Translocation of isotopically distinct macroalgae: a route to low-cost biomonitoring?

Darren R. Gröcke • Blanca Racionero-Gómez • James W. Marschalek • H. Chris Greenwell

Department of Earth Sciences, Durham University, Durham, DH1 3LE, UK

d.r.grocke@durham.ac.uk

Abstract

Nitrogen stable isotope ratios ($\delta^{15}N$) in macroalgae are often used to identify sources of nitrogenous pollution in fluvial and estuarine settings. This approach assumes that the macroalgal $\delta^{15}N$ is representative of the sources of the pollution averaged over a timespan in the order of days to weeks, but the preferential uptake of a particular nitrogen compound or potential for fractionation in the water column or during uptake and assimilation by the macroalgae could make this assumption invalid. Laboratory studies were therefore performed to investigate the uptake and assimilation of both nitrate and ammonium at a variety of concentrations using the vegetative (non-fertile) tips of the brown macroalgae, *Fucus vesiculosus.* Nitrate appeared to fractionate at high concentrations, and was found to be taken up more rapidly than ammonia; within 13 days, the macroalgae tips were in isotopic equilibrium with the nitrate solution at 500 µM. These experiments were complemented by an investigation involving the translocation of macroalgae collected from a site enriched in ^{15}N relative to natural levels (Staithes, UK), to the

River Tees, Middlesbrough (UK), a site depleted in ^{15}N relative to natural levels. The nitrogen isotope signature shifted 50% within 7 days, with samples deployed nearer the surface subject to greater change. These findings suggest that the translocation of macroalgae with isotopically distinct signatures can be used as a rapid, cost-efficient method for nitrogen biomonitoring in estuarine environments.

Keywords Nitrogen • Isotopes • Pollution • Environmental monitoring • Macroalgae • Seaweed

Introduction

Stable isotope ratios are an excellent tool to discern, or ascertain, biological, ecological and environmental processes. The modern nitrogen cycle has been heavily influenced by human activity. Waste products, such as sewage and fish farm effluent, are normally more enriched in ^{15}N than seawater (Vizzini and Mazzola, 2004), whereas agricultural waste products are normally more depleted in ^{15}N (Heaton, 1986). This has led to the application of using nitrogen stable isotope ratios ($\delta^{15}N$) of marine sediments, marine organisms and macroalgae to monitor nitrogen pollution/contamination (e.g., McClelland et al. 1997; Savage 2005).

$\delta^{15}N$ can also be measured in dissolved inorganic nitrogen (DIN: $\delta^{15}N_{DIN}$) taken directly from the water (Deutsch et al. 2006; Korth et al. 2014). Unfortunately, in systems such as estuaries with very complex flow regimes, spot sampling does not always represent the true average concentrations because of high temporal variability; it is also more time-consuming and costly to do isotopic analysis of DIN. To address this difficulty, nitrogen isotope ratios in macroalgal

44 tissues are often utilised to discern sources of excess nutrients (Costanzo et al. 2001, 2005;
45 Savage and Elmgren, 2004; Derse et al. 2007; Dailer et al. 2012).

46 However, this methodology assumes that macroalgal $\delta^{15}N$ values are representative of an
47 integrated $\delta^{15}N$ value of nitrogen inputs over a time period, which implies that the $\delta^{15}N$ values of
48 the source(s) are the sole contributors to the $\delta^{15}N$ of the macroalgae. This does not account for
49 the potential for fractionation during nitrogen transformations in the water column, or in the
50 processes of uptake and assimilation, which can lead to $\delta^{15}N$ values in algal biomass different to
51 that of the ambient nitrogen source (Viana et al. 2011; Swart et al. 2014). Nitrogen uptake by
52 macroalgae is influenced by morphological factors, metabolism, tissue type, age and nutrition
53 (e.g., Rosenberg and Ramus 1984; Pedersen 1994; Neori et al. 2004). During this process,
54 nitrogen is transported from the water through the cell membrane and assimilated into organic
55 compounds, such as proteins (McGlathery et al. 1996). Macroalgal $\delta^{15}N$ values are also
56 significantly influenced by the enrichment of ammonium in a river, as originally documented by
57 Minagawa and Wada (1984), and more recently by Savage and Elmgren (2004), Savage (2005)
58 and Viana et al. (2011). However, to more accurately interpret macroalgae $\delta^{15}N$, a good
59 understanding of the fractionation processes taking place is required (Viana et al. 2011).

