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Taxon-specific responses of Southern Ocean diatoms to Fe enrichment revealed by synchrotron radiation FTIR microspectroscopy

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Abstract

Photosynthesis by marine diatoms contributes substantially to global biogeochemical cycling and ecosystem productivity. It is widely accepted that diatoms are extremely sensitive to changes in Fe availability, with numerous in situ experiments demonstrating rapid growth and increased export of elements (e.g. C, Si and Fe) from surface waters as a result of Fe addition. Less is known about the effects of Fe enrichment on the phenotypes of diatoms, such as associated changes in nutritional value, furthermore data on taxon-specific responses is almost non-existent. Enhanced supply of nutrient-rich waters along the coast of the subantarctic Kerguelen Island provide a valuable opportunity to examine the responses of phytoplankton to natural Fe enrichment. Here we demonstrate the use of synchrotron radiation Fourier Transform Infrared (SR-FTIR) microspectroscopy to analyse changes in the macromolecular composition of diatoms collected along the coast and plateau of Kerguelen Island, Southern Ocean. SR-FTIR microspectroscopy enabled the analysis of individual diatom cells from mixed communities of field-collected samples, thereby providing insight into in situ taxon-specific responses in relation to changes in Fe availability. Phenotypic responses were taxon-specific in terms of intraspecific variability and changes in proteins, amino acids, phosphorylated molecules, silicate and carbohydrates. In contrast to some previous studies, silicate levels increased under Fe enrichment, in conjunction with increases in carbohydrate stores. The highly abundant taxon *Fragilariopsis kerguelensis* displayed a higher level of phenotypic plasticity than *Pseudo-nitzschia* spp., while analysis of the data pooled across all measured taxa showed different patterns in macromolecular composition compared to those for individual taxon. This study demonstrates that taxon-specific responses to Fe enrichment may not always be accurately reflected by bulk community measurements, highlighting the need for further research into taxon-specific phenotypic responses of phytoplankton to environmental change.

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1 Introduction

Growth of diatoms in the global ocean is estimated to contribute ~ 20 % of the total primary productivity on Earth, thereby supporting substantial marine and terrestrial ecosystems, including fisheries which supply 15 % of the world's animal protein for human consumption (Armbrust, 2009; Mora et al., 2009; Nelson et al., 1995). As their frustules rain down to the deep ocean, diatoms export substantial quantities of C, Fe and Si from surface waters and determine the nutrient budget of the global ocean (Arigo, 2005; Ingall et al., 2013; Le Quere et al., 2005). Diatoms are very sensitive to changes in Fe availability and rapidly form expansive blooms when the trace metal becomes available (Marchetti and Cassar, 2009). Fe limitation is known to cause changes in diatom morphology (such as reduced cell size), reduced photosynthetic efficiency, pigment levels and growth rates (Marchetti and Cassar, 2009). The critical role of diatoms in ocean biogeochemistry and ecosystem functioning merits further investigation of their phenotypic responses to environmental change.

Naturally Fe-enriched water near the Kerguelen Island supports the largest annual phytoplankton bloom (45 000 km²) in the high nutrient, low chlorophyll Southern Ocean (Quéguiner et al., 2007). The natural phenomenon of Fe enrichment in this region presents a valuable opportunity to study the effects of Fe perturbations on diatom growth, productivity, community composition, biogeochemistry and carbon export (Blain et al., 2007). Little is currently known about the effects of Fe on the macromolecular composition (i.e. carbohydrates, lipids, proteins and nucleic acids) of diatoms. The macromolecular composition is an important component of the phenotype, determining energy and nutrient fluxes available for higher trophic levels and influencing cellular carbon productivity (Andersen et al., 2004; Kroon and Thoms, 2006; Sackett et al., 2013). Given the importance of diatoms to elemental cycling and marine ecosystem productivity, this study aimed to investigate the macromolecular responses of major groups of diatoms in waters near Kerguelen Island in relation to differences in Fe availability. Further, we demonstrate a novel, microspectroscopy-based approach to *phenomics*,

