Anonymous Referee #1

General comments of referee 1

The authors have put together a strong manuscript that presents novel analyses of a dataset of phytoplankton taxonomy and pigments from the Beaufort Sea. They were cautious in their use of CHEMTAX as an approach to determine the relative presence of different phytoplankton types, in that they optimized the input ratio matrix for their phytoplankton communities. A comparison of the CHEMTAX results to both cell abundance and carbon biomass is useful in the interpretation of the pigment data, and highlights the differences in phytoplankton community descriptions that arise from the use of different measurement techniques. The authors could focus more on the importance of using different methods to characterize the phytoplankton community, and it seems that even after pointing out some of the misinterpretations and challenges with using CHEMTAX they still promote it as the most accurate method. The manuscript will be stronger with less emphasis on CHEMTAX as a way to monitor phytoplankton populations, and more as a component in the suite of measurements that are needed to characterize phytoplankton communities accurately and for diverse applications. Overall the work is thorough and relevant, and will potentially be useful for future analyses of Arctic Ocean pigment data.

Author's general response

We would like to thank referee 1 for his report about our paper. We greatly appreciate the positive and constructive comments and will take care to answer each of them. The referee feels that we could focus more on the importance of using different methods to characterize the phytoplankton communities. We fully agree with this point and will put more emphasis on the importance of using other, complementary, approaches to accurately characterize the phytoplankton communities. We will modify the introduction and conclusion accordingly, to avoid overselling the merits of the CHEMTAX method. Our aim was not to present pigments as a method that outperforms other approaches of characterizing phytoplankton communities. Some critical information such as the carbon content or species composition cannot be obtained with pigments. Nevertheless, we thought that the development of pigment-based method like CHEMTAX should be encouraged as it provides an especially well-adapted method, if regionally calibrated, to monitor the dominant phytoplankton groups from year to year with good reproducibility. Please note that to answer a comment of the referee 2, a table was added as supplementary material to provide a statistical testing of the difference between the environmental conditions of the clusters.

We greatly appreciate the time taken by referee 1 to point out the grammatical and conjugational mistakes in order to improve the manuscript. The detailed comments of referee 1 have helped to improve the manuscript. Below we address all of the specific and technical comments raised by referee 1 and highlight the changes made in the revised manuscript with a yellow overlay.

Specific comments of the referee 1 (in blue) and associated author's changes in manuscript (in black)

14489, 20-22: Given the previous statement about the uncertainties in the CHEMTAX method regarding dinoflagellates that lack peridinin and heterotrophic prey pigments, it may be more accurate to say something like "...variability in several different phytoplankton populations that are not affected by these misinterpretations".

We modify accordingly (L.36-37).

14490, 17: Although, different measurement approaches provide different informatio on the phytoplankton community – so while they are hard to compare, only using one approach will limit the breadth of knowledge. 26: And, all four of the satellite methods listed were developed using in situ data that were not from the Arctic (i.e., the Arctic may require its own regional tuning).

We underlined the importance of using various approaches (L73-76). We indicate the fact satellite methods were tuned in non-polar regions. (L83-85)

14491, 22: An "Arctic-specific" parameterization may not be realistic… maybe it would be more appropriate for the parameterization to be for a region and season, and could be used as a starting point for other Arctic CHEMTAX work.

We understand the hesitancy to create an "Arctic-specific" parameterization as the ratios and species require regional calibration. As proposed by the referee, we specified that our parameterization is adapted for use in the Beaufort Sea but can be used as a starting point for future Arctic CHEMTAX work (L131-134).

14497, 14-15: The difference in scales for TChl a values is not immediately clear in Figure 2... maybe make a note in the Fig. 2 caption alerting the reader to this fact.

We add a caption alerting the different scale in the Fig.2 (L.946-947).

14499, 2: "a greater contribution of Pras during the relatively icy summer of 2002" is vague – was it found near the ice, near shore, which part of the Arctic, etc.

We change the sentence to precise on which part of the Arctic this assumption is related (L.369-371).

14508, 7: The last sentence of the paper seems to make a claim that was not supported throughout the manuscript – it implies that CHEMTAX is the accurate approach while others (microscopy, flow cytometry) are not. However, earlier in the paper the limitations of using pigments only (C:Chl a variations, detection of ingested pigments by heterotrophs) were discussed. So, there may be a better way to end the manuscript that emphasizes the need for multiple measurement types, or at least the consideration of these uncertainties when using HPLC pigments and CHEMTAX "for detecting seasonal or interannual changes in phytoplankton communities".