60 It has therefore been suggested that variability in $\delta^{15}N$ due to isotopic fractionation may be an
61 important factor controlling macroalgal tissue $\delta^{15}N$ (e.g., Viana and Bode 2015; Swart et al.
62 2014), as macroalgal $\delta^{15}N$ could be modified by environmental parameters such as oxygen
63 concentration, microbe concentration, pH, temperature, light and DIN concentration (Raimonet
64 et al. 2013; Jona-Lasinio et al. 2015). Furthermore, reduced N, as NH_4^+, is preferred to NO_3^- as a
65 nitrogen source by some macroalgal species (Cohen and Fong 2005), thus the $\delta^{15}N$ of the
66 macroalgae could be strongly influenced by a NH_4^+ signal independent of NO_3^-. Bacterial

67 populations could therefore affect $\delta^{15}N_{DIN}$ (Korth et al. 2014; Ochoa-Izaguirre and Soto-Jiménez
68 2015).
69 Riera (1998) and Riera et al. (2000) reported that *Fucus* from natural (uncontaminated) sites
70 have $\delta^{15}N$ values of around +6 ‰. Savage and Elmgren (2004) and Savage (2005) reported
71 significant increases in $\delta^{15}N$ (greater than 7 ‰) from *Fucus* that were impacted by sewage
72 pollution. Notwithstanding the subtle difference between each site, this could be explained
73 simply by background oceanographic factors independent of human activity (Viana and Bode,
74 2013); thus, every site being investigated should be treated independently. Deutsch and Voss
75 (2006) indicated that *in situ* incubation experiments in an unpolluted brackish location could be
76 suitable as a simple monitoring tool, but their data were inconclusive for *Fucus vesiculosus*.
77 Viana et al. (2011) measured $\delta^{15}N$ in macroalgal tissues in coastal areas between 1990 and 2007
78 and found a decrease in $\delta^{15}N$ from ~ +8 ‰ to ~ +5 ‰, which they related to a reduction in
79 human activities and the level of contamination, and/or other environmental factors.
80 In the present study, we aim to assess the usefulness of $\delta^{15}N$ in the macroalgae, *Fucus*
81 *vesiculosus* (hereafter, *Fucus*) as a low-cost, easily deployed biomonitor for nitrogen pollution.
82 We use *Fucus* as it is near ubiquitous in United Kingdom coastal waters, and has been shown to
83 be a species where the isotopic composition of the tissues is linked to that of the environment
84 (e.g. Viana et al., 2015). In the first instance, laboratory incubation experiments of vegetative
85 *Fucus* tips, in the presence of different concentrations of nitrate and ammonia, were undertaken
86 to determine the nitrogen isotope response. In addition, this study involved the novel
87 translocation of vegetative tips of *Fucus* from one site (Staithes, UK) that has an enriched ^{15}N
88 signature, to an industrial site (River Tees, UK) which has a depleted ^{15}N signature. In this case,
89 the nitrogen isotope response of vegetative *Fucus* tips was used to determine whether short-term

90 or long-term field experiments are required to assess nitrogen pollution. The usefulness of $\delta^{15}N$
91 in macroalgae as a pollution biomonitor has been argued by different authors, but until now, it
92 has not hitherto been proven that translocation of vegetative tips of *Fucus* could be a useful tool
93 for nitrogen monitoring. Therefore, we aim to determine whether the isotopic signal of seaweed
94 can be modified to give a signature significantly shifted from any environment where we may
95 wish to deploy environmental monitoring.
96