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nating the spectra (Fig. 2). Differences in the height and position of the peaks indicated that the macromolecular composition varied between taxa. Cell spectra from sites E-1 and E-5 had the strongest absorbance by bands associated with proteins (1540 cm⁻¹), lipids (3050–2800 cm⁻¹, 1745 cm⁻¹, and 1450 cm⁻¹), phosphorylated molecules (~ 1240 cm⁻¹) and carbohydrates (~ 1150 cm⁻¹) compared to stations EW-4 and TEW-8 (Fig. 2a and b). At station E-1 (Fig. 2a), *Pseudo-nitzschia* spp. showed the highest absorbance by proteins and lipids and *E. antarctica* the lowest. Also at station E-1, *F. kerguelensis* cells had the highest absorbance by phosphorylated molecules, followed by *E. antarctica*, *Pseudo-nitzschia* spp. then *Chaetoceros* spp. At station E-5 (Fig. 2b), *Pseudo-nitzschia* spp. and *F. kerguelensis* cells showed the highest absorbance by proteins and lipids, whereas *E. antarctica* had the lowest. Another notable feature of the cell spectra from station E-5 (Fig. 2c) was the relatively low variability in the region 3050–2800 cm⁻¹, as indicated by small error bars compared to cell spectra from the other stations.

3.2 Stations E4-W and TEW-8 (higher Fe availability)

Cells from the stations with higher levels of Fe availability (E4-W and TEW-8) showed spectra with weaker absorbance from bands above 1250 cm⁻¹ compared to those from stations E-1 and E-5 (Fig. 2c and d). As with the other stations, *F. kerguelensis* cell spectra showed the strongest absorbance by phosphorylated molecules (~ 1240 cm⁻¹) and the weakest by silicate and carbohydrates (1160–1040 cm⁻¹) relative to the other taxa.

3.3 Multivariate modeling and taxonomic classification

Partial Least Squares Discriminant Analysis (PLSDA) is a multivariate modeling process enabling the modeling and classification of spectral datasets. In this study, PLSDA served to both validate the visual observations made from the average second derivative spectra and to assess the power of SR-FTIR microspectroscopy to taxonomically

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ing weights plots (Fig. 5c and f) showed similar trends for both the pooled taxon and *F. kerguelensis* with regard to trends in protein, phosphorylated molecules, silicate and carbohydrate levels between sites. In spite of this, the contribution of phosphorylated molecules to variation between cell spectra was stronger for *F. kerguelensis* alone.

The PLSDA by station for *F. kerguelensis* also served to examine the phenotypic plasticity of the species between high and moderate Fe stations (Fig. 5d–f). Models that can robustly discriminate between cell spectra from different stations, should indicate that the macromolecular composition of those cell spectra vary distinctly with station (Sackett et al., 2013). Factors 1 and 3 of the scores plots from PLSDA models explained 49% of the variation in the spectral dataset, with good clustering by station. *Pseudo-nitzschia* spp. did not show a similar pattern of clustering and model discrimination accuracy was poor (Fig. 6). This indicated that *F. kerguelensis* demonstrated a higher level of phenotypic plasticity under conditions of high and moderate Fe than did *Pseudo-nitzschia* spp.

4 Discussion

The taxon-specific analysis of *F. kerguelensis* cell spectra revealed that stations with lower Fe availability demonstrated higher levels of intraspecific variability. The sources of variation between cell spectra were multivariate, including most of the major macromolecular bands (proteins, phosphorylated molecules, amino acids, silicate and carbohydrates). Differences in variability were not observed when data was pooled across the four taxa, suggesting that community averages may not necessarily reflect the macromolecular composition of *F. kerguelensis*, despite its relatively high abundance within the assemblage. Although studies of intraspecific variability in microalgae are rare, higher levels of population variability have been previously reported for laboratory cultured microalgae under nutrient stress using SR-FTIR microspectroscopy (Heraud et al., 2008). Also, in a previous study of the Southern Ocean diatoms *Fragilariopsis cylindrus*, *Chaetoceros simplex* and *Pseudo-nitzschia subcurvata*, higher intraspecific

