778	A	0.32	294.07	94.1	6.1	56.2	11	8
FOGG	50.54503333, 4.02438333	A	0.3	59.82	17.95	4.02	36.6	25	10
SHAUG	50.45411111, 4.04022222	S	0.28	46.9	13.13	4.7	39.2	29	8
WHITE	50.52058056, 3.97186667	A	0.12	24.43	2.93	5.89	53.1	7	4
YELV_A	50.51971944, 4.03115556	S	0.13	39.98	5.2	5.4	53.6	8	4
YELV_B	50.51783611, 4.03679167	A	0.21	697.81	146.54	5.86	36.9	49	7
YELV_C	50.51586111, 4.03990833	A	0.13	17.45	2.27	5.35	44.7	13	4

Notes IC, invasion category; D, dominant; S, sparse; A, absent

Biotic sampling

Macroinvertebrates were sampled at each pond from submerged and marginal vegetation. Each sample was obtained with eight back-and-forth 1 m sweeps of a standard pond net (200 × 250 mm, mesh size 1 mm) and stored in individual containers with 90% industrial denatured alcohol (IDA). Samples were taken from six haphazard points within ~3 m of the shoreline, ensuring that all "Dominant" samples came from pure *C. helmsii* stands. In total, the sampling procedure yielded 72 samples – 36 from "Absent" ponds, 18 from "Dominant" ponds, and 18 from "Sparse" ponds. At each pond, a specimen of each macrophytes species present was also collected and preserved in IDA.

In the laboratory, macroinvertebrates were sorted from detritus and plant material with a timed approach (20 minutes per sample). This procedure was deemed appropriate because for the first 10 samples nearly all animals were sorted within the first 15 minutes of sorting, and few to none were found by the 30th minute. Animals were examined under a dissection microscope and identified to the lowest recognisable taxon using keys to adult and larvae of freshwater macroinvertebrates using a range of standard taxonomic keys. Most animals were identified to species, some only to genus, but all dipteran larvae were identified to family or subfamily. When identification was not possible due to damage or poor preservation, taxa were assigned to operational taxonomic units. Animal abundance (number of individuals), geographic origin (native/non-native, National Biodiversity Network, 2021) and functional feeding groups (Cummins, 2019) were recorded. Of the macrophytes collected, some were identified to species, but this was not always possible, in which case only genus was recorded. Macroinvertebrates (taxon, occurrence, origin, functional feeding group) and macrophytes (taxon, occurrence) are listed, respectively, in Tables 8 and 9 (Appendix).

Data analyses
In order to explore differences in macroinvertebrate diversity, four measures were used: abundance (*N*), taxon richness (*S*), Shannon-Wiener Index (*H'*) and Pielou's evenness (*J*). *N* and *S* represent the total number of individuals and the number of taxa in each category, respectively. *H'* reflects the entropy (i.e., the degree of randomness) of an ecosystem, whereby the higher the value, the lower capacity to predict information (i.e., taxal occurrence) in that system. By complementing *N* and *S* measures, *H'* is an excellent tool to detect change in biological assemblages (Fedor & Zvaríková, 2018) and thus highly appropriate to study the impacts of *C. helmsii*. *H'* was used to obtain *J* with the following formula:

$$J = \frac{H'}{\ln(S)}$$

These diversity measures were obtained for each invasion category to test for differences between ponds and to determine how different levels of invasion affect macroinvertebrate diversity.

As the data comparing differences in diversity between invasion categories were unbalanced ("Absent", n = 36; "Dominant", n = 18; "Sparse", n = 18), a parametric analysis of variance was not a robust approach (Shaw & Mitchell-Olds, 1993; Hector et al., 2010). Consequently, non-parametric Kruskal-Wallis tests were used to assess differences between invasion categories for all diversity parameters. When statistically significant differences were found, multiple comparison Dunn's tests were used for post-hoc pairwise comparisons and Bonferroni corrections were applied to the resulting *p*-values.

To summarise the aggregate differences of taxa and functional feeding guilds counts for all pairwise comparisons between invasion categories, matrices of Bray-Curtis dissimilarity were produced for both taxonomic and functional feeding guild data.

However, prior to this, two subsets of 18 randomly selected “Absent” samples were created for both taxa and functional feeding guild data such that the number of samples in each invasion category would be 18, to prevent heterogeneity of multivariate dispersion caused by unbalanced designs (Anderson, 2017). To check if there were differences in taxonomic and functional feeding guild composition between the three invasion categories, permutational analyses of variance (PERMANOVAs) with 999 permutations were performed on both balanced datasets. Furthermore, in order to determine whether environmental variables contributed to the dissimilarity between assemblages, BIOENV analyses (Clarke & Ainsworth, 1993) were performed to compare taxon abundance and functional feeding guild matrices with environmental data. Results of BIOENV analyses revealed the set of environmental variables that best correlated with dissimilarities between groups, based on Spearman’s rank-order correlation. Finally, similarity percentages (SIMPER) analyses were used to identify the taxa and guilds that most influenced the dissimilarity between assemblages.

All PERMANOVAs were performed on fourth-root-transformed data to reduce the influence of highly abundant taxa or guilds. Assumptions of homogeneity of multivariate dispersion were met in all cases. Results were summarised graphically by ordination plots using non-metric multidimensional scaling (NMDS), including superimposed vectors to show the environmental factors that had the best correlation with changes in assemblage composition, based on the results of the BIOENV analysis. Further, two stacked bar plots were produced in order to highlight the relative proportion of non-native/native taxa and of functional feeding guilds across the three invasion categories.

