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DIATOMS AS INDICATORS OF FINE SEDIMENT STRESS

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ABSTRACT

Excessive delivery of fine sediments to waterbodies has a detrimental impact on the biotic elements used for waterbody status classification. Although diatoms are typically used to assess stress from eutrophication, as fine sediment has the potential to impact diatoms in many ways, it is not surprising that an index based on benthic diatom assemblages has been proposed: the relative abundance of motile species. This measure is based on the fact that many raphid diatom species are capable of migrating through deposited sediment to avoid negative impacts. However, the use of such an index has yet to be fully tested.

Various data analysis techniques were used to explore how indices based on diatom assemblages (related to eutrophication and siltation), diatom species, and the traits motility and nutrient affinity responded to a gradient of percentage cover of fine sediment. Although diatom species showed marked variation in their affinity for percentage cover of fine sediment, the relationship between motility (both percent motile and the trait motility) and deposited fine sediment is not sufficiently strong to be used as a reliable indicator of fine sediment stress. We present an approach which could potentially be used to develop a new index (DISCO - Diatom Indictor of Sediment COnditions) based on the response of diatoms to fine sediment, but caution that this index requires further development before use. Despite hydromorphology having considerable potential to affect benthic diatoms, existing indices designed to assess eutrophication were robust to hydromorphological modification, reducing the possibility of false diagnosis of impacts.

INTRODUCTION

Diatom assemblages, as either phytobenthos or phytoplankton, are typically used to assess the extent of stress from eutrophication (nutrient pollution as dissolved inorganic phosphorus or to a lesser extent dissolved inorganic nitrogen (e.g. Kelly *et al*., 2001; Kelly & Whitton, 1995). However, it has been suggested that benthic algae, in addition to sensitivity to nutrients, are also particularly prone to the impacts of increased fine sediment loads (Jones et al., 2014). As benthic algae are photosynthetic, they are dependent upon light; any increase in the turbidity of the water column caused by suspended fine sediment will reduce light availability and, hence, reduce photosynthesis and biomass of benthic algae. Nevertheless, increased delivery of fine sediment to rivers has the potential to impact diatom assemblages in many ways, both direct (e.g. scouring by saltating particles: Okada, 2009) and indirect (e.g. through changes to herbivorous invertebrates: Jones *et al*., 2012b). One of the most profound effects of fine sediment occurs as a consequence of deposited material smothering benthic algae and the substrata to which they attach (Jones *et al*., 2014). Hence, it is not surprising that an index of sediment pressure based on benthic diatom assemblage structure has been proposed. This index comprises simply the relative abundance of motile species (Bahls, 1993). This measure is based on the fact that many species of raphid diatoms are capable of migrating through deposited sediment and, thus, avoid the negative effects of burial. There is clear utility of such an index for assessing the impact of hydromorphological modifications to rivers, particularly those that alter the rate of delivery and retention of fine sediment. Hence, this index (relative abundance of motile species) has been variously adopted by regulatory authorities worldwide to interpret the impact of siltation on diatom communities.

Negative effects of hydromorphological modification could be expected through both direct and indirect impacts on the substrate on which benthic algae grow. For example, direct modification of in-stream and marginal habitat will alter substrate composition, whereas reductions in flow velocity, caused by impoundments, tend to increase the deposition of fine sediment altering both bed substrate and the potential for planktonic algae to thrive. There is also the potential for hydromorphological modifications to affect diatom assemblages in ways other than through changes of the substrate, for example through modification of near-bed flow velocity which is known to influence boundary layers and, hence, growth and photosynthesis of primary producers (Finlay *et al*., 1999; Schneck *et al*., 2011).

As with all attempts to link ecology to hydromorphological alterations, there is a potential issue of scale (Larsen *et al*., 2009). It is typical for hydromorpholgical assessments to be undertaken at the reach scale, whilst biota are frequently sampled at a patch scale: the degree to which biological communities are nested between these two scales will influence how community composition reflects pressures (Larsen and Ormerod, 2010), as will the mechanism by which hydromorphological stress impacts upon the community (Jones *et al*., 2012b). Diatoms are affected by fine sediment in various direct and indirect ways (Jones *et al*., 2014), and it cannot be assumed that by sampling patches of hard substrate any impact of fine sediment will be avoided other than immediate patch-scale effects (e.g. abrasion, burial, loss of substrate for attachment). At a community level, species (and traits) are lost as the proportion of "good" patches diminishes (Larsen and Ormerod, 2010), and colonizer effects occur as the community in the surrounding habitat changes. Sediment-induced changes to the macrophyte flora influence flow, shade and water chemistry (Jones *et al*., 1996; Jones *et al.*, 2012a), and will affect the diatom assemblage where sampled directly from macrophytes (Jones *et al.*, 2000). Further, indirect impacts will occur as changes to the invertebrate and fish community cascade down to their food resources (Jones *et al*., 2012b).

With such pronounced potential effects of hydromorphology on diatom assemblages it is possible that diatom-based indices (other than relative abundance of motile species) may be sensitive to hydromorphological impacts. As these indices were developed largely to assess eutrophication stress, it is critical to determine if any change in the benthic algal community associated with hydromorphological alteration influences the relationship between these indices and nutrient stress, otherwise a false diagnosis of the issues acting on a site could be returned. Nevertheless, a diatom-based index capable of detecting stress from hydromorpological modification would be particularly useful as it would provide a measure of the impact at the base of the food web, and would add to the arsenal of tools available, further increasing the confidence of any assessments made (Johnson *et al*., 2006).

The primary objective of this work was to establish if the relative abundance of motile species is a valid measure of stress from fine sediment: despite being in use for over 20 years this index has yet to be fully tested. We were also interested to determine if hydromorphological alteration confounds interpretation of diatom-based indices. We worked from the hypothesis that hydromorphological alteration would influence diatoms traits, particularly motility, as this would confer an advantage to species that could migrate to avoid the impact of increased deposition of fine sediment or thicker benthic boundary layers. In addition, we hypothesized that the traits of motility and nutrient affinity would not be linked to each other, which would confer independence to diatom-based indices for assessing eutrophication and hydromorphological stress. In order to achieve these objectives we used existing data to address three key questions,

a) Are diatom indices sensitive to hydromorphological alteration?

b) Does percent motile taxa respond to variation in cover of fine sediment?

c) Does the diatom assemblage vary with cover of fine substrate?