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5 variability was shown, particularly for *F. cylindrus*, under extreme salinity and temperature conditions (Sackett et al., 2013). Given that dissolved and particulate Fe levels appeared to be decreasing during the time series stations (indicated by a decrease from E-3 to E-5; Bowie et al., 2014), high intraspecific variability in *F. kerguelensis* could be indicative of a population in transition towards Fe-limitation. Thus, the increased intraspecific variability may have been a sign that the population was in transition, relative to the Fe-enriched stations; however, further studies are required with controlled laboratory trials to confirm that the observed macromolecular changes were due solely to the influence of Fe supply.

10 Based on the SR-FTIR spectra, levels of silicate were higher in *F. kerguelensis* cells from the Fe-enriched stations (E4-W and TEW-8) compared to the moderate Fe stations (E-1 and E-5). This result contradicts some previous studies which have shown an opposite correlation between Fe availability and silicification (Marchetti and Cassar, 2009), but is not unprecedented. During the SOFeX experiment, Twining et al. (2004a) observed a 16% increase in cellular Si after Fe addition for individual *Fragilariopsis* spp. cells, and Hoffmann et al. (2007) measured an increased ratio of biogenic silica (BSi) to particulate organic phosphate with bulk measurements of Fe-enriched Southern Ocean diatoms during the EIFEX project.

20 At the Fe-enriched stations (E4-W and TEW-8), the growth rate of the bulk population was substantially elevated compared to the moderate Fe stations (E-1 and E-5; Table 1). In their review of silicon metabolism in diatoms, Martin-Jézéquel et al. (2000) reported that the widely accepted inverse relationship between silicification and growth rate does not always hold, particularly when extracellular silicon is not limiting (Martin-Jézéquel et al., 2000). Thus, it is possible that Fe enrichment, in the presence of adequate ambient Si levels, stimulated both high growth rate and high levels of biogenic silica. In conjunction with silicate, absorbance from phosphorylated molecules and starch was also higher in cell spectra from the moderate-Fe stations (E-1 and E-5; Fig. 2a and b). This is interesting because a previous spectroscopic study found that absorbance from phosphorylated molecules was positively correlated with growth rate (Jebsen

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ally, samples from the Fe enriched stations were observed to have substantially higher carbon productivity, which could account for increased concentrations of cellular carbon (Jacquet et al., 2014). If the decrease in BSi/POC was caused primarily by a large increase in cellular C concentrations, calculations of cellular C from cell volume may provide underestimates under Fe enrichment, because cell size is constrained by the frustule (Twining et al., 2004a).

Such changes in cellular carbon and Si content are consistent with changes in macromolecular composition observed by SR-FTIR microspectroscopy. The PLSDA loading weights plot for *F. kerguelensis* shows that cell spectra from station TEW-8 had the highest absorbance from silicate ($\sim 1080 \text{ cm}^{-1}$) and carbohydrates ($\sim 1040 \text{ cm}^{-1}$) among the four stations (Fig. 5f). Southern Ocean diatoms have been observed to accumulate carbohydrates to a maximum in the evening, providing a source of carbon skeletons for protein production over night (van Oijen et al., 2004). Given that station TEW-8 was sampled at 10 PM, it is possible that cellular carbohydrate levels were at a maximum at this time. Although carbohydrates contain a relatively lower proportion of carbon (g C (g DW)^{-1}) compared with lipids and proteins (~ 0.4 , ~ 0.76 and ~ 0.53 respectively), it is plausible that a substantial increase in carbohydrate storage levels could cause an increase in cellular carbon (Geider and La Roche, 2011). The pattern of changes in silicate and carbohydrate levels between stations was more complex for the pooled data set than for *F. kerguelensis* alone, indicating that community average measurements may not always reflect the composition of the dominant taxa, which can differ widely, and should therefore be interpreted cautiously.