97 ## Material and methods

98 ### Macroalgae Selection

99 Macroalgae of the genus *Fucus* belong to the brown macroalgae family Phaeophyceae. *Fucus* is
100 commonly found along sheltered shores of the North Sea, Baltic Sea, Atlantic Ocean and Pacific
101 Ocean. *Fucus* is a tethered macroalgae, with a growth rate ranging between 0.05 – 0.80 cm/day
102 and a life span on the order of 3 – 5 years (Strömgren 1977; Carlson 1991). The species is
103 annually episodic, gonochoristic and highly fecund (i.e., prolific). Gametes are released into the
104 seawater and the eggs are fertilized externally to form a zygote that starts to develop as soon as it
105 settles into a substrate. The gametes are released from receptacles found in the fertile tips of the
106 macroalgae. However, *Fucus* also have vegetative tips that do not contain these structures and
107 are composed of a parenchymatous thallus (Hiscock 1991). The meristematic zones of the
108 vegetative tips of *Fucus* have a significantly greater uptake of nitrogen than in older parts of the
109 macroalgae tissue (Savage and Elmgren 2004; Viana et al. 2015), and hence vegetative tips of
110 *Fucus vesiculosus* were used in this study.
111

112 ### Study Area

113 Two sites were chosen for this study: Staithes, North Yorkshire, UK (54°33'N 00°47'W) and the
114 River Tees, Borough of Teeside, Middlesbrough, UK (54°35′20″N 1°11′15″W) (Fig. 1). Both
115 locations are affected by eutrophication. Staithes was selected as a non-industrial site, compared
116 to the River Tees estuary, which has experienced intensive industrialization since the 1830's,
117 predominantly through iron manufacturing, ship building, engineering and, recently, chemical
118 industries, with inputs that include surfactants and polybrominated diphenyl ethers (Allchin et al.
119 1999; Lye et al. 1999). Moreover, mining activities in the upper catchment prior to 1955 released
120 metals such as copper and lead into the river that contaminated sediments at least 70 km
121 downriver (Hudson-Edwards et al. 1997). Thus, as the River Tees became one of the most
122 heavily industrialized regions of Britain (Environment Agency North East Region, 1996) the
123 lower river and estuary became heavily polluted. Industrial pollution may have enhanced the
124 excessive growth of certain problem macroalgae (*Ulva* spp.) in specific locations of the Tees
125 estuary causing sandbar accretion and loss of unique wading habitat and migratory species of
126 fish (Williams et al. 2009). From the 1970's onwards there have been major steps taken to reduce
127 the quantity of pollutants delivered to the river (i.e., a 70% reduction in ammonia), resulting in a
128 significant decline of macroalgae blooms and the return of salmon and trout (Environment
129 Agency North East Region, 1996; Williams et al. 2009).
130 *Fucus* vegetative tips were collected from Staithes in July, August and September 2015 (Fig.
131 1). A random suite of mixed samples were used to culture *in situ* the macroalgae in the River
132 Tees and another part of the sample set were used for *in vitro* culturing using different nitrate and
133 ammonia concentrations. Moreover, in order to generate a background $\delta^{15}N$ value, *Fucus*
134 growing in the River Tees were collected and their structures separated (i.e. tips and blades) in
135 May, June and July in 2015 during both low and high tide periods.