METHODS

Are diatom indices sensitive to hydromorphological alteration?

Data from 1578 sites in Germany, Austria and the Netherlands, compiled from national monitoring agencies during the WISER project (Moe *et al*., 2013), were used to establish the impact of hydromorphological pressure on the relationships between indices based on phytobenthos and phosphorus concentration using ANCOVA. Standard Water Framework Directive protocols were used to collect and process samples of phytobenthos: samples were collected from stone scrapes or plant stems, digested using hydrogen peroxide or acid permanganate and mounted on a slide where 300 valves were identified and counted (Kelly et al. 1998). Twelve indices of phytobenthos were calculated from the assemblage recorded at each site, namely Descy (Descy's pollution metric), Watanabe (Watanabe's Diatom community index), TDI (Trophic Diatom Index), % planktonic (centric) taxa, IPS (Indice de Polluo-Sensibilité), IDAP (Artois-Picardie Diatom Index), EPI-D (Diatom-based Eutrophication/Pollution Index), D-CH (Swiss Diatom Index), IDP (Biological Diatom Index), LOBO (Lobo's Biological Water Quality Index), TID (Trophic Index) and % motile taxa (all indices were calculated using Omnidia version 3, see Birk *et al.* (2010) for full details). Nutrient concentrations were derived from chemical monitoring data collected by the national agencies, where standard analytical techniques were used: annual mean orthophosphate concentration (derived colourimetrically using molybdenum blue) was used as a measure of nutrient availability. The influence of six hydromorphological alterations was investigated, namely channel modification, artificial embankment, impoundment, modification of instream habitat, modification of riparian vegetation and velocity increase. Based on observations at the time of sampling, each site was categorized according to the extent of hydromorphological alteration, with 2 to 4 categories used for each modification type to describe increasing severity of alteration.

For each index, the influence of hydromorphological alteration on the relationship with annual mean orthophosphate concentration was determined using general linear models in SAS, where extent of hydromorphological alteration was a fixed class variable and $\log_{10}$ orthophosphate concentration a continuous variable. Where significant effects of hydromorphological alteration on the relationship between the index and $\log_{10}$ orthophosphate concentration were found, relationships were checked to establish if the results were trivial, i.e. data from modified sites were all within the range of scatter of unmodified sites and relationships explained less than 5% of the variance.

Does percent motile taxa respond to variation in cover of fine sediment?

Data collected from 182 sites across Europe during the STAR project, which aimed to standardize biological assessment protocols (Furse *et al*., 2006), were used. At each site samples of phytobenthos were collected from stone scrapes or plant stems in spring, digested using hydrogen peroxide or acid permanganate and mounted on a slide where 300 valves were identified and counted (Kelly *et al*., 1998). The percent motile taxa was determined following Jones *et al*. (2014). Substrate composition, as percent cover of size classes of the international scale (ISO 14688-1:2002), was estimated visually at each site: deposited fine substrate was considered to be sand and silt, clay, and the sum of both these categories. Both percent motile and percent cover of fine substrate were transformed using arcsin to normalize the data. Annual mean orthophosphate and total phosphate concentrations were derived colourimetrically using molybdenum blue (after digestion using hot persulphate for total). Conductivity was determined using a dip probe. The relationship between % motile taxa, deposited fine substrate and water chemistry variables was investigated using linear regression using SAS. Where significant relationships with bed composition were detected, analysis was repeated where all sites with zero fine substrate were excluded to determine if the results were trivial, i.e. the influence of zero recorded fines was driving the relationship.

Does the diatom assemblage vary with cover of fine substrate?

Data were compiled from surveys undertaken on behalf of the Welsh Government to assess the effectiveness of agri-environment schemes in Wales (Agri-environment Monitoring and Services

Contract Lot 3 183/2007/08 (Anthony *et al*., 2012) and the Glastir Monitoring and Evaluation Programme (CEH, 2016)). Sites were scattered across Wales, covering a wide range of physico-chemical conditions. In spring, samples of the diatom assemblage at each site were collected from 5 replicate stones (or macrophytes where suitable stones were lacking) randomly selected from the benthos: attached algae were removed from the surface with a toothbrush, rinsed with stream water into clean HDPE bottles and preserved with Lugol's iodine. On return to the laboratory, samples were digested with hydrogen peroxide and mounted on microscope slides. The slides were examined under x 1000 magnification, with 300 diatom valves from random fields of view in each sample being identified to species level following Kelly and Yallop (2012). The method, a standard approach for diatom samples (Kelly *et al*., 2008), provides an estimate of relative abundance of taxa. Data on the trait of interest (i.e. mobility) were acquired from Jones *et al*. (2014) and on nutrient affinity (TDI score) from Kelly and Yallop (2012). The physical characteristics of each river reach from which diatom samples were collected was assessed either in the field or from maps, together with visual assessments of substrate composition as percentage cover within size classes of the international scale (ISO 14688-1:2002). Percentage cover of fine substrate was determined as the sum of sand, silt and clay. Conductivity and pH were determined in the field with dip probes. Nutrient concentrations were determined by standard analystical techniques on water samples collected at the time of sampling or modelled using frameworks capable of estimating pollutant loading from land use within each of the selected catchments (Gooday *et al*., 2014).

Here the objective was to quantify the association between variation in the diatom assemblage and the gradient of percentage cover of fine-grained sediment having first factored out that portion of the biological variation correlated with natural background variation between streams. Data were analysed using partial ordination, which involved a two-step process. The first step was to determine the main drivers of assemblage composition, the second step was to establish the variation in assemblage composition that was attributable to the parameter of interest (i.e. percentage cover of fine-grained sediment) once the influence of the main drivers has been removed: In simple terms this analytical process is equivalent to establishing: "When all other things are equal, what is the response of diatoms to fine sediment?" The critical step in the process is establishing statistically robust and biologically relevant main drivers. The approach has been used previously to develop robust invertebrate-based biotic indices to determine the level of stress from acidification (Acid Waters

Indicator Community Index: Murphy *et al*., 2013) and fine sediment (Combined Fine Sediment Index: Murphy *et al*., 2015). The AWIC index thus developed has been shown to be as effective as 6 months of fortnightly pH measurement using conventional probes (Ormerod *et al*., 2006), and is now adopted by the UK environmental agencies for use in WFD assessments.