This study adds to the growing evidence that SR-FTIR microspectroscopy has great value as a tool for quantifying various traits (e.g. growth rate, nutrient status, nutritional value, taxonomic identification) in phytoplankton and other microorganisms (Heraud et al., 2005, 2007; Jebson et al., 2012; Ngo-Thi et al., 2003; Sackett et al., 2013). Trait-based approaches are a powerful, relatively untapped resource for improving predictions of phytoplankton community composition and dynamics in response to environmental change (Litchman and Klausmeier, 2008). For example, phenotypic

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5 plasticity provides great potential for a species to cope with rapid and prolonged environmental changes (Charmantier et al., 2008). Accurate, taxon-specific models that describe a broad range of phenotypic parameters, referred to here as *phenomic models*, will enhance our ability to predict the response of the phytoplankton community to future climate scenarios. The power of these multivariate phenomic models depends largely on our ability to collect phenotypic data using high-throughput, robust and inexpensive techniques. Multivariate modeling approaches are recognised as integral tools for the analysis of ecophysiological traits (Litchman and Klausmeier, 2008), however less attention has been paid to the power of multivariate *measurement* techniques, such as SR-FTIR microspectroscopy and X-ray Fluorescence Microprobe Analysis, for data collection. Encouraging the use of such multivariate measurement techniques, which can be used to predict multiple traits simultaneously, will dramatically increase the speed of trait data collection for microorganisms. Moreover, the advent of commercially available portable infrared and Raman spectrometers means that spectroscopic measurements can now be made in real time in the field.

5 Conclusions

This study demonstrated the taxon-specific responses of four dominant diatom taxa in association with natural Fe enrichment. These responses were not entirely reflected by the pooled data which was viewed as analogous to bulk community averages. *Fragilariopsis kerguelensis* showed distinct phenotypic differences (i.e. plasticity) between high and moderate Fe stations, whereas *Pseudo-nitzschia* spp. did not (Figs. 5 and 6). Understanding differences between taxa is important for improving our ability to predict phytoplankton community composition and dynamics in response to environmental change (Arrigo, 2005). The current lack of taxon-specific data is related to methodological challenges that limit in situ studies to bulk-community measurements. Methods which permit the analysis of individual cells, such as SR-FTIR microspectroscopy, present a pathway for the collection of taxon-specific data to fill this gap. Such data is in

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great demand from carbon cycle and ecosystem modelers, particularly for the Southern Ocean (Carr et al., 2006), and is necessary for advancing our ability to predict the effect of environmental changes, including climate change, on Earth's marine and terrestrial ecosystems.

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Table 1. Description of sampling stations and associated biogeochemical characteristics.

Station	Dissolved Fe	POC (μM)	PBSi (μM)	BSi/POC (mol)	^{a,b} $\delta^{13}\text{C}$ -POC (‰VPDB)	^b Growth rate (%)	Productivity _{E_z} ($\text{mg C m}^{-2} \text{d}^{-1}$)	Notes
E-1	Moderate	0.57	0.36	0.63	+1.63	25	578	Complex recirculation system located in a stationary meander of the Polar Front. Time series data. Receiving Plateau waters. Receiving Kerguelen Island waters.
E-5	Moderate	0.44	0.28	0.63	+1.46	25	1064	
E4-W	Higher	1.18	0.67	0.57	+2.83	50	3287	
TEW-8	Higher	7.1	1.65	0.23	+4.67	75	No data	
Ref.	Trull et al. (2014)	Trull et al. (2014)	Trull et al. (2014)	Trull et al. (2014)	Trull et al. (2014)	Trull et al. (2014)	Jacquet et al. (2014)	Blain et al. (2014a); Trull et al. (2014)

NB: values reported from Trull et al. (2014) are for size fraction 20 μm , which is composed of diatoms and other microalgae.

^a isotopic indices of growth rate,

^b increase relative to reference station,

E_z euphotic zone,

^{VPDB} Vienna Pee Dee Belemnite standard.

Abbreviations: Particulate Organic Carbon (POC), Particulate Biogenic Silicate (PBSi).

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Table 2. Infrared band assignments.