This last paragraph was modified. We highlights the importance of coupling pigments information with information from others methods (L.683-689).

Figures 8 & 9: It may be useful to look not only at the correlation values but also at the predictive capabilities (RMSE) of cell abundance and carbon biomass from the CHEMTAX-derived algal groups.

We added the root-mean square error (RMSE) in Fig. 8 and Fig. 9. Explanations are included in the figure captions (L.996-999 and L.1008-1012).

Technical comments of the referee 1 (in blue) and associated author's changes in manuscript (in black)

Throughout the manuscript, "matrix ratio" should be "ratio matrix"; also all genus and species names should be italicized.

We changed for ratio matrix

14489, 15: Should be “Microscopic counts” 24: First sentence of the introduction is a little awkward. Perhaps replace “experiences” with “is undergoing”. 26: Can leave out “in terms of” in this sentence.

Corrections done

14490, 2: Can leave out “sized” in this sentence. 4: “Ice free” should be “ice-free” 22: “Other techniques...” is vague; perhaps list them.

Corrections done. We list the methods, i.e. flow cytometry and molecular analysis.

14491, 5: “to characterize” should be “characterization of” 13: Remove “Only” (or use “Only a few”) 20: “underscored” should be “underscores” 23: Should be “CHEMTAX in the Arctic Ocean”

Corrections done

14492, 8: Maybe rephrase to “The pigment ratios of these dominant Arctic groups were then found...” 10: This final sentence is vague, maybe say something more specific about the study being presented, such as “This work demonstrates the use of CHEMTAX to describe phytoplankton populations, and similar studies conducted in the future could be used to investigate changes in populations over time”.

We modify the sentence accordingly to the referee suggestion (L.142-145).

14493, 28: Could be “phytoplankton were distributed among 10 classes. . .”

Corrections done

14494, 3: Could be “unidentified cells were < 5µm” 3: The sentence starting with “Microscopic analysis...” is not clear – it could just start with “Enumeration of picophytoplankton… ”

Corrections done

14495, 10: Should be “Two inputs...” 14: Chlorophyllide has not yet been spelled out (add the full name and the abbreviation in parentheses) 17: Should be “allowed us to accurately” or “allowed accurate definition of...” 18: Should be “due to the fact that their specific pigment...”

Corrections done

14496, 1: Should be “raphidophytes and dictyochophytes” 2: “Allo” should be spelled out (this goes for other pigments throughout as well, at least the first time they are mentioned they should be spelled out) 7: Should be “containing the pigment Pras” 8: Should be “associated with” (this comes up multiple times; change throughout) 19: Should be “The ratio of pigment/Chl a” 25: Probably should not be a new paragraph

We take care to spell out the full pigment name each time it appears for the first time.

14497, 5: “ie” should be “i.e.” 13: Should be “twice as high” (and again later in the manuscript) 23: It would be clearer to add commas: “These two pigments, characteristic of diatoms, represented...”

Corrections done

14498, 9: Can remove the word “pigments” 15: “at the expense of” implies that one only increased because the other decreased (which may be true, but no real evidence of it) – it might be more accurate to say “...increased while diatom pigments decreased”

We change accordingly.

14499, 4-6: Should be “characteristic of...” 22: Replace “matix” with “matrix”

We change accordingly.

14500, 13: Replace “the cluster 3” with “cluster 3” 14: Should be “no longer present” 20: Maybe instead of “It is consistant” use “This is consistent” (note spelling change as well)

Corrections done

14501, 1: Replace “underlines” with “underlined”, or “described” 26: The y-axis label of Fig. 5 should be “Nitracline” to match the caption and text; if all nutrients and not just nitrite/nitrate are being considered, then “nutricline” could be used, but it should be made clear which is represent in Fig. 5.

Corrections done. We change the y-axis label of Fig. 5 for nitracline because we consider nitrate as the limitant nutrient.

14502, 15: Replace “provide” with “provides” 16-18: Maybe rephrase this sentence it currently sounds like CHEMTAX can be used to monitor environmental changes (which may be true indirectly, but it is not a first order application). 19: Maybe replace “footprint” with “indication”

Corrections done. We change accordingly the sentence (L.574-575) to avoid the confusion mentioned by the referee.

14503, 15: “Fig. 3a and c” is meant to be Fig. 4a and c? 22: Fig. 8 and Fig. 9 are switched

Yes, the figures called are wrong; actually it was 6a and 6c. We switched Fig. 8 and 9 in the text.

14504, 26: For consistency, label figures 8d and 9d the same.