136

137 ***In Vitro* Cultures**

138 To investigate nitrogen uptake by *Fucus*, vegetative tips (length = 1.5 cm; wet weight = 0.12 –
139 0.15 g) without visible microalgae (i.e., epiphytes) collected from Staithes were cultured in
140 seawater —modified after Gustow et al. (2014). Ten tips from separate individuals growing in
141 the same area were placed into separate 250 mL glass jars containing two mesh shelves. Four
142 tips were placed in the bottom of the jar and three tips above each mesh layer (Fig. 2). All jars
143 were filled with sterile, filtered (0.7 μm) seawater also collected from Staithes. Each set of three
144 replicate jars were doped using a known volume of nitrate (HNO_3) or ammonia (NH_4OH).
145 Doped seawater nitrogen concentrations in the cultures were 10 μM, 50 μM, 100 μM and 500
146 μM. Although diluting a solution rather than using a salt led to slight differences in salinity and
147 pH, these effects were measured as small enough to be considered negligible. It was also
148 assumed that no other nutrients or trace metals were limiting, and that the water used had very
149 little nitrogen in it initially. Identical tips incubated in just the filtered seawater (0.7 μm) were
150 used to set three control replicates and record the 0 μM $\delta^{15}N$ values of the macroalgae.

151 To remove the effects of a closed atmospheric environment, all jars were loosely covered with
152 lids to allow gaseous exchange with the atmosphere. No additional nutrients were added into the
153 seawater, except the natural gaseous exchange. The vegetative tips inside the bottles were
154 transferred into an incubator with a set light/dark rhythm of 16:8 h, light intensity of 125 μmol
155 photons/m^2s^2 and a temperature of 11 ºC. For the ammonia and nitrate solutions, after 3 days had
156 elapsed half the tips were removed, weighed and then analyzed for $\delta^{15}N$, with the remainder
157 were weighed and processed after 13 days of incubation. The pH and salinity of the jars were

158 measured every 2–3 days, and at the same time the medium was changed (2 times for all
159 cultures) to avoid the accumulation of any metabolites.
160
161 ***In Situ* Cultures – River Tees**
162 In order to monitor changes in $\delta^{15}N$ in *Fucus* arising due to the industrial processes impacting the
163 estuary, vegetative tips were collected and transferred from Staithes, where they were cultured *in*
164 *situ* at four buoy locations in the River Tees (Fig. 1) during July and August. Buoy locations
165 were selected to span an 8 km to 10 km section of the river where the mean water depth is
166 around 15 m. All vegetative apical thallus tips (the specimens) were kept in a plastic container
167 filled with seawater from Staithes for transportation. A random selection of these vegetative tips
168 from individuals growing in the same area as the ones used for the *in vitro* cultures were placed
169 in nylon fruit bags and cable tied. Four navigation buoys were used in this study, and at each
170 buoy a chain weighed down by a 1 kg weight was attached. The fruit bags, containing the
171 vegetative tips, were attached to the chain at each buoy at two depths below the water surface:
172 0.2 m and 1.0 m. Two simultaneous *in situ* experiments were undertaken at the same buoy
173 location and heights, as detailed below.
174 At each depth two fruit bags were attached containing the two types of experiment: (i) a long-
175 term (continuous) one, denoted Experiment 1; and (ii) a short-term culturing experiment
176 (Experiment 2). Experiment 1 used 200 vegetative tips in total (i.e., 25 tips per fruit bag), with
177 five tips collected every seven days over three weeks to monitor long term isotopic changes in
178 the River Tees. Experiment 2 consisted of a total of 40 vegetative tips (i.e., five tips per fruit bag)
179 and was used to monitor short-term (7 days) isotopic changes in the River Tees. After a week of
180 *in situ* culturing all the tips of Experiment 2 were collected and replaced with fresh vegetative

tips collected from Staithes that same morning. Experiment 2 was repeated every week for four weeks.