Wavenumber (cm ⁻¹)	^a Assignment	Reference (and refs. therein)
1200–900	ν (C–O–C) of polysaccharides, dominated by ring vibrations of carbohydrates	Giordano et al. (2001); Naumann (2000)
1080	ν (Si–O) from the silicate frustule	Giordano et al. (2001)
1150	ν (C–O) from carbohydrate moieties	Giordano et al. (2001); Heraud et al. (2007)
1230	ν_{as} (P=O) from phosphorylated molecules (includes nucleic acids, phosphorylated proteins and phosphorylated lipids)	Giordano et al. (2001)
1516	Phenol group (ring vibration), possibly from Tyrosine	Naumann (2000); Venyaminov and Kalnin (1990)
1400	ν_s (COO ⁻) carboxylate stretching mode, primarily from free amino acids	Fraille et al. (2010)
1450	δ_{as} (CH ₃) and δ_{as} (CH ₂) mainly from lipids and proteins	Heraud et al. (2005)
1540	δ (N–H) of amides associated with proteins	Giordano et al. (2001); Heraud et al. (2007)
1735	ν (C=O) of ester functional groups, from lipids and fatty acids	Vongsvivut et al. (2012)
1708	ν (C=O) of free fatty acids	Murdock and Wetzel (2009)
2850	ν_s (C–H) from methylene (–CH ₂) groups, primarily from lipids	Vongsvivut et al. (2012)
2920	ν_{as} (C–H) from methylene (CH ₂) groups, primarily from lipids	Vongsvivut et al. (2012)
2960	ν_{as} (C–H) from methyl (CH ₃) groups, primarily from proteins	Vongsvivut et al. (2012)
3015	ν (C–H) of cis-alkene –HC=CH–, from unsaturated fatty acids	Vongsvivut et al. (2012)

^a ν_{as} – asymmetric stretch; ν_s – symmetric stretch; δ_{as} – asymmetric deformation (bend); δ_s – symmetric deformation (bend).

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[Title Page](#)[Abstract](#)[Introduction](#)[Conclusions](#)[References](#)[Tables](#)[Figures](#)[Back](#)[Close](#)[Full Screen / Esc](#)[Printer-friendly Version](#)[Interactive Discussion](#)**Table 3.** Taxonomic Classification Summary Statistics for PLSDA models.

Station	No. Taxa	<i>n</i> (cal)	<i>n</i> (val)	Factors	Sensitivity	Specificity
E-1	4	94	54	3	91 %	97 %
E-5	3	85	48	5	94 %	97 %
TEW-8	2	29	19	4	100 %	100 %
E4-W	2	71	45	1	98 %	98 %