We label figures 8d and 9d.

14505, 10: “ingested it” should be “ingested them” 14: Should be “likely to be significant...”

Corrections done.

14506, 12: Should be “observed in the Arctic Ocean” 25: Remove “availability” 24: “at deep” should be “at a deep”, or if kept “at deep” then “maximum” should be “maxima”

Corrections done.

14507, 1: Remove the second “of the” i.e. should be “of a deepening nutricline. . .” Also replace “since a decade” with “over the past decade” 4: “induced” is a bit awkward, maybe use “introduced” 25: Should be “in the Arctic Ocean”, also “averaged” should be “average”

Corrections done.

14508, 5: This sentence makes it sound a bit like the alternative being suggested is not a blind use of CHEMTAX, implying that earlier it was suggested to do so – obviously that is not the case so it may be worthwhile to rephrase the sentence.

We rephrase the sentence.

14517: In the first sentence of Table 3, the word “light” should follow “(surface samples)”

Corrections done.

14520: First sentence in Table 4 caption should have “mean ± standard deviation”. Also, “The cluster 1” should be “Cluster 1”. Also, the “:” after “radiation” should be “;”

Corrections done.

Anonymous Referee #2

General comments of referee 2

This paper describes the use of pigment signatures and microscopy to identify phytoplankton communities and link distribution to water column characteristics in the Beaufort Sea. The authors use HPLC pigments and microscopy to develop pigment ratios with which to initialize the CHEMTAX program. The outcome of the research was the identification of 4 clusters of phytoplankton communities described in the first order by nutrient availability. I appreciate the authors discussing the impacts of differences in phytoplankton type on carbon cycling (sinking etc). Overall this work provides the community with localized CHEMTAX tunning for the Beaufort Sea. I would like the authors to provide a threshold value for changes in starting pigment ratios.

Author's general response

We would like to thank referee 2 for his relevant comments. According to his comments, we modified some points of the manuscript and add information that will certainly improve the manuscript.

Referee 2 asks us to provide a threshold value for changes in initial pigments ratios. If the ratios and criteria are well defined on the basis of regional phytoplankton knowledge, reasonable variations of the ratios will not strongly affect the output of CHEMTAX in terms of phytoplankton abundance. We tested the sensitivity of CHEMTAX by multiplying each number of the ratio matrix by a random factor. It appears that by independently and randomly varying the ratios until 35% of their initial values, the final abundance varies by only 2% on average. We suggest that a threshold value of 50% ensures confidence in the CHEMTAX output. By testing the sensitivity of CHEMTAX to the different ratio matrix found in the literature (see figure 3), we understand that the difference in CHEMTAX interpretation is mainly due to the choice of pigments attributed to each group. For example, Suzuki et al. (2002) by characterizing the cryptophytes group with only "alloxanthin" obtained a greater contribution of this group compared to other studies in which cryptophytes were characterized by both "alloxanthin" and "chlorophyll *c1c2*". In fact, in the second case, the group must satisfy two conditions to be identified. A second important source of discrepancy is to which group the highest ratio of a given pigment is associated. This group will more likely be dominant if the concerned pigment is highly concentrated in the sample. For example, in Not et al. (2005), a higher "fucoxanthin" ratio was attributed to haptophytes (0.676) than to diatoms (0.421) while other studies attributed the higher "fucoxanthin" ratio to diatoms. This choice results in a low contribution of diatoms and a high contribution of haptophytes in Not's study.

Below we address all of the specific comments raised by referee 2 and highlight the changes made in the revised manuscript with a yellow overlay.

Specific comments of the referee 2 (in blue) and associated author's changes in manuscript (in black)

Abstract: Line 15: Spelling, "Microscopic count" replace with counts.

We modify accordingly (L.29).

Introduction: 14492 Line 5: Grammar replaces "allows to characterize" with "allows for the characterization of" 14493 Line8 thr 11: Last sentence is not a very good end to this section. Why is CHEMTAX critical? Why not use microscopy, HPLC is also time consuming and expensive. I would like the authors to try harder to convince me that I should care about CHEMTAX results.