All taxa that were found in less than 3 % of samples were excluded from analyses. Canonical Correspondence Analysis (CCA) was used to establish the relationship between diatom assemblage composition and a number of candidate environmental variables characterising river condition and type. The environmental variables offered to the analysis included physical (e.g. distance from source, altitude, slope, cross-sectional area) and chemical (nutrient concentrations, pH, alkalinity) parameters, and the percentage cover of fine sediment (sand, silt and clay). These variables were chosen as they are likely to include the main drivers of diatom assemblage compostion. Variables were selected from this suite sequentially for inclusion in the model after testing the significance of their influence using Monte Carlo simulation tests. CCA was undertaken with Hill's scaling of ordination scores, with focus on inter-species distances, and manual forward selection (n = 999 permutations, $P < 0.05$ as the significance threshold for inclusion in the model) to determine the optimal subset of variables that accounted for the gradients in the diatom assemblage. The next step in the analysis was to remove the influence of the environmental variables that described river type, leaving only the relationship between fine sediment and diatom taxa. This was done by partial CCA, using the physical and chemical variables associated with river type, which had been identified as significant above, as covariables. The variation in diatom taxa that remained was that which was explained by the amount of deposited fine sediment. All ordinations were undertaken using CANOCO 4.5 software (ter Braak and Šmilauer, 2002). The output of the analysis was a single ranking of sensitivity of taxa to fine sediment irrespective of river type. Logistic regression was used in SAS to determine the probability of occurence of the traits of interest, mobility and nutrient affinity, relative to the distribution of the species scores on pCCA axis 1, defined by the gradient of deposited fine sediment cover.

RESULTS

Are diatom indices sensitive to hydromorphological alteration?

There was a significant relationship with $\log_{10}$ orthophosphate for almost all indices tested (Table I). However, hydromorphological alteration had no effect on this relationship (Table I and Figure 1): the only significant interaction effects detected, suggesting an effect of hydromorphology on the relationship with $\log_{10}$ orthophosphate, were trivial (i.e. the relationships explained little of the variation and the scatter of points was within that of the unmodified sites: see Figure 1). It should be noted that percent motile showed a significant relationship with $\log_{10}$ orthophosphate for three out of the six tests.

Does percent motile taxa respond to variation in cover of fine sediment?

In the STAR data, weak relationships were found between the percent motile taxa and the percent cover of clay and of total fine sediment in the substrate. However, these relationships appeared to be trivial, driven by sites where zero fines had been recorded, which encompassed the full range of values for all other sites. No relationship between percent motile taxa and any measure of percent cover of fine sediment in the substrate was found when the sites with zero fines were excluded (Figure 2 a-c). On the other hand, percent motile taxa showed a strong response to conductivity, orthophosphate and total phosphate concentration (Figure 2 d-f).

Does the diatom assemblage vary with cover of fine substrate?

The initial CCA on the Welsh data indicated that alkalinity, percentage fine sediment cover, orthophsophate concentration and river slope at the site were best at describing the variation in the diatom taxa. Whilst these results do not necessarily imply that these are the drivers of change in the diatom assemblages, simply that they were the best statistically at describing the observed variation in the assemblages, it is highly likely that these environmental parameters are the main determinants of diatom assemblage composition, i.e. water chemistry, nutrients and river type (i.e. background expected sediment/flow conditions). The response of diatoms to nutrients, particularly orthophosphate concentrations, is well known and the basis for the TDI index (Kelly and Whitton, 1995). Similarly, the influence of alkalinity (or the related variables pH and conductivity) on diatom assemblages is well documented and, indeed, is used to predict reference condition when interpreting TDI (Kelly *et al*., 2001). River slope, describes background flow conditions and, hence, retention of sediment (Naden *et al*., 2016). The amount of deposited fine sediment at a site is determined by both the sediment load (amount of sediment entering the river) and retention (proportion of load that is deposited). Sediment load is highly influenced by human activities in the catchment (e.g. agricultural practices), which influences the amount of deposited sediment in the river. The likelihood of further underlying master variables influencing the results is negligible. It should be noted that all samples were collected in spring so any influence of seasonal variation was obviated. Hence, alkalinity, orthophsophate concentration and river slope at the site were used as covariables in the partial ordination, leaving only the influence of percentage fine sediment cover.

The first axis of the pCCA was correlated with percentage fine sediment cover. The distribution of the taxa along the first axis, an gradient of increasing percentage cover of fine sediment, was used to rank the diatom taxa from most to least sensitive to fine sediment (Figure 3). The taxa most strongly correlated with a low percentage cover of fine sediment were *Brachysira*, *Frustulia krammeri*, *Nitzschia tubicola*, *Diadesmis contenta*, *Nitzschia gracilis*, and *Surirella crumena*, whilst those most strongly associated with a high cover of fine sediment were *Cocconeis*, *Luticola mutica*, small *Navicula* species, *Navicula capitatoradiata* and *Gyrosigma acuminatum*.

Despite there being a strong influence of percentage cover of fine sediment on diatom assemblage composition, the prevalence of motility appeared to be distributed across the gradient of fine sediment (Figure 4a): there was no significant relationship between occurence of motility and the species pCCA axis 1 scores. Both motile and non-motile taxa were found throughout the gradient of percentage cover of fine sediment. In contrast, nutrient affinity had a significant realtionship with the gradient of percentage cover of fine sediment, with higher scoring taxa (higher affinity to nutrients) tending to have an association with a high percentage cover of fine sediment (Figure 4b).

DISCUSSION

Are diatom indices sensitive to hydromorphological alteration?