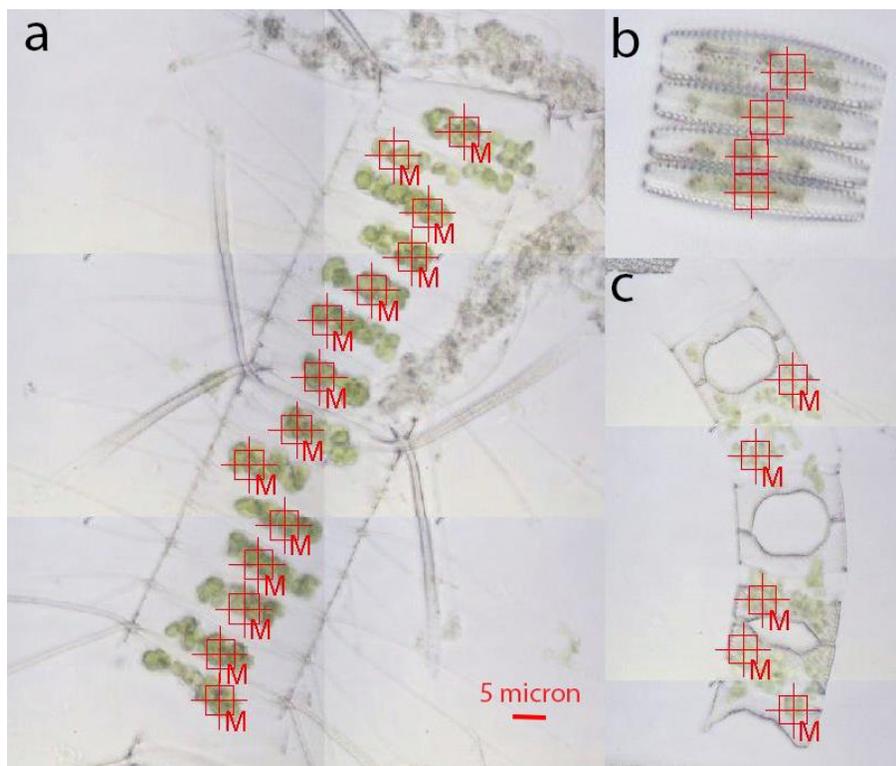


Figure 1. Visible images of *Chaetoceros* spp. (a), *F. kerguelensis* (b) and *E. antarctica* (c) cells showing the infrared measurement positions (indicated by cross hairs). The red square around the crosshairs indicates that the aperture size on the infrared microscope was set to $5 \times 5 \mu\text{m}$.

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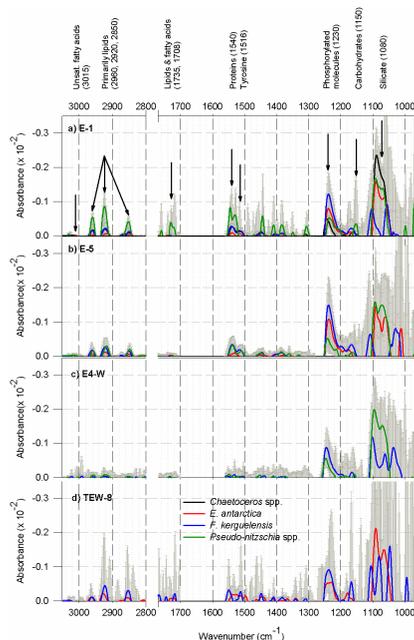


Figure 2. Average second derivative cell spectra from four common genera: *Chaetoceros* spp., *Eucampia antarctica*, *Fragilariopsis kerguelensis* and *Pseudo-nitzschia* spp. Taxonomic abundance varied between stations hence there is no data for *Chaetoceros* spp. and *Eucampia antarctica* at station E4-W, or *Chaetoceros* spp. and *Pseudo-nitzschia* spp. at station TEW-8. **(a–d)** show four different stations. Peaks show the macromolecular composition of cells which is dominated by phosphorylated molecules (1240 cm^{-1}), carbohydrates ($\sim 1150\text{ cm}^{-1}$) and silicate (1080 cm^{-1}). Peaks found in the spectral region $3050\text{--}2800\text{ cm}^{-1}$, $\sim 1735\text{ cm}^{-1}$ and $\sim 1450\text{ cm}^{-1}$ indicate the presence of lipids. Additionally, peaks at 1540 and 1515 cm^{-1} indicate the presence of proteins and the amino acid tyrosine, respectively. Error bars indicate one standard deviation from the mean. Note: the y-axis has been reversed to ease examination of the negative peaks (which are the relevant peaks after second derivative transformation).

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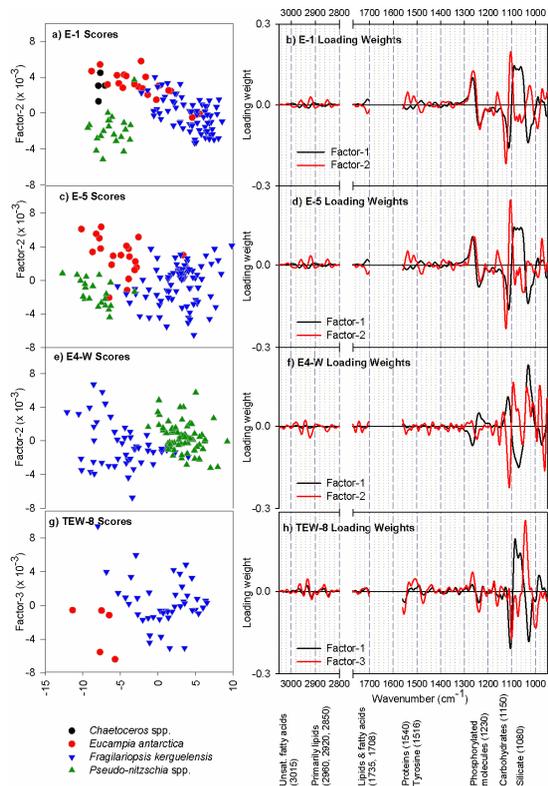