The grammar was corrected (L.92). We modified the last sentence of the introduction. Referee 2 pointed out that too much emphasis was attributed to CHEMTAX as the most accurate method for monitoring phytoplankton populations. Our intention was not to discard other methods; we agree that the use of various measurement techniques increases the accuracy of phytoplankton studies. We modified the introduction (L. 72-76) and conclusion (L.767-771) to highlight the importance of using complementary approaches. Nevertheless, we introduce the pigments and CHEMTAX as a suitable method to provide an overview of phytoplankton populations when accuracy at a species level is not needed. The critical benefits of CHEMTAX for monitoring studies rely in the ability of pigments to characterize small and large phytoplankton equally, while microscopy is effective primarily for large cells. HPLC analysis shows good reproducibility in comparison to microscopy (Hooker et al., 2005) facilitating the detection of year-to-year changes in the communities (L.765-766). Note that CHEMTAX must be seen as a tool to convert pigments into phytoplankton groups. Pigments alone are of limited utility when working on population ecology, diversity and repartition.

Methods 14497 Line 7: First use of Pras, please use the full spelling first i.e Prasinoxanthin (Pras) 14497 Line10: Lut same issue as above 14497 Line 12: "two matrix ratio" replace with "ratios"

We take care to spell out the full pigment name each time it appears for the first time. We change for "ratios".

Results 14500 Line 4: Grammar, "was twice higher" replace with "was twice as high" 14500: I appreciate the authors attempts to discuss the impact of differences in starting ratios, more useful here would be a threshold over which changes in starting ratios would render the CHEMTAX output significantly wrong. How big of a deviation in ratios can the method take?

The grammar correction was done. We discuss the sensibility of CHEMTAX when changing the initial ratio and propose a threshold value (L.432-433) based on the sensitivity test (L424-432).

14501: Looking at Table 4, the conditions of cluster 1 and 2 don't appear to be different, some statistical testing here would be informative.

Because clusters 1 and 2 are clearly different for most of the parameters, we presume that the comment refers to cluster 1 (surf) and cluster 2. We performed a Student's test (t-test), presented in Table 1 of supplementary material, to examine if the average conditions are significantly different between the clusters. The t-test highlighted that clusters 1, 2, 3, 4 are significantly different from each other by a minimum of 3 environmental parameters (L492-493, L520-523) as well as clusters 1 (surf) and 1 (SCM) (L507-508). But, as highlighted by referee 2, cluster 1 (surf) and cluster 2 didn't exhibit significant differences in their environmental conditions (L523-526) (p-value of the t-test was higher than 0.1 for all parameters). Therefore, according to k-means testing these two clusters have significantly different pigment compositions. We suppose that the different community (high diatoms) observed in cluster 1 surface stations could be a remnant of a past event whose specific conditions are no longer visible, possibly an upwelling as previously observed (Comeau et al., 2011; Forest et al., 2014).

14501 Line 25-28: chlorophytes are not just a freshwater species, at PSU 24 to 26 the freshwater term doesn't make sense. The discussion here about the phytoplankton present is not well described.

We replace « freshwater » by « low salinity waters » (L499-503)

14504 Line 22: Fig 9 is referenced before Fig 8. 14506 Line 17: (Brugel et al 2009) should be Brugel et al (2009)

We correct it (L.503).

Comeau, A. M., Li, W. K., Tremblay, J. E., Carmack, E. C., and Lovejoy, C.: Arctic Ocean microbial community structure before and after the 2007 record sea ice minimum, PLoS ONE, 6, e27492, 2011.

Forest, A., Coupel, P., Else, B., Nahavandian, S., Lansard, B., Raimbault, P., Papakyriakou, T., Gratton, Y., Fortier, L., and Tremblay, J.-É.: Synoptic evaluation of carbon cycling in the Beaufort Sea during summer: contrasting river inputs, ecosystem metabolism and air–sea CO 2 fluxes, Biogeosciences, 11, 2827-2856, 2014.

Hooker, S. B., Van Heukelem, L., Thomas, C. S., Claustre, H., Ras, J., Barlow, R., Sessions, H., Schlüter, L., Perl, J., and Trees, C.: Second SeaWiFS HPLC Analysis Round-robin Experiment (SeaHARRE-2), National Aeronautics and Space Administration, Goddard Space Flight Center, 2005.

Not, F., Ramon, M., Latasa, M., Marie, D., Colson, C., Eikrem, W., Pedrós-Alió, C., Vaulot, D., and Simon, N.: Late Summer Community Composition and Abundance of Photosynthetic Picoeukaryotes in Norwegian and Barents Seas, Limnology and Oceanography, 50, 1677-1686, 2005.

Suzuki, K., Minami, C., Liu, H., and Saino, T.: Temporal and spatial patterns of chemotaxonomic algal pigments in the subarctic Pacific and the Bering Sea during the early summer of 1999, Deep Sea Research Part II: Topical Studies in Oceanography, 49, 5685-5704, 2002.