All statistical analyses were performed using *R* (R Core Team, 2020) in RStudio 1.3.1093 (RStudio Team, 2020). *H’* was obtained using the function *diversity* from the *R* package “vegan” (Oksanen et al., 2015). Kruskal-Wallis tests were carried out with the native *R* statistical package, followed by the function *dunn.test* of the “FSA” package (Ogle et al., 2021) for post-hoc multiple comparisons. PERMANOVA, BIOENV and SIMPER were performed using the functions *adonis*, *bioenv* and *simper*, respectively, from the *R* package “vegan”. RStudio was also used to produce all plots.

Results

Diversity measures

A total of 73 different taxa were found across all ponds (Table 8, Appendix). The most abundant taxon was the non-native gastropod *Physella acuta* with 514 individuals in total. Of this total, the highest number occurred in “Dominant” ponds which harboured an average of 110.7 (SD ± 44.6) individuals, followed by 29.7 (SD ± 42) in “Sparse” ponds, and 15.5 (SD ± 35.7) in “Absent” ponds. *N* was significantly different across invasion categories (Kruskal-Wallis $\chi^2 = 21.95$, $p < 0.0001$; Figure 2), this being driven by “Dominant” sites having significantly greater *N* than both “Absent” and “Sparse” (Table 2).

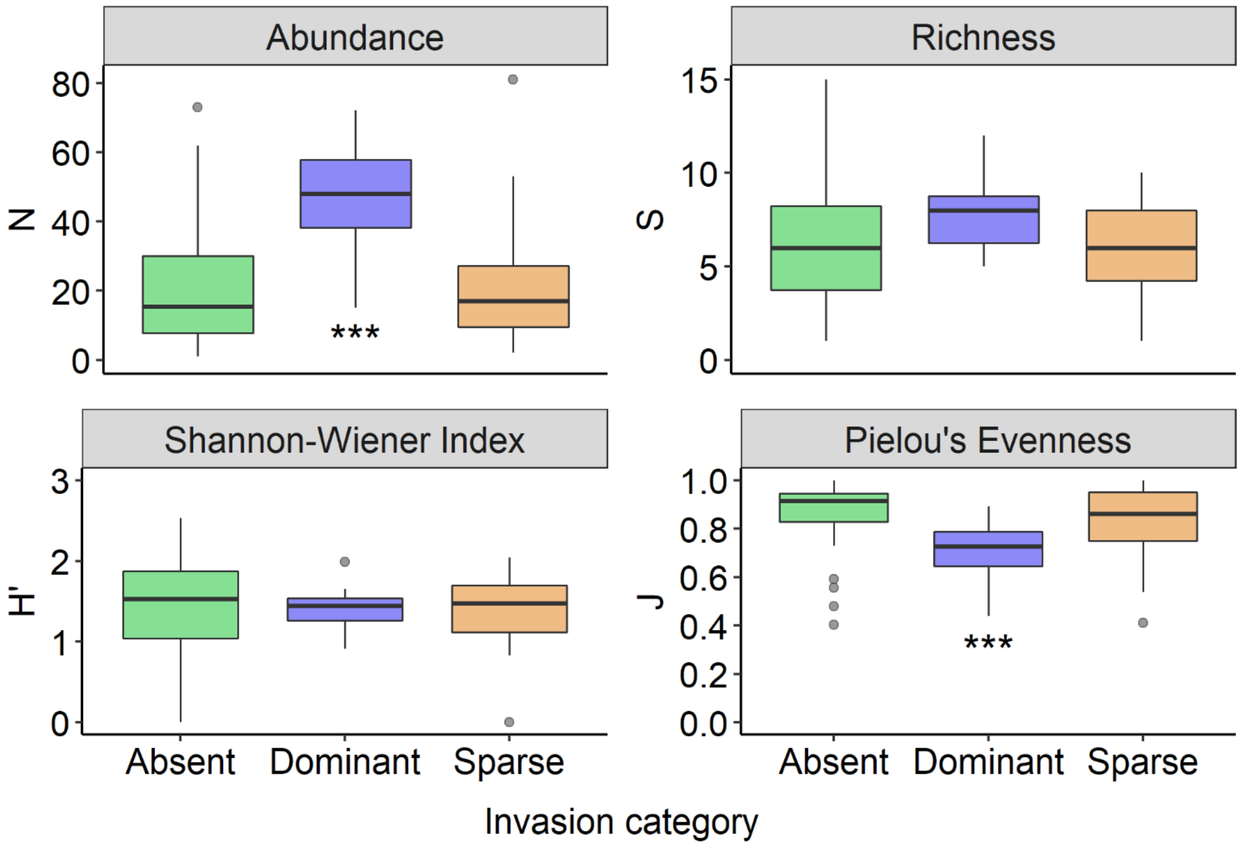

Figure 2: Diversity parameters for the three invasion categories. Boxplots with median, quartiles, and ranges for each of the diversity parameters measured; grey points are outliers. Asterisks (***) denote statistically significant differences (p ≤ 0.001)

Table 2: Multiple comparison Dunn's test for pairwise comparisons of *N* between invasion categories

Comparison	*Z*	*p* (Bonferroni-adjusted *p*)
Absent vs. Dominant	-4.5	**< 0.0001 (< 0.0001)**
Absent vs. Sparse	-0.27	0.78 (1)
Dominant vs. Sparse	3.66	**< 0.001 (< 0.001)**

Note Statistically significant differences ($p \leq 0.05$) are denoted by bold-faced type

There was no significant difference between invasion categories for both *S* (Kruskal-Wallis $\chi^2 = 5.08$, $p = 0.078$; Figure 2) and *H'* (Kruskal-Wallis $\chi^2 = 0.46$, $p = 0.79$; Figure 2). However, *J* was significantly different across invasion categories (Kruskal-Wallis $\chi^2 = 16.56$, $p < 0.001$; Figure 2), with "Dominant" having the lowest evenness value of all three categories (Table 3).