Nitrogen Isotope Analysis

Nitrogen isotope ratios were measured in the Stable Isotope Biogeochemistry Laboratory (SIBL) at Durham University. Each sample was oven-dried at 60 °C for 24 h and ground into a powder with an agate mortar and pestle. Aliquots of the powder, weighing between 1.3 mg and 1.6 mg were placed into tin capsules and stored in a desiccator prior to isotopic analysis. Homogenized vegetative macroalgae tips were analyzed using a Costech Elemental Analyzer (ECS 4010) connected to a Thermo Scientific Delta V Advantage isotope ratio mass spectrometer. Nitrogen isotope ratios are reported in standard delta (δ) notation in per mil (‰) relative to atmospheric nitrogen. Data accuracy is monitored through routine analyses of in-house and international standards: the in-house standards (e.g., Urea, Glutamic Acid) are stringently calibrated against international standards (e.g., USGS 40, IAEA 600, IAEA N1, IAEA N2). Analytical uncertainty for $\delta^{15}N$ measurements is typically ± 0.1 ‰ for replicate analyses of in-house and international standards, and typically < 0.2 ‰ on replicate sample analysis. Total nitrogen was obtained as part of the isotopic analysis using an in-house standard (i.e., Glutamic Acid, 9.52 % N). The nitrogen isotope analysis of the nitrate and ammonia solutions were achieved by placing the solution in smooth walled tin capsules and placed directly into the zero-blank autosampler. The tin capsules were left open and analyzed through the ECS 4010 in the same manner as the vegetative tips.

Statistical Analysis

Statistical t-tests using a significance level of 0.05 were performed using R Studio software. For testing the statistical hypothesis, *p*-values are used. The *p*-value is defined as the probability of obtaining a result more extreme or equal to what was actually observed, thus, if the *p*-value is smaller or equal to the significance level, it suggests that the observed data are consistent.

Results

In Vitro Cultures from Staithes (Nitrate)

The starting $\delta^{15}N$ value of the *Fucus* vegetative tips in this experiment was +8.7 ‰ (Table 1). The longer the period of exposure to the introduced nitrate, the more the *Fucus* vegetative tips integrated the nitrogen isotope signature of the added solution. After culturing *Fucus* for 13 days under 500 µM of nitrate, the $\delta^{15}N$ values (+6.5 ‰ ± 0.2 ‰) of the tips were statistically similar to the $\delta^{15}N$ value of the nitrate solution used (+7.1 ‰ ± 0.4 ‰) (Fig. 3). Within three days the *Fucus* vegetative tips only shifted ~ 1 ‰ (~ 50%) under a N nutrient concentration of 500 µM.

In Vitro Cultures from Staithes (Ammonia)

The starting $\delta^{15}N$ value of the *Fucus* vegetative tips in this experiment was +10.6 ‰ ± 0.1 ‰ (Table 2). After culturing *Fucus* vegetative tips for 13 days under 500 µM of ammonia, the $\delta^{15}N$ values (+6.8 ‰ ± 0.2 ‰) were significantly different from the initial $\delta^{15}N$ value (p-value < 0.05), but did not reach the $\delta^{15}N$ value of the ammonia solution used (+2.4 ‰ ± 0.5 ‰) (Fig. 3). The 13-day experiment at 500 µM only represented approximately 45% of isotopic exchange with the *Fucus* vegetative tips.

Native Populations and Translocation from Staithes to the River Tees (Short-Term and Long-Term Experiments)

Fucus $\delta^{15}N$ measurements from Staithes, 2015 (n = 27) had an average value of +10.0 ‰ ± 0.5 ‰ (Table 3). On the other hand, *Fucus* samples from the River Tees in 2015 (n = 94) recorded an average $\delta^{15}N$ value of –1.7 ‰ ± 4.3 ‰ (Table 4), which was statistically different (p-value < 0.001) to that from Staithes, 2015. Dividing the *Fucus* samples into blades (–2.5 ‰ ± 4.2 ‰), fertile (–1.2 ‰ ± 3.9 ‰) and vegetative tips (–1.6 ‰ ± 4.8 ‰) showed no statistically significant difference between macroalgae sub-structures.