It was not possible to detect any effect of the hydromorphological modifications tested on indices of phytobenthos, despite alterations that influence flow velocity, the rate of sedimentation and in-stream habitat being included in the analysis. Although this result may be perceived as negative in the search for a diatom-based indicator of hydromorphology, it is an encouraging result: indices developed to assess the impact of nutrient pollution on phytobenthos should be robust to hydromorphological alteration, otherwise false diagnoses could result. Nevertheless, it was assumed that general descriptors of phytobenthos, such as percent planktonic taxa and percent motile taxa, would respond to hydromorphological alterations. Retention time is thought to be one of the main constraints on how rivers respond to eutrophication (Hilton *et al*., 2006), and it was assumed that any modifications that influence this (e.g. impoundment) would have an effect on the algal community and how it would respond to nutrient availability. Furthermore, it was assumed that any hydromorphological modification that influenced substrate would affect phytobenthos: substrate is thought to have a substantial influence on benthic algal community composition (Biggs *et al*., 1998; Schneck *et al*., 2011). Percent motile taxa has been proposed as an index of deposited fine sediment (Bahls, 1993) and, due to the effect of fine sediment on the response of diatoms to nutrients, it is recommended that percent motile taxa is used when interpreting indices such as TDI (Kelly *et al*., 2001). In these data nutrients (log_{10} orthophosphate) had a more pronounced effect on percent motile taxa than did any of the hydromorphological modifications investigated.

Does percent motile taxa respond to variation in cover of fine sediment?

It is possible that the categorizations of hydromorphological modification used in the WISER data did not adequately describe the extent of change imposed upon the river sites, thus obscuring any relationships. However, the STAR data indicated that percent motile taxa was not related to visual estimates of the percentage cover of fine sediment in the bed substrate. Rather, percent motile taxa appeared to be related to nutrient conditions, as was found in the WISER data. Although motile taxa do thrive in fine substrates (Dickman *et al*., 2005) there may be competitive advantage to this trait under other conditions. The relationship between percent motile and nutrients could be a consequence of competition for light between algal species favouring those taxa that can migrate to the top of the layer of benthic algae when nutrients are abundant, or simply that many species with these characteristics (small, rapidly growing, motile) are indicative of high nutrient conditions (Kelly *et al*., 2001).

Does the diatom assemblage vary with cover of fine substrate?

Despite the lack of a relationship between percent motile and substrate composition, the Welsh data indicated that percentage cover of fine sediment had a strong influence on diatom assemblages. This pCCA took into account variation due to natural gradients in river type and nutrient concentrations, leaving only that variation attributable to differences in cover of fine sediment, and it was possible to rank the taxa according to their affinity to this gradient. Despite a clear taxonomic response to sediment, motility did not show any association with the gradient of precentage cover of fine sediment. It appears that motility is a trait characteristic of taxa associated with a wide range of fine sediment conditions and cannot be reliably attributed to any part of the gradient of sediment pressure. Hence, it is recommended that percent motile taxa is not used as an index of fine sediment. On the other hand, the other trait investigated, nutrient affinity, did show a significant relationship with the gradient of precentage cover of fine sediment. As the partial analysis took into account that portion of the variation that was due to river type when ranking the taxa against the gradient of fine sediment, this response was not due to rivers with fine substrate tending to have higher nutrient concentrations. Specifically, orthophsophate concentration in the water was one of the covariables used in the analysis. As finer substrates are more strongly associated with anoxic conditions within the substrate and nutrient recycling (Pretty *et al*., 2006), it is possible that within-river sources of nutrients encourage those taxa with high nutrient affinity where fine sediment dominates the substrate.

Despite the failure to confirm percent motile as a diatom-based index of fine sediment, the strong influence of percentage cover of fine sediment on diatom assemblages suggests that there is potential to develop a robust metric relating diatoms to fine sediment pressure using the approach outlined here. Excess fine sediment has a variety of both direct and indirect impacts on diatoms (Jones *et al*., 2014) which may influence the ranking of taxa along the axis of percentage cover of fine sediment. Whilst motility may confer an advantage with respect to burial, taxa with small stature, robust frustules and/or strong adherance structures are more resitant to the scouring associated with excess fine sediment. Nevertheless, the analysis undertaken here does not seek to attribute causal mechanisms, which may be various and involve multiple traits, rather to establish a statistically robust ranking of the relative abundance of taxa along the gradient of fine sediment pressure. In Table II we have made the provisional next step in the development of such an index by assigning tolerance scores to the taxa based on their relative position along pCCA axis 1, with the most fine sediment-tolerant taxon (*Cocconeis* sp.) being scored 1 and taxa in successively more distant 10 percentile bands (percent of the axis 1 distance between the highest and lowest scoring taxa) along pCCA axis 1 being assigned scores of 2, 3, 4, etc. We suggest that this index (DISCO – Diatom Indictor of Sediment COnditions) should be calculated as an average weighted by percent occurence similar to TDI. However, we would caution that this should be considered a provisional diatom index to fine sediment stress for the following reasons. A) Visual assessments of percent cover of fine sediment are not a good estimate of the pressure from excess fines (Naden *et al*., 2015), particularly as they exclude any fine sediment entrained within the river bed (Duerdoth *et al*., 2015), which can have pronounced ecological impacts (Jones *et al*., 2014; Murphy *et al*., 2015): when considering the pressure from excess fine sediment, it is preferable to include some measure of the rate of retention relative to the expected retention if the site were in reference condition. B) A more extensive dataset would be preferable so that more species could be included and scores based on responses over a wider range of conditions, and include any influence of seasonal variation. C) Any new index should be tested against an independent dataset to confirm its performance. Hence, we suggest that the index is not used until more rigorous testing has been undertaken with an independent test dataset, in particular to determine any influence of seasonal changes in diatom assemblage composition.

CONCLUSIONS

Although benthic diatoms have been used primarily as indicators of eutrophication, deposition of excess fine sediment has the potential to cause a significant impact on benthic diatoms (Jones *et al*., 2014). Here we have tested the suggestion that the relative proportion of motile taxa can be used as an index of stress from fine sediment. Although diatoms did show a distinct response to percent cover of fine sediment, we found that percent motile taxa and the trait motility were not correlated with percentage cover of fine sediment. Rather, the percent motile index appears to be correlated with nutrient concentration. Hence, we recommmend that percent motile taxa is not used as an index of fine sediment, and suggest that a new index should be developed. We suggest that the approach described here has the potential to be developed into an index of sediment conditions, and present a provisional version of such an index (DISCO - Diatom Indictor of Sediment COnditions) based on the response of diatoms to fine sediment. However, we caution that this index requires considerable further development and testing before use.