Table 3: Results of the multiple comparison Dunn's test for pairwise comparisons of *J* between invasion categories

Comparison	*Z*	*p* (Bonferroni-adjusted *p*)
Absent vs. Dominant	4.02	**< 0.0001 (< 0.001)**
Absent vs. Sparse	0.8	0.43 (1)
Dominant vs. Sparse	-2.77	**< 0.01 (< 0.05)**

Note Statistically significant differences ($p \leq 0.05$) are denoted by bold-faced type

Assemblage composition

The taxonomic composition differed significantly between ponds under different invasion pressures (Table 4). Invasion category alone contributed significantly to variation between assemblages, accounting for 20% of the variation. However, conductivity, macrophyte richness, turbidity, pond volume, and the interaction between conductivity and plant richness, conductivity and turbidity, and plant richness and turbidity also contributed significantly to the difference between assemblages, and accounting, in total, for 35% of the variation.

Table 4: PERMANOVA results of taxonomic composition (abundances) based on Bray-Curtis dissimilarity

Factor(s)	DF	MS	*F*	R2	*p*
Invasion category	2	1.71	9.51	0.2	**0.001**
Conductivity	1	0.82	4.57	0.05	**0.001**
S_m	1	1.36	7.57	0.08	**0.001**
Turbidity	1	0.94	5.21	0.05	**0.001**
Pond volume	1	0.85	4.76	0.05	**0.001**
Conductivity × S_m	1	0.59	3.27	0.03	**0.003**
Conductivity × Turbidity	1	0.44	2.46	0.03	**0.015**
S_m × Turbidity	1	1.03	5.74	0.06	**0.001**
Residuals	44	0.18		0.45	
Total	53			1	

Notes S_m, plant richness. Bold-faced type indicates significant values ($p \leq 0.05$)

The NMDS ordination based on taxa abundances showed overall separation between “Dominant” samples and both “Sparse” and “Absent”, with a more conspicuous overlap between “Sparse” and “Absent” samples (Figure 3). Nonetheless, there was some overlap of pond assemblages between all three categories. Conductivity, macrophyte richness, turbidity and pond volume were detected by the BIOENV analysis as the parameters with the best correlation with assemblage data (Spearman’s ρ = 0.47). Accordingly, these factors were incorporated into the NMDS plot (Figure 3).