Moreover, the vegetative tips that were collected from Staithes shifted by 50% from those recovered from the River Tees buoys (short-term experiment, +4.1 ‰ ± 1.3 ‰; long-term experiment, +3.9 ‰ ± 1.7 ‰) (p-value < 0.05), although they do not reach the background levels of *Fucus* growing in the river (–1.7 ‰) (Fig. 4, 5). The long-term *Fucus* vegetative tips from Buoy 4 after 21 days reached the closest to the average background $\delta^{15}N$ value of the River Tees. After seven days all the transferred vegetative tips had significantly depleted $\delta^{15}N$ values compared to their original values from Staithes. Significant differences (p-value < 0.01) were observed for the short-term Experiment 2 samples, depending on the depth that the macroalgae was placed at each buoy (Fig. 4). Significant differences (p-value < 0.01) were also observed for the long-term Experiment 1 depending on sample depth (Figure 5).

Discussion

Assessment of *Fucus* to Incorporate Nitrogen Isotope Sources

Many studies have been designed to discover whether macroalgae $\delta^{15}N$ values are a reliable tracer of nitrogen pollution of the marine environment (Savage and Elmgren 2004; Lapointe and

249 Bedford 2007; Piñón-Gimate et al. 2009; Carballeira et al. 2013; Ochoa-Izaguirre and Soto-
250 Jiménez 2015; Wang et al. 2016). However, the direct link between anthropogenic nitrogen
251 inputs and $\delta^{15}N$ values in macroalgae is still not fully understood. In most cases macroalgal $\delta^{15}N$
252 values are therefore inferred to be directly related to inorganic nitrogen inputs. Nevertheless,
253 Viana and Bode (2013) analyzed $\delta^{15}N$ from macroalgae, nitrate and ammonia in different
254 environments and concluded that it was not possible to establish a simple relationship between
255 macroalgae $\delta^{15}N$ with the concentration and $\delta^{15}N$ value of nitrate and/or ammonia. Therefore,
256 Viana and Bode (2013) proposed that due to variability in inorganic nitrogen inputs, local
257 environmental factors and coastal upwelling were all contributing to macroalgae $\delta^{15}N$ values.
258 More recently, Swart et al. (2014) has shown that the concentration of nitrate can produce
259 significant isotopic fractionation (up to 6 ‰) in a green and rhodophyte algae uptake when of the
260 order of 500 µM concentration.
261 In this study, we show that *in vitro* cultures of *Fucus* grown under different concentrations of
262 nitrate reach isotopic equilibrium in under 13 days, whereas the same experimental procedure
263 with ammonia showed that isotopic equilibrium was not reached after 13 days (Fig. 3). The
264 isotopic value of the solution was closer to the initial value of the macroalgal tissue than the
265 ammonia isotopic signature, which might explain this observation. Macroalgae with increased
266 nutrient supply have elevated uptake rates and increased tissue nutrient contents (Valiela et al.
267 1997; Fong et al. 2004), thus the cultures with higher nitrate/ammonium concentrations could
268 have gained more nitrogen and become isotopically lighter than their lower concentration
269 counterparts. Although this study shows a clear relationship between macroalgae $\delta^{15}N$ and
270 source $\delta^{15}N$, this may not be true in a natural environment, as shown by the high degree of
271 scatter in macroalgae $\delta^{15}N$ from the River Tees background dataset (–1.7 ‰ ± 4.3 ‰, Table 4).

To establish whether concentration-dependent isotopic fractionation occurs, a simple two end-member mixing model was used as a first order approximation (Kaldy 2011). These end members comprised of the initial algal nitrogen pool ($\delta^{15}N$, depending on date) and the nitrate/ammonium solutions added (with $\delta^{15}N$ values of +7.1 ‰ and +2.4 ‰ respectively). The following equation was used to model the mixing:

$$\delta_{sample} = \delta_{source1} \times f_1 + \delta_{source2} \times f_2$$

where source 1 is the initial algal pool and source 2 is the added nitrogen source, with their relative fractions f_1 and f_2 such that $f_2 = 1 - f_1$. This was used to model the expected $\delta^{15}N$ of the sample if no fractionation was occurring. The f values are calculated from the change in total nitrogen, with growth correction. The deviations from the expected values based on a simple mixing model should theoretically represent fractionation. Assuming this to be correct, the nitrate solutions appear to fractionate considerably, whereas the ammonium solutions appear to fractionate very little, even after 13 days at high concentrations (Fig. 3).