Despite hydromorphology having considerable potential to affect benthic diatoms, the existing indices tested, which have been designed to assess stress from eutrophication, were robust to hydromorphological modification, thus reducing the possibility of false diagnosis of impacts.

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Table I. Results of ANCOVA investigating the influence of hydromorphological alteration on the relationship between phytobenthos indices and log_{10} orthophosphate concentration using WISER data. P values of the relationship between indices and log_{10} orthophosphate and the interaction with modification. Significant values shown in bold, trivial results (i.e. data from modified sites were all within the range of scatter of unmodified sites and relationships identified explained less than 5% of the variance) shown in square brackets

	Impoundment		Channel modification		Modification of instream habitat		Embankment		Riparian vegetation		Velocity modification	
Levels of modification	2		4		3		4		4		2	
	PO_4	PO_4 * Impoundment	PO_4	PO_4 *Channel	PO_4	PO_4 * Instream	PO_4	PO_4 * embankment	PO_4	PO_4 * Riparian Vegetation	PO_4	PO_4 * Velocity
Descy	0.543	0.959	**0.005**	0.511	0.721	0.506	0.193	0.215	**0.003**	0.962	0.709	[0.017]
Watanabe	**0.002**	0.497	**<.001**	0.242	**<.001**	0.515	**<.001**	0.408	**<.001**	0.136	0.172	0.769
TDI	**<.001**	0.157	**<.001**	0.250	**<.001**	0.861	**0.023**	0.679	**<.001**	[0.003]	**<.001**	0.806
% planktonic	**0.001**	0.607	**0.010**	[0.038]	**<.001**	[0.014]	**0.016**	0.362	**<.001**	0.133	**<.001**	0.859
IPS	**<.001**	0.097	0.430	0.086	**<.001**	[0.033]	**<.001**	0.756	0.186	0.108	**0.002**	0.268
IDAP	**<.001**	[0.059]	**<.001**	0.319	**<.001**	0.545	**<.001**	0.816	**<.001**	0.569	**0.006**	0.766
EPI-D	**<.001**	[0.035]	**<.001**	[0.004]	**<.001**	0.391	**<.001**	0.782	**<.001**	0.211	**<.001**	0.962
D-CH	**<.001**	0.361	**0.004**	[0.022]	**<.001**	0.673	**0.011**	0.062	**0.014**	0.772	**<.001**	0.558
IDP	**0.028**	0.350	**<.001**	0.219	**0.002**	0.775	**0.002**	0.742	**<.001**	0.163	**0.001**	0.366
LOBO	**<.001**	[0.008]	**<.001**	[0.006]	**<.001**	0.961	**<.001**	0.300	**<.001**	[0.008]	0.392	0.114
TID	**<.001**	0.917	0.071	0.128	**<.001**	[0.063]	**<.001**	0.654	**0.036**	0.121	**<.001**	0.631
% motile	0.107	0.261	**<.001**	0.477	0.071	0.119	0.589	0.660	**<.001**	[0.011]	**<.001**	0.842

Table II. The assignment of provisional DISCO (Diatom Indicator of Sediment COnditions) scores for for diatom taxa based on pCCA axis 1 of the Welsh agri-environment monitoring data (see Figure 3).

Taxon	Score	Taxon	Score
Brachysira sp.	10	*Achnanthidium* sp.	6
Frustulia krammeri	10	*Nitzschia paleacea*	6
Nitzschia tubicola	9	*Navicula angusta*	6
Diadesmis contenta	9	*Diploneis* sp.	6
Nitzschia gracilis	9	*Nitzschia dissipata* subsp. *media*	6
Surirella crumena	9	*Stauroneis* sp.	6
Fragilariforma sp.	8	*Diatoma mesodon*	6
Navicula claytonii	8	*Nitzschia perminuta*	6
Nitzschia hantzschiana	8	*Eucocconeis laevis*	6
Gomphonema olivaceoides	8	*Encyonema 'ventricosum'* agg.	6
Achnanthidium pyrenaicum	8	*Nitzschia sigma*	6
Encyonopsis sp.	8	*Melosira varians*	6
Eunotia sp.	8	*Navicula lanceolata*	6
Bacillaria paradoxa	8	*Frustulia* sp.	6
Nitzschia pusilla	8	*Encyonema gracile*	6
Nitzschia capitellata	8	*Navicula tripunctata*	5
Achnanthes oblongella	7	*Navicula capitata*	5
Meridion circulare var. *constrictum*	7	*Diploneis petersenii*	5
Tabellaria sp.	7	*Surirella angusta*	5
Peronia fibula	7	*Cocconeis pediculus*	5
Frustulia vulgaris	7	*Nitzschia archibaldii*	5
Nitzschia fonticola	7	*Amphora* sp.	5
Gomphonema clavatum	7	*Navicula cryptotenella*	5
Fragilaria capucina	7	*Navicula tenelloides*	5
Stauroneis anceps	7	*Diploneis oblongella*	5
Sellaphora pupula	7	*Psammothidium* sp.	5
Surirella roba	7	*Navicula* sp.	5
Neidium sp.	7	*Geissleria acceptata*	5
Nitzschia palea	7	*Surirella* sp.	5
Planothidium frequentissimum	7	*Tryblionella* sp.	5
Fragilaria sp.	7	*Psammothidium lauenburgianum*	5
Gomphonema parvulum	7	*Psammothidium grishunun* fo. *daonensis*	5
Denticula tenuis	7	*Caloneis* sp.	5
Gomphonema 'intricatum' type	7	*Amphora pediculus* agg.	5
Pinnularia sp.	7	*Nitzschia recta*	5
Eolimna minima	7	*Surirella brebissonii*	5
Gomphonema truncatum	7	*Gomphonema olivaceum*	5
Nitzschia linearis	7	*Luticola* sp.	5
Fragilaria vaucheriae	7	*Rhoicosphenia abbreviata*	5
Gomphonema clevei	7	*Cyclotella* sp.	5
Pseudostaurosira/Staurosira agg.	7	*Planothidium lanceolatum*	5
Planothidium rostratum	7	*Nitzschia* sp.	5
Fistulifera/Mayamaea spp.	7	*Encyonema* sp.	4
Brachysira vitrea /neoexilis	7	*Navicula radiosa*	4
Nitzschia dissipata	7	*Synedra ulna*	4
Pennate undif.	7	*Navicula menisculus*	4
Nitzschia sociabilis	6	*Reimeria* sp.	4
Adlafia suchlandtii	6	*Stephanodiscus* sp.	4
Gomphonema sp.	6	*Psammothidium helveticum*	4
Meridion circulare	6	*Navicula cincta*	4
Adlafia bryophila	6	*Nitzschia amphibia*	4
Chamaepinnularia	6	*Sellaphora seminulum*	4
Synedra sp.	6	*Navicula viridula*	4
Craticula molestiformis	6	*Stauroforma exiguiformis*	4
Navicula veneta	6	*Cocconeis placentula*	3
Psammothidium subatomoides	6	*Gomphonema angustatum*	3
Diatoma sp.	6	*Surirella minuta*	3
Achnanthes sp.	6	*Caloneis silicula*	3
Hannaea arcus	6	*Hantzschia amphioxys*	3
Navicula cryptocephala	6	*Gyrosigma acuminatum*	3
Navicula gregaria	6	*Navicula capitatoradiata*	2
Navicula rhynchocephala	6	*Navicula* [small species]	2
Stauroneis kriegeri	6	*Luticola mutica*	1
Gomphonema acuminatum	6	*Cocconeis* sp.	1