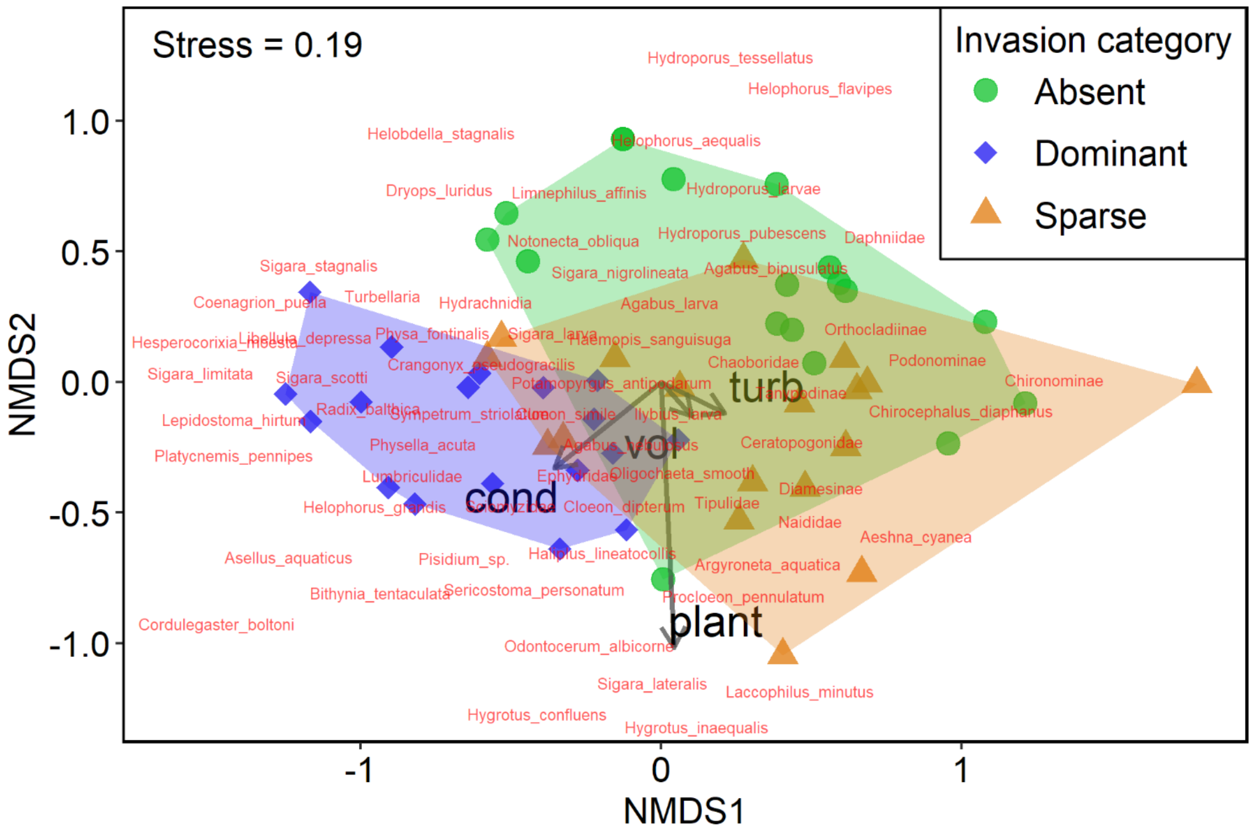

Figure 3: NMDS ordination of macroinvertebrate assemblage taxonomic composition under different *Crassula helmsii* invasion pressures. Symbols represent different invasion categories: green circles for uninvaded ponds ("Absent"), blue diamonds for ponds where *C. helmsii* was dominant ("Dominant"), orange triangles for ponds where *C. helmsii* was patchy ("Sparse"). Labelled vectors indicate correlation with change in assemblage composition (vol, pond volume; turb, turbidity; plant, macrophyte richness; cond, conductivity). Annotations show taxa and their occurrence in relation to samples. Polygons envelop samples from each invasion category and are colour-coded accordingly

SIMPER analysis revealed that the dissimilarities between the "Dominant" and "Sparse" macroinvertebrate assemblages were driven mostly by *P. acuta*, *Crangonyx pseudogracilis* and *Radix balthica*, whose greater average abundance in "Dominant" sites contributed altogether to 24.61% of the difference between assemblages (Table 5). Of these three species, *P. acuta* and *C. pseudogracilis* are non-native to Britain, contributing to the greater abundance of NNI taxa found in "Dominant" ponds (Figure 4a). Although not as markedly, *Cloeon dipterum* was also significantly more abundant in "Dominant" assemblages when compared to "Sparse" ones. *P. acuta*, *C. pseudogracilis* and *R. balthica* were also found to drive statistically significant differences between "Dominant" and "Absent" assemblage, contributing, in total, to 25.82% of the variation between assemblages. There was a significantly higher average abundance of Naididae in "Sparse" than in "Absent" assemblages, which accounted for nearly 8% of the dissimilarity. Tanypodinae, Chironominae and Orthocladiinae also contributed significantly to the difference between macroinvertebrate assemblages (17.22%), but all three taxa had greater average abundances in "Absent" sites. Table 5 summarises SIMPER results for the taxa that contributed ≥ 5% to differences between assemblages; complete results can be found in Table 10 (Appendix).

Table 5: Results of SIMPER analysis for the taxa contributing ≥ 5% to the dissimilarity between macroinvertebrate assemblages (ponds grouped by invasion category). Taxa are listed in order of their contribution to the differences between assemblages

Taxon	Category		AD	SD	AD/SD	AA		Contribution (%)	*p*
	x	*y*				*x*	*y*		
Physella acuta	D	S	0.09	0.05	1.60	1.91	0.60	10.71	**0.001**
Crangonyx pseudogracilis	D	S	0.06	0.05	1.24	1.09	0.23	7.48	**0.002**
Radix balthica	D	S	0.05	0.05	1.00	0.89	0.42	6.72	**0.004**
Naididae	D	S	0.04	0.04	1.19	0.31	0.88	5.6	0.158
Agabus sp. (larva)	D	S	0.04	0.03	1.27	0.40	0.89	5.24	0.703
Cloeon dipterum	D	S	0.04	0.05	0.85	0.64	0.37	5.15	**0.023**
Physella acuta	D	A	0.11	0.05	2.24	1.91	0.25	12.01	**0.001**
Crangonyx pseudogracilis	D	A	0.07	0.05	1.26	1.09	0.18	7.37	**0.001**
Radix balthica	D	A	0.06	0.06	0.95	0.89	0.00	6.44	**0.001**
Naididae	S	A	0.06	0.06	1.13	0.88	0.19	7.94	**0.001**
Tanypodinae	S	A	0.05	0.05	1.05	0.40	0.73	6.66	**0.003**
Agabus sp. (larva)	S	A	0.05	0.05	0.95	0.89	0.83	5.98	0.09
Physella acuta	S	A	0.05	0.06	0.73	0.60	0.25	5.78	1
Chironominae	S	A	0.04	0.05	0.77	0.32	0.34	5.28	**0.002**
Orthocladiinae	S	A	0.04	0.05	0.84	0.31	0.48	5.28	**0.003**

Notes AD, average dissimilarity; SD, standard deviation; AA, average abundance; D, dominant; S, sparse; A, absent. Bold-faced type indicates significant values ($p \leq 0.05$)