Translocation of *Fucus* as a Biomonitor

The complexity of natural environments makes identifying and tracing nitrogen pollution sources difficult, as the $\delta^{15}N$ values will be dependent on the isotopic source of the nitrogen, as well as complex organo-mineral interactions and chemistry occurring in the water column. This complexity will be especially pronounced in a river-estuary setting, where the salt-wedge of intruding sea water may result in colloid phenomena such as flocculation. Since the background levels of $\delta^{15}N$ in *Fucus* are reported to be between +4 ‰ and +6 ‰ (e.g., Riera 1998; Riera et al. 2000; Savage and Elmgren 2004), the values observed in *Fucus* from Staithes (+10.0 ‰) and the River Tees (–1.7 ‰) indicate that different anthropogenic inputs of nitrogen have affected them.

294 The more elevated values in Staithes in 2015 compared to 2014 (~ +8.5 ‰, Gröcke et al.
295 unpublished data) may be related to a reported spillage in August 2015 of sludge from
296 Hinderwell Wastewater Treatment Works into Dales Beck at Dalehouse, near Staithes. In fact,
297 the degree of variation (standard deviation = 4.3 ‰, values ranging from –8.5 ‰ to +10.8 ‰) in
298 $\delta^{15}N$ *Fucus* from the River Tees suggest that this area is affected by multiple nitrogen sources,
299 which is also evident in the translocation experiments presented in this study. Conversely, $\delta^{15}N$
300 values from Staithes *Fucus* are tightly constrained (standard deviation = 0.5 ‰), suggesting a
301 consistent nitrogen isotope source during the entire collection period in 2015. In areas with high
302 natural variation in $\delta^{15}N$, *in situ* incubation of macroalgae can therefore be considered more
303 representative of $\delta^{15}N_{DIN}$ than native macroalgae. This study therefore supports previous research
304 suggesting that isotopic studies of indigenous macroalgae are unsuitable for retrospective
305 monitoring of nitrogen pollution (Carballeira et al. 2014; Viana et al. 2015).
306 $\delta^{15}N$ values of *Fucus* samples from Staithes changed significantly within the first week of
307 translocation to the River Tees (Fig. 4) showing the rapid uptake or exchange of nitrogen. During
308 the short-term translocation experiment, nearly all vegetative tips of *Fucus* isotopically shifted
309 by 50% from the background value for Staithes towards the background value of the River Tees
310 in 2015 (Fig. 4, Table 5). However, there are subtle differences between each week of
311 translocation and collection, between each buoy, and the depth of the samples in the water
312 column. The *Fucus* bottom samples (1 m) did not isotopically shift as much as the samples
313 located at the top of the chain suspended from the buoy (20 cm). Overall, the range in isotopic
314 exchange between the Staithes and River Tees background $\delta^{15}N$ values ranges from 30–80%
315 using a simple two end-member mixing model. The depth variation observed in this
316 translocation study can be explained by:

317 (a) A depth stratification of nitrogen pollution sources. The River Tees has a tidal range of >5
318 m. However, compared to macroalgae growing on the banks and sea walls of the River Tees the
319 buoy samples remained at the same water depths during the entire tidal cycle (i.e., 20 cm and 1
320 m). Therefore, the macroalgae at those depths experienced no periods above sea level, and
321 maintained the same environmental conditions for certain parameters (light, temperature)
322 through 24 h, though the relative depth profiles of fresh and saline water may change. The River
323 Tees $\delta^{15}N$ data would suggest that surface waters were ^{15}N-depleted in comparison to deeper
324 water. This is consistent with major industrial effluent discharges being released with fresh
325 water, giving a relatively buoyant waste field (Warwick et al. 2002).
326 (b) Isotopic fractionation in macroalgae as a result of varying environmental parameters (e.g.,
327 salinity, light, temperature etc.) with depth and at different spatial points (buoys). Although
328 fractionation processes in macroalgae are poorly understood, light levels appear to have the
329 opposite effect to what was observed in the present study, especially if the nitrogen source is
330 ammonia (Dudley et al. 2010).
331
332 Few studies have been performed on the effects of other environmental parameters, but it is
333 possible that these also contribute to the observed trend as the processes of nitrogen uptake,
334 fractionation and assimilation can be affected by the environment. Complexity in the system's
335 nitrogen pools would be expected to be exacerbated in intertidal macroalgae, as these are
336 exposed to atmospheric inputs and other local edge effects (Ochoa-Izaguirre and Soto-Jiménez
337 2015). For example, Kim et al. (2013) suggest that periodically immersed *Porphyra umbilicalis*
338 individuals have a higher $\delta^{15}N$ than ones that are continuously submerged.