Figure 3. Taxonomic Classification by PLSDA results for four common genera: *Chaetoceros* spp., *Eucampia antarctica*, *Fragilariopsis kerguelensis* and *Pseudo-nitzschia* spp. (a–h) show four different stations. Scatter plots on the left hand side shows scores and line plots on the right hand side show the associated loading weights. The scores plots indicate that the cell spectra cluster well by taxonomic grouping at each site. The loading weights plots indicate that the source of the variation between cell spectra is largely from the lower wavenumber region ($\sim 1240\text{--}1000\text{ cm}^{-1}$).

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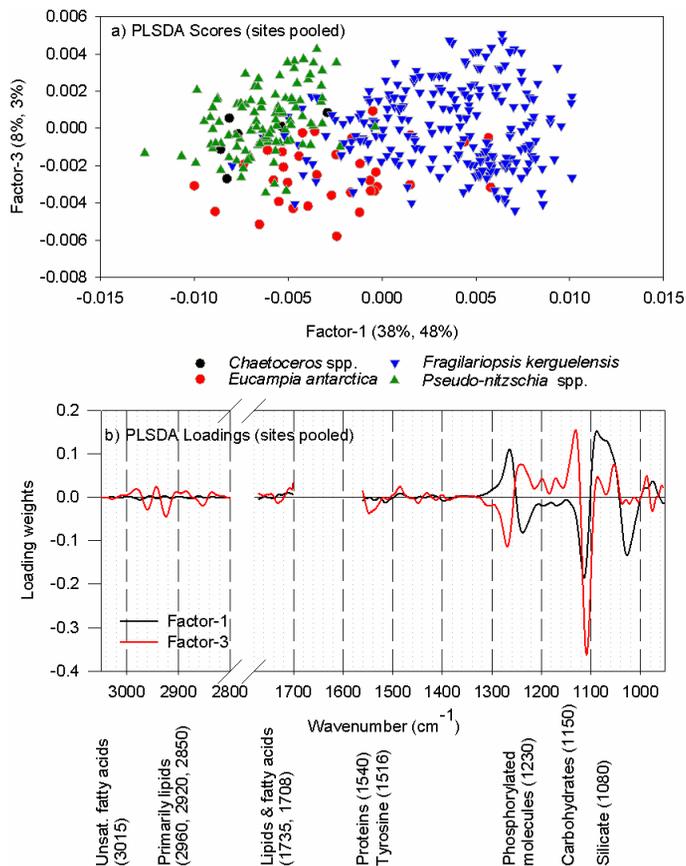


Figure 4. Classification of cell spectra by PLSDA with data pooled across stations. PLSDA scores plot (a) shows clustering of cell spectra by taxon. PLSDA Loading weights plot (b) shows spectral bands which drive variation between cell spectra – primarily phosphorylated molecules (1230 cm^{-1}), carbohydrates (1150 cm^{-1}) and silicate (1080 cm^{-1}).