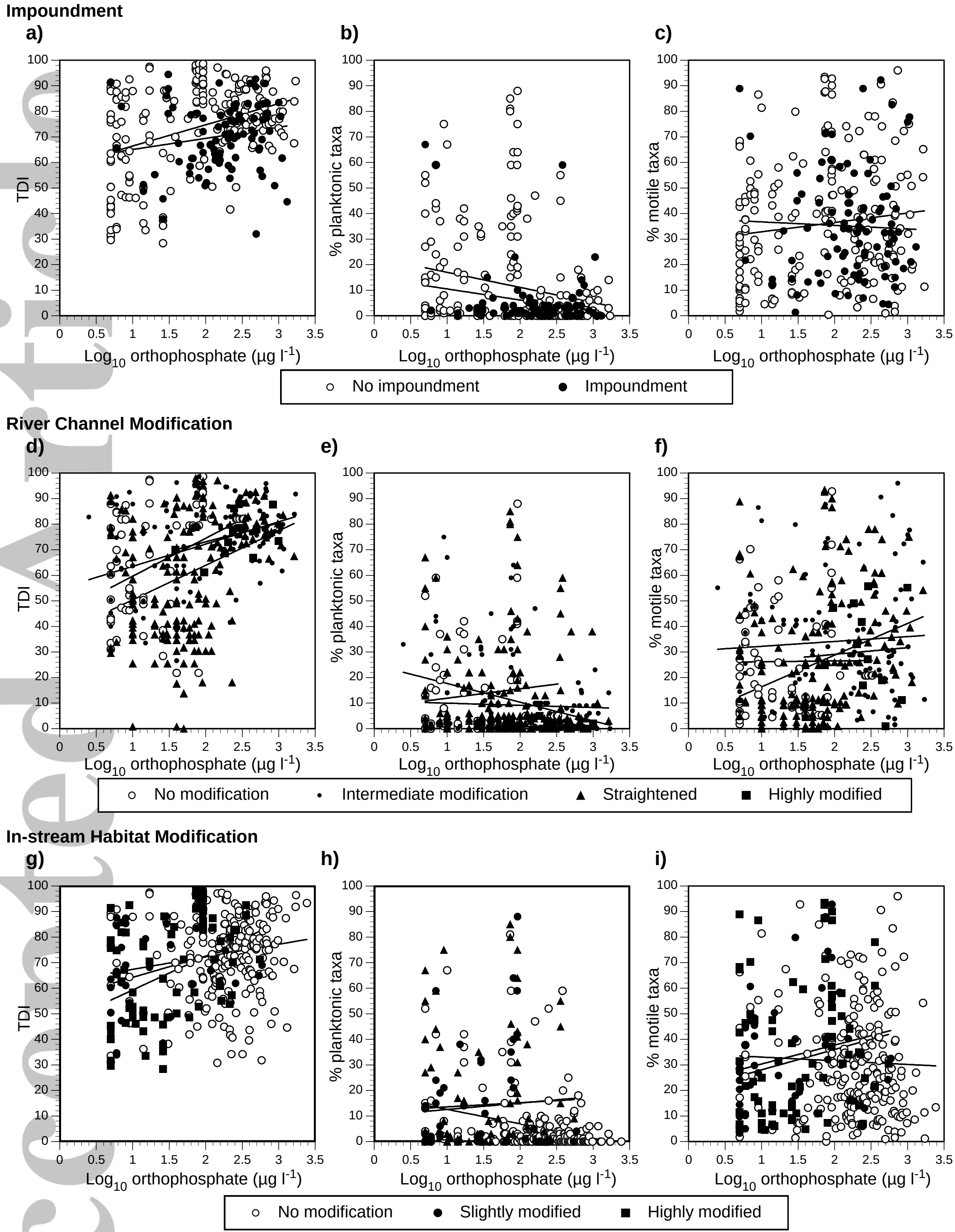

Figure 1. The influence of impoundments (a, b, c), channel modification (d, e, f), and in-stream habitat modification (g, h, i) on the relationship between $\log_{10}$ orthophosphate concentration and three indices of phytobenthos, TDI (a, d, g), % planktonic taxa (b, e, h), and % motile taxa (c, f, i). Influence of hydromorphological modification assessed by ANCOVA, see Table 1 for statistical significance of relationships.