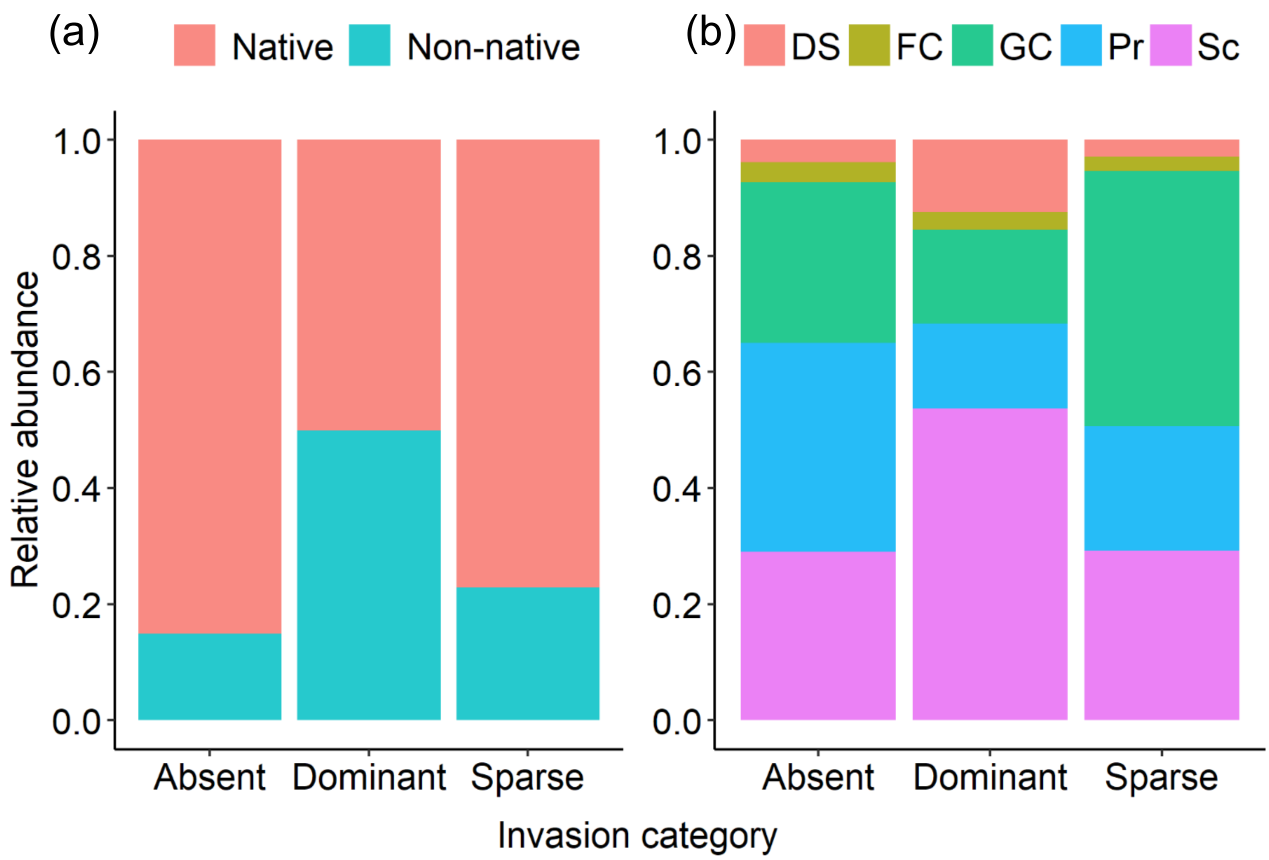

Figure 4: (a) Stacked bar plot of the relative abundance of native and non-native macroinvertebrate taxa in the assemblages examined. (b) Stacked bar plot of the relative abundance of each functional feeding guild in the assemblages examined (DS, detrital shredder; FC, filtering collector; GC, gathering collector; Pr, predator; Sc, scraper)

There was a significant difference in functional feeding guild composition between macroinvertebrate assemblages (Table 6). Significant differences in assemblages were primarily driven by invasion category, which explained 36% of the dissimilarity. But conductivity, macrophyte richness and the interaction between conductivity and pH also had a significant effect on functional feeding guild composition, cumulatively explaining 23% of the dissimilarity between assemblages.

The NMDS ordination showed distinct separation of “Dominant” from “Absent” assemblages, whilst overlapping marginally with “Sparse” assemblages (Figure 5). In contrast, most “Sparse” samples overlapped with “Absent” ones, with only few isolated samples. In general, more scrapers and detrital shredders were present in “Dominant” assemblages, but this relationship was also influenced by macrophyte richness. BIOENV analysis corroborated PERMANOVA results, indicating a correlation between pH, conductivity and macrophyte richness and functional feeding guild composition (Spearman’s ρ = 0.45).

Table 6: PERMANOVA results of functional feeding guild composition (abundances) based on Bray-Curtis dissimilarity

Factor(s)	DF	MS	*F*	R^2	*p*
Invasion category	2	0.88	21.71	0.36	**0.001**
Conductivity	1	0.14	3.52	0.03	**0.038**
pH	1	0.1	2.34	0.02	0.12
$\boldsymbol{S_m}$	1	0.56	13.87	0.11	**0.001**
Conductivity × pH	1	0.46	11.29	0.09	**0.001**
pH × S_m	1	0.05	1.27	0.01	0.29
Conductivity × pH × S_m	1	0.1	2.45	0.02	0.077
Residuals	45	0.04		0.36	
Total	53			1	

Notes S_m, plant richness. Bold-faced type indicates significant values ($p \leq 0.05$)

The guilds contributing most to the differences between invaded and uninvaded ponds were scrapers and detrital shredders (Table 7). Scrapers contributed to nearly 40% of the dissimilarity between “Dominant” and both “Sparse” and “Absent” communities. Detrital shredders were significantly more abundant in “Dominant” ponds, contributing significantly to the dissimilarity between “Dominant” and both “Sparse” and “Absent” ponds; ~22% contribution. There was a significant difference between the average abundance of predators in “Sparse” and “Absent” ponds, with the latter harbouring the greatest number. Predators were also the most abundant guild overall in “Absent” ponds. Gathering collectors were relatively abundant in all ponds, whereas filtering collectors were the least abundant guild in all ponds. Accordingly, there were no significant differences of gathering collectors and filtering collectors’ average abundances in all pairwise comparisons between assemblages. SIMPER results for differences in feeding guild composition are summarised in Table 7. Figure 5b shows the relative abundance of each guild across the three invasion categories.