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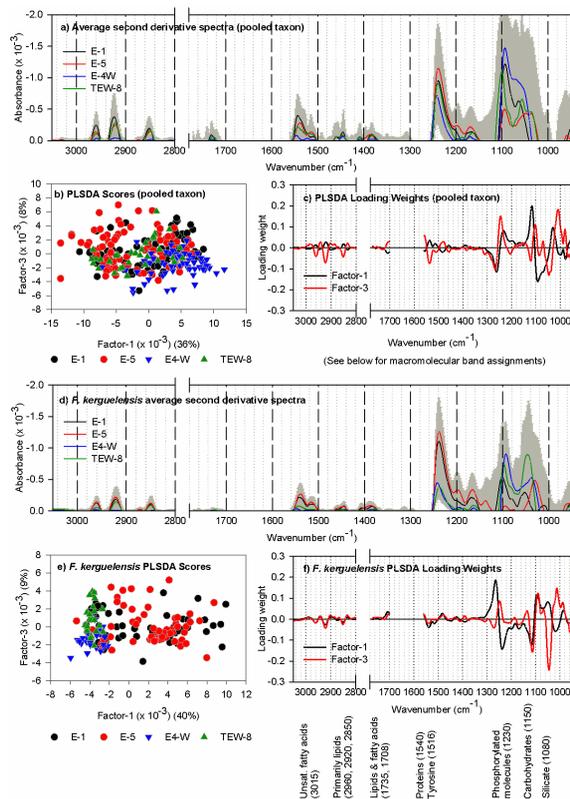


Figure 5. Variations in macromolecular composition for four taxon pooled (**a–c**) and separately for *F. kerguelensis* (**d–f**). Average second derivative spectra (**a** and **d**) and loading weights plots (**c** and **f**) indicate the source of the variation between the taxonomic groups, including strong bands related to phosphorylated molecules, silicate and carbohydrates (~ 1240 – 1000 cm^{-1}). Weaker contributions from lipids and proteins are indicated by the presence of bands at 1730 cm^{-1} and 1540 cm^{-1} , respectively. Scores plots (**b** and **e**) show clustering by station and differences in intrapopulation variability.

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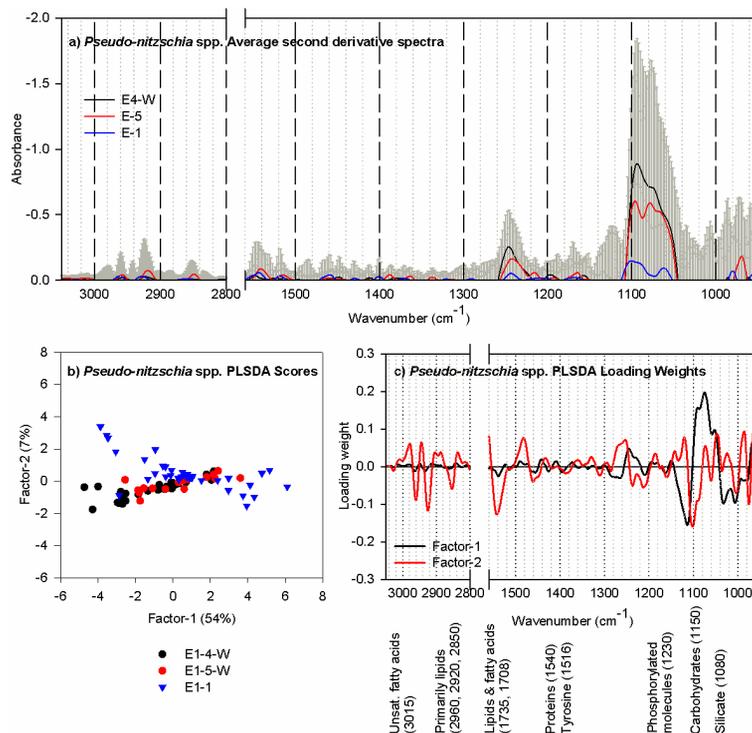


Figure 6. Average second derivative spectra (a), PLSDA scores plot (b) and PLSDA loading weights plot for *Pseudo-nitzschia* spp. at stations E-1, E-5 and E4-W. Although the average second derivative spectra show differences between treatments, intra and interspecific variability are both high, resulting in weak clustering by station in the scores plot. The loading weights plot shows that variability between cell spectra is largely driven by protein levels ($\sim 1540 \text{ cm}^{-1}$) carbohydrates (1150 cm^{-1}) and silicate (1080 cm^{-1}).