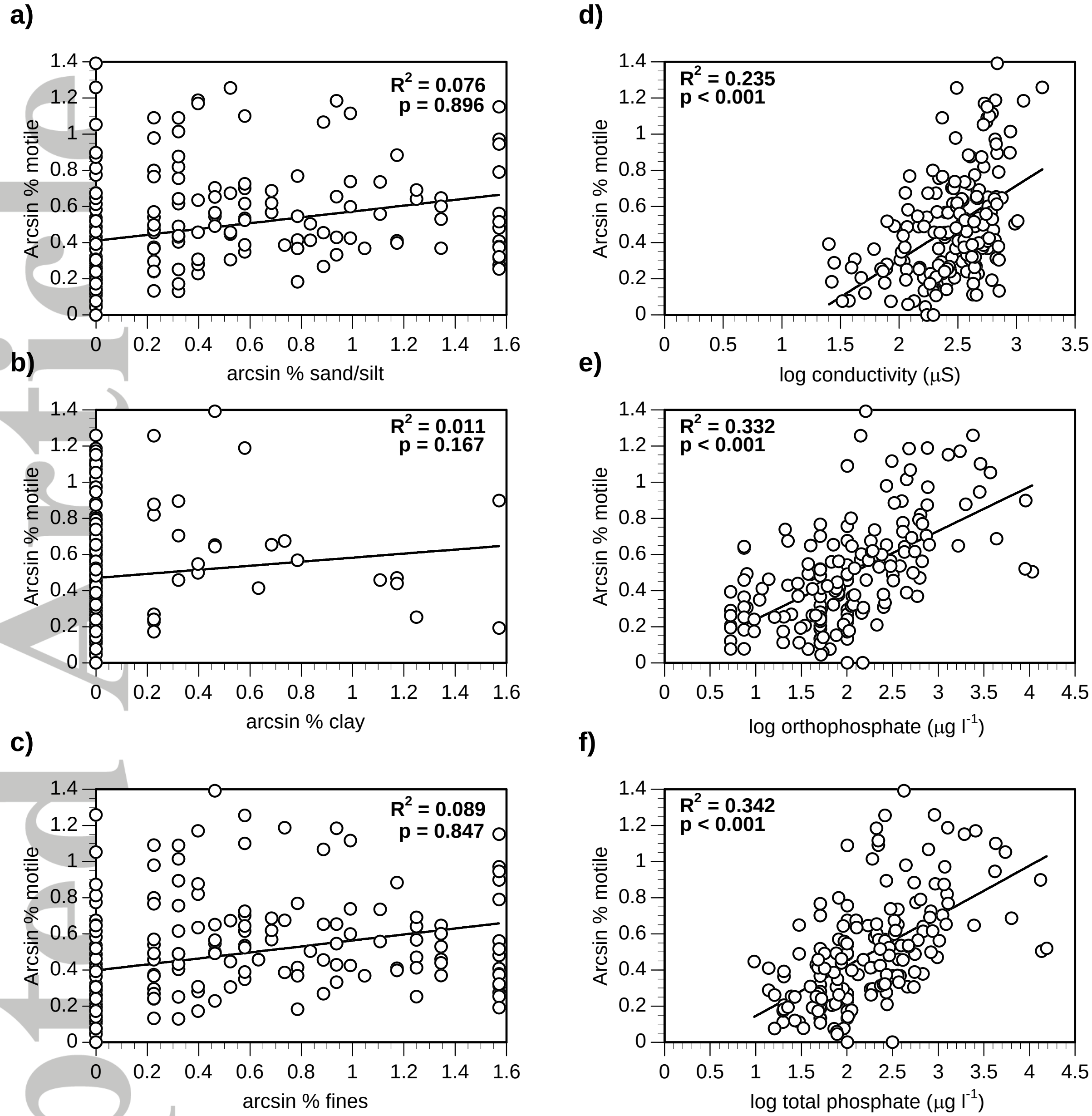

Figure 2. Relationships between the relative abundance of motile diatom taxa and measures of deposited fine sediment and water chemistry. a) % sand and silt (6 µm - 2 mm), b) % clay (< 6 µm), c) % fine sediment (sand, silt and clay), d) conductivity (µS), e) orthophosphate (µg l^{-1}), and f) total phosphate (µg l^{-1}). R^2 and p shown, zero values for % clay and % fine sediment bed composition have been excluded as trivial results were returned (see text).