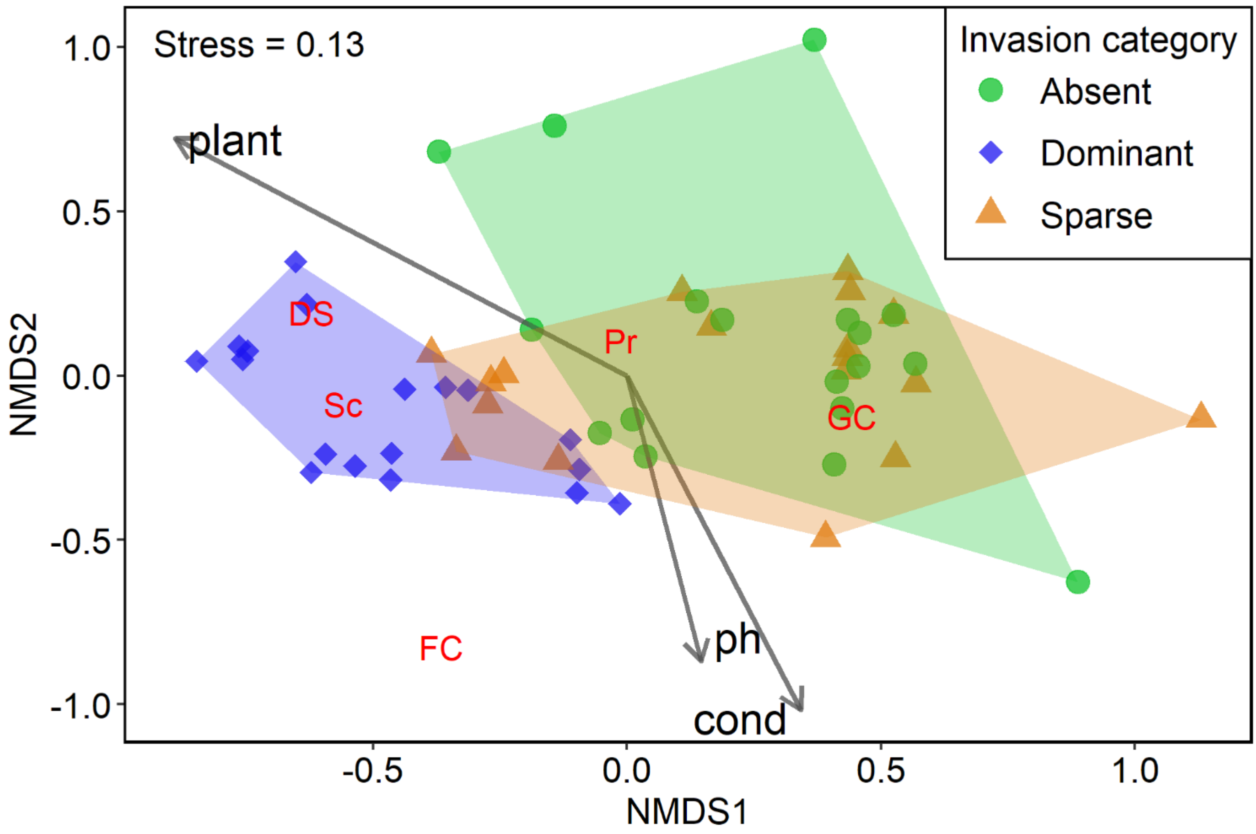

Figure 5: NMDS ordination of freshwater macroinvertebrate assemblage functional feeding guild composition in ponds under different Crassula helmsii invasion pressures. Symbols represent different invasion categories: green circles for uninvaded ponds ("Absent"), blue diamonds for ponds where C. helmsii was dominant ("Dominant"), orange triangles for ponds where C. helmsii was patchy ("Sparse"). Labelled vectors indicate correlation with change in assemblage composition (plant, macrophyte richness; cond, conductivity; ph, pH). Annotations show functional guilds and their occurrence in relation to samples (DS, detrital shredders; FC, filtering collectors; GC, gathering collectors; Pr, predators; Sc, scrapers). Polygons envelop samples from each invasion category and are colour-coded accordingly

Table 7: Results of SIMPER analysis for the functional feeding guilds (FG) showing the contribution of each guild to dissimilarity between macroinvertebrate assemblages (ponds grouped by invasion category). FG are listed in order of their contribution to the differences between assemblages

FG	Category		AD	SD	AD/SD	AA		Contribution (%)	*p*
	x	*y*				*x*	*y*		
Sc	D	S	0.17	0.11	1.58	2.2	0.68	38.39	**0.001**
DS	D	S	0.1	0.07	1.29	1.16	0.43	21.96	**0.006**
GC	D	S	0.09	0.07	1.22	1.04	1.56	20.91	0.663
FC	D	S	0.04	0.06	0.69	0.41	0.16	9.58	0.242
Pr	D	S	0.04	0.05	0.81	1.54	1.35	9.16	0.883
Sc	D	A	0.19	0.09	1.97	2.2	0.55	39.18	**0.001**
DS	D	A	0.11	0.08	1.39	1.16	0.28	22.2	**0.002**
GC	D	A	0.1	0.08	1.17	1.04	1.26	20.84	0.398
Pr	D	A	0.04	0.04	0.97	1.54	1.4	8.92	0.863
FC	D	A	0.04	0.06	0.7	0.41	0.13	8.86	0.234
Sc	S	A	0.1	0.11	0.94	0.68	0.55	27.73	1
GC	S	A	0.1	0.1	0.98	1.56	1.26	28.04	0.323
Pr	S	A	0.07	0.08	0.8	1.35	1.40	18.32	**0.011**
DS	S	A	0.06	0.07	0.89	0.43	0.28	17.02	1
FC	S	A	0.03	0.06	0.48	0.16	0.13	7.89	0.886

Notes AD, average dissimilarity; SD, standard deviation; AA, average abundance; D, dominant; S, sparse; A, absent; Sc, scraper; GC, gathering collector; DS, detrital shredder; Pr, predator; FC, filtering collector. Bold-faced type indicates significant values ($p \leq 0.05$)