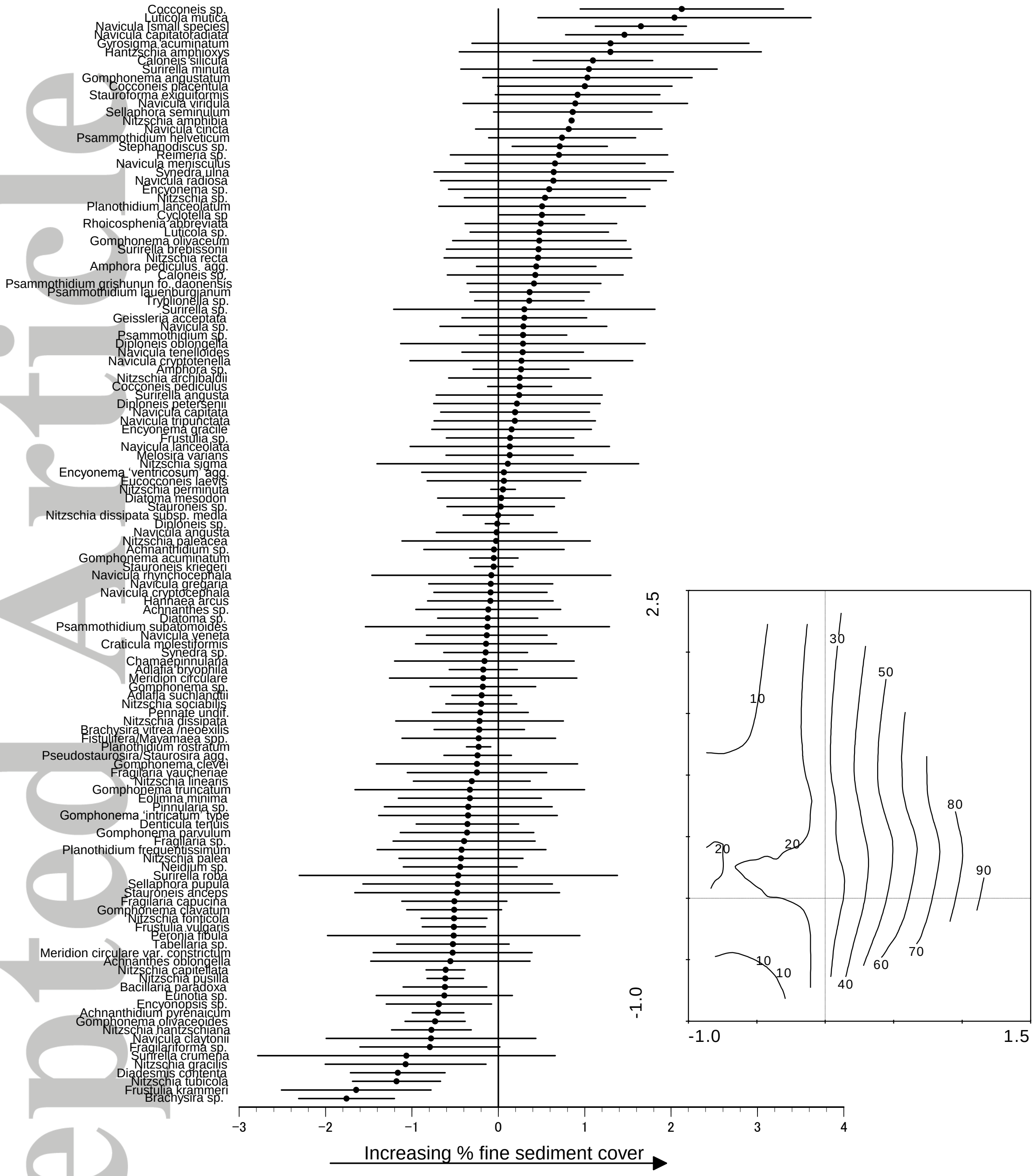

Figure 3. Optimum (point) and amplitude (line) of diatom taxa along the first canonical axis of pCCA, correlated with increasing % fine sediment cover. Taxa are ranked from least sensitive to most sensitive to fine sediment (top to bottom). Inset shows contour gradients of percentage fine sediment cover with respect to axis 1 of the pCCA ordination space.

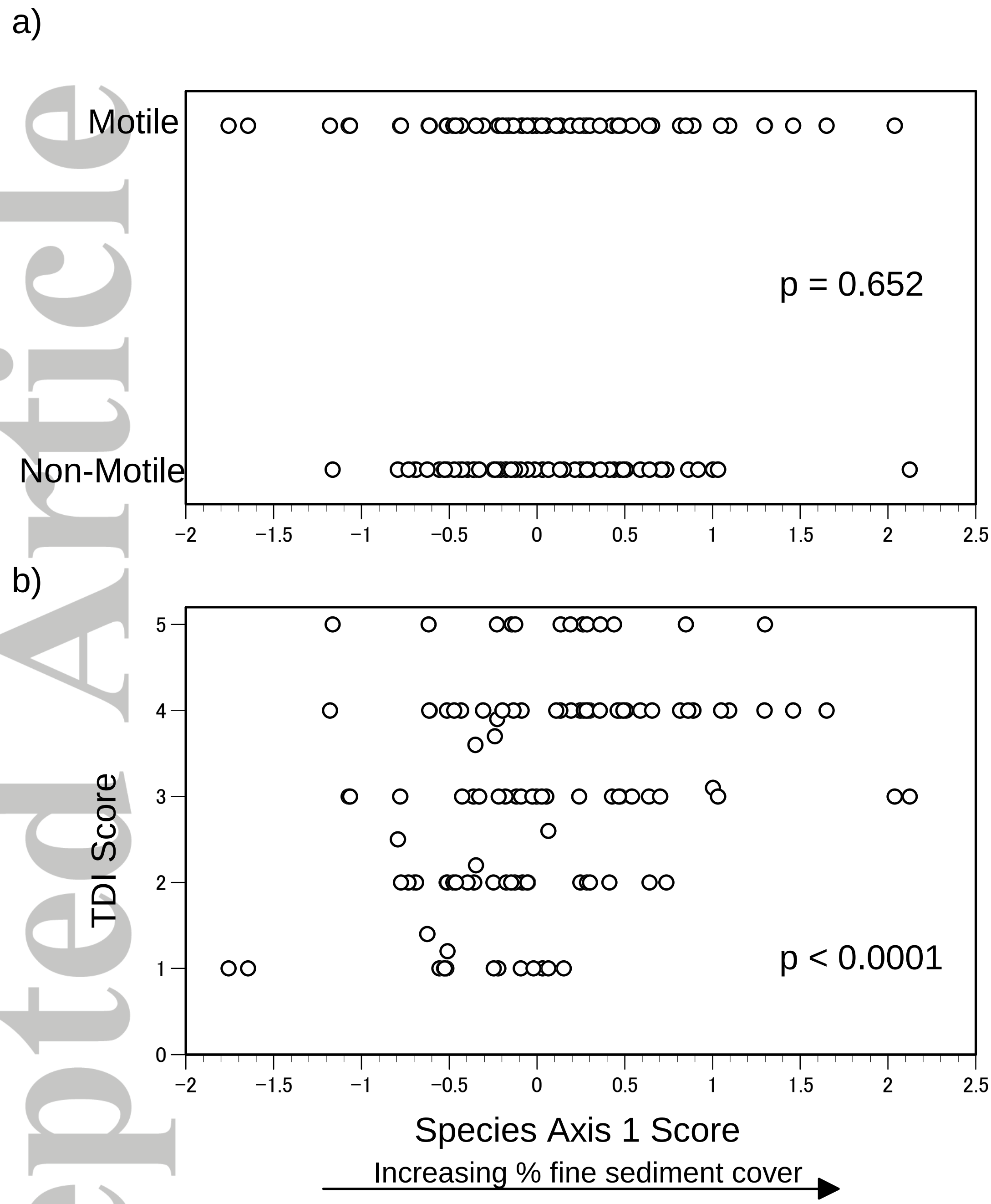

Figure 4. Distribution of two diatom traits, a) motility and b) nutrient affinity (as TDI score) along the first canonical axis of a pCCA, correlated with increasing % fine sediment cover (see Figure 3). The optima of taxa, and their corresponding trait characteristic, are plotted by their pCCA axis 1 scores. Significance of relationships determined by logistic regression.