Discussion

Non-native invasive (NNI) macrophytes are often associated with negative ecological impacts on recipient environments. However, the effects of biological invasions are not always predictable and need to be evaluated considering local factors. Here, macroinvertebrate assemblages from freshwater ponds under different invasion pressures by the NNI macrophyte *C. helmsii* were compared. In accordance with the findings of other researchers (Ewald, 2014; Smith, 2015; Smith & Buckley, 2015), the results of this study revealed no significant effect of *C. helmsii* on macroinvertebrate diversity indices – i.e., *S* (richness) and *H'* (Shannon-Wiener Index), regardless of the extent of invasion. By contrast, ponds where *C. helmsii* was predominant had a significantly higher *N* (total individual abundance) and lower *J* (Pielou's Evenness), indicating unequal relative contribution of some groups to the faunal assemblage. In terms of composition, assemblages differed significantly across invasion categories. The NMDS ordinations showed a greater degree of variation between individual "Sparse" and "Absent" ponds than "Dominant" ones, for both taxonomic and guild composition. These differences were partly due to the set of unique environmental variables that characterised the ponds sampled. Nonetheless, there was a significant effect of invasion category, indicating that *C. helmsii* influences macroinvertebrates assemblage composition.

The effect of the structuring role of macrophytes on taxonomic diversity in aquatic environments is recognised by several authors, both in freshwater (McAbendroth et al., 2005; Thomaz et al., 2008; Mormul et al., 2011) and marine systems (Norderhaug, 2004; Hauser et al., 2006; Graham & Nash, 2013). The general assumption is that by increasing structural complexity, macrophytes provide a variety of spatial niches, facilitating the coexistence of many species. Accordingly, vegetated areas are often associated with higher diversity than unvegetated areas, where bare substratum and the water column may not offer the same ecological value, especially for taxa vulnerable to predation. In this study, because all ponds were sampled from vegetated areas where macrophytes formed intricate architectures (e.g., *Myriophyllum spicatum*, *Hypericum erodes*, *Callitriche palustris*), the lack of a measurable effect on *S* and *H'* could indicate that *C. helmsii* is similar to other macrophytes, at least in terms of habitat provision. In addition, as most macroinvertebrates tend to accumulate on the edges of freshwater ponds (Gee et al., 1997), the margins of similar ponds are expected to harbour similar diversity.

Because macrophyte structure provides a three-dimensional structure where many animals can co-occur, vegetated patches are also associated with higher densities of individuals. The highly branched and dissected structure of *C. helmsii*, therefore, could also explain why a significantly higher *N* was found in “Dominant” ponds. However, if this assumption were true, it would imply that dense monotypic *C. helmsii* stands have a greater ecological value than other similarly complex macrophytes, also resulting in higher diversity. Instead, here, there was no effect on both *S* and *H'* and, in fact, the higher *N* of “Dominant” ponds was coupled with the lowest *J* value. Thus, a more likely explanation for what the data suggest is that, when the dominant macrophyte, *C. helmsii* affects the relative proportion of taxa inhabiting its canopies by favouring, or otherwise hindering, the proliferation of some taxa. And indeed, the relative proportion of native and non-native taxa found in “Dominant” ponds indicated that non-native macroinvertebrates were more numerous where *C. helmsii* was predominant than elsewhere.
The effect of dense *C. helmsii* stands on macroinvertebrate taxa may be linked to the potential of the plant to exude allelopathic compounds. Allelopathic compounds are often bioactive secondary metabolites (e.g., phenolic compounds) that plants produce to deter herbivores, epiphytes, parasites or even the growth of other plants. Whether *C. helmsii* exudes secondary metabolites affecting the surrounding environment has not been directly tested yet, but Grutters et al. (2017) highlighted the allelopathic potential of *C. helmsii* and, recently, Reynolds & Aldridge (2021) confirmed this hypothesis reporting negative effects of crushed plant material on the growth of phytoplankton (*Chlorella* and *Synechocystis*). In addition, *C. helmsii* is known to accumulate high levels of Cu (copper) and store it in a non-detoxified form (Küpper et al., 2009), which could indicate allelopathy by means of metal hyperaccumulation – a strategy employed by a range of plant species as a defensive mechanism against herbivory and parasitism (Ernst, 1987; Boyd & Martens, 1994; Boyd et al., 1994; Martens & Boyd, 1994; Pollard & Baker, 1997). Given that “Dominant” ponds had on average a substantially higher number of the Cu-tolerant *P. acuta* (Spyra et al., 2019) than “Sparse” and “Absent” ponds, it is highly probable that metal allelopathy plays a role in structuring macroinvertebrate assemblages. The high abundance of the non-native *P. acuta* would not only explain the significantly higher *N* of “Dominant” ponds but also the significantly lower *J* resulting from the disproportionate contribution of the gastropod to the assemblage. In addition, it would justify the higher relative abundance of NNI macroinvertebrate taxa observed in “Dominant” ponds.

The influence of environmental variables on the composition of freshwater macroinvertebrate assemblages is well-documented in the literature (e.g., Tucker, 1958; Friday, 1987; Williams et al., 2004; Gutiérrez-Estrada & Bilton, 2010; Sun et al., 2019) and the effect of the factors considered in this study on assemblage composition are in agreement with available literature. For example, all chironomids larvae except Ceratopogonidae were in significantly higher numbers in "Absent" than "Sparse" and "Dominant" ponds and, as highlighted by the NMDS ordination, conductivity had a negative effect on their occurrence. Chironomids are known to be highly sensitive to pollution (Nicacio & Juen, 2015) and, particularly, heavy metal contamination (Armitage & Blackburn, 1985; Sheehan & Knight, 1985; Di Veroli et al., 2014). As conductivity is often a good proxy for water quality (e.g., Loock et al., 2015; Meland et al., 2020), the negative relationship between conductivity and chironomids in this study can be linked to their preference for less polluted waters. Furthermore, the selection of water bodies with less *C. helmsii* would also reinforce the hypothesis of metal allelopathy.

Other than conductivity, pH, turbidity and water volume – a parameter related to pond permanence – are also known to have a measurable effect on macroinvertebrates assemblages. Similarly, macrophyte richness is also an important factor and can be even used to predict macroinvertebrate diversity (Law et al., 2019). However, whilst these factors undoubtedly played a crucial role in the ecological dynamics underpinning macroinvertebrate assemblage composition, they only explained partially the differences observed. Importantly, PERMANOVA revealed a significant effect of invasion category on assemblage composition, suggesting that *C. helmsii* had an impact on macroinvertebrates. This effect could, again, be attributed to the physical structure of *C. helmsii*. Because the leaves and branches of *C. helmsii* are stiffer than similarly dissected macrophytes (e.g., *C. palustris*), it is likely that its canopies can hold a higher load of detritus and periphyton. In fact, in general, *C. helmsii* stands in "Dominant" ponds had a far greater cover of filamentous algae on the submerged branches than stands from "Sparse" ponds (pers. obs.). The high density of branches and filamentous algae can substantially restrict space available within canopies, favouring smaller species, potentially reducing water flow and, also, trapping high quantities of detritus. As assemblages from "Dominant" ponds were characterised by a lower number of predators, it is likely that dense *C. helmsii* stands favour the settlement of small scrapers and detrital shredders at the expense of large swimming predators (e.g., *Agabus bipustulatus*, *Notonecta obliqua*). This was evident not only by the taxonomic composition of "Dominant" ponds, where periphyton- and detritus-feeding species like *R. balthica*, *P. acuta* and *C. pseudogracilis* abounded but also by the difference in functional feeding guild composition between ponds, which indicated an overall scarcity of scrapers and detrital shredders in assemblages from "Sparse" and "Absent" ponds. Finally, the fact that the sediment-dwelling detritivores Naididae occurred in "Absent" ponds more than in invaded ones is also indicative of the detritus-trapping capacity of *C. helmsii*, which would otherwise prevent detritus from reaching the bottom of ponds where the anellids feed. Thus, it appears that the effect of *C. helmsii* stands on assemblage composition may be ascribed to a potential to obstruct water circulation and impede the passage of relatively large free-swimming species, whilst increasing the availability of certain food items (e.g., periphyton).

One possible shortfall of this study is the short distance between "Dominant" ponds if compared to the distance between all "Sparse" and "Absent" sites. Given that wetland ponds are known to be influenced by metacommunity dynamics (Meutter et al., 2007; Heino et al., 2015), whereby spatial processes and biological dispersal are critical in structuring biological communities, the potential for spatial autocorrelation between "Dominant" ponds was high. Thus, the possibility that the similarity between assemblages from "Dominant" ponds was driven by a combination of spatial processes and macroinvertebrates' dispersal limitation cannot be excluded. It is noteworthy, however, that larvae of some winged taxa (e.g., *Coenagrion puella*, *Cloeon* spp., *Sciomyzidae*) were found to drive significant differences between "Dominant" and both "Sparse" and "Absent" ponds, suggesting that dense *C. helmsii* mats do not hinder oviposition of these taxa. By contrast, chironomids appeared to exhibit a preference for uninvaded ponds. Therefore, though the limitation of this study could be addressed with a study of the effects of *C. helmsii* at different spatial scales (e.g., local vs nationwide), these findings also underline that this non-native macrophyte may act as an environmental filter for some species. Whether this is due to increased mortality of larvae or reduced hatching cannot be determined here. Thus, it would be extremely useful if future researchers focussed on the effects of *C. helmsii* on selected species during various life stages, with emphasis on the potential for metal allelopathy.

Conclusions

Even though this study demonstrated no effect of *C. helmsii* on taxonomic diversity, other ecologically significant effects were discovered. In summary, it seems that dense *C. helmsii* stands favour the proliferation of some taxa, particularly scrapers and gathering collectors. Of these taxa, three were non-native macroinvertebrates, but the non-native Cu-tolerant *P. acuta* was the most abundant. The effects of *C. helmsii* may be attributed to the effect of metal allelopathy, as well as the capacity to trap detritus and favour the growth of periphyton. In contrast, where *C. helmsii* was not the dominant macrophyte, there was no effect on any of the diversity parameters measured and a marginal effect on assemblage composition, primarily driven by differences in abundances of chironomid larvae and Naididae.

Whilst this study was conducted in a small area of the Southwest of the UK, it complements the general notion that the effects of non-native species are not always predictable. Importantly, it underscores the fact that taxonomic diversity is not necessarily a good indicator of the status of an ecosystem and thus, comprehensive, multi-layered approaches are needed to assess the impacts of invasive species. These findings also indicate that the already widespread distribution of *C. helmsii* in Britain may be already altering freshwater ecosystems without necessarily reducing diversity. From a management perspective, this means that continual monitoring of taxonomic composition is required where the plant is the dominant feature of ponds. Because manual eradication is unlikely to be achievable on a large scale, it would be useful to find species as potential candidates for biological control. In view of this, studies aimed at understanding the allelopathic potential of *C. helmsii* and its effects on herbivores would be extremely helpful.

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