In the River Tees estuary, the background noise in the $\delta^{15}N$ values appears to be very high. Suspending transplanted samples in the water column appears to remove most of this natural variation, allowing greater precision when monitoring pollutants. It is also interesting to note that at Staithes, the standard deviation and range of $\delta^{15}N$ in *Fucus* vegetative tips is small despite the fact that these samples were harvested from different positions on the shore face, and thus exposed differentially to the atmosphere. Combined with findings that sites exposed to higher nutrient levels have a higher seasonal variation, this could suggest that environmental controls on fractionation are far more important when nutrient levels are high (Carballeira et al. 2014; Wang et al. 2016).

A similar $\delta^{15}N$ offset between bottom and top buoy samples was also observed for the long-term translocation experiment (Fig. 5). In fact, even after 21 days of incubation in the River Tees, the Staithes *Fucus* samples show an isotopic exchange of between 30–80% using a simple two end-member mixing model. This is identical to the short-term translocation experiment. However, some buoys did show a consistent change throughout the experimental period. For example, the top *Fucus* samples from Buoy 4 record a consistent shift towards the River Tees 2015 background $\delta^{15}N$ value of –1.7 ‰ (see Fig. 5, Table 6): 50 % change in the first 7 days, 20 % from day 7 to day 14; and 10 % from day 14 to day 21.

River Tees Spatial Trends

Due to the complexity of the River Tees with respect to flow patterns, nitrogen stratification and human activity (i.e., dredging) it is difficult to explain why this site (i.e., Buoy 4) and depth (i.e., top) is the only place to record a pattern expected through Rayleigh fractionation. Other sites, such as Buoy 1 top, show a reverse trend through the 21-day translocation experiment (Figure 5).

362 This suggests that the translocation of vegetative *Fucus* tips from Staithes would require longer
363 than 21 days to equilibrate with the ambient $\delta^{15}N$ value for the River Tees. Viana et al. (2015)
364 suggested a period of around 16 days would be required for a complete turnover of nitrogen in *F.*
365 *vesiculosus*. However, the degree of isotopic change between Staithes and the River Tees is
366 much larger than that applied in Viana et al. (2015), hence the amount of isotopic change
367 required in this study would be energetically demanding and unlikely to benefit the *Fucus*
368 samples (see Raven 2003). Instead, the majority of the nitrogen transfer seems to have occurred
369 within 7 days (see Fig. 4, 5). This fairly rapid uptake and assimilation of the local nitrogen
370 isotope signature into macroalgae suggests that the translocation of isotopically distinct
371 macroalgae can be used as an efficient and cost-effective method to trace and monitor short-term
372 nitrogen pollution sources.
373 Despite the macroalgal $\delta^{15}N$ being far less variable than in native populations, no clear trends
374 in the $\delta^{15}N$ of vegetative tips are apparent along the river channel (see Fig. 1, 5). The seemingly
375 random differences between buoys and weeks suggests local factors such as fluvial inputs, tides,
376 drainage and upwelling could all be playing a minor role in the $\delta^{15}N$ values. Further
377 complications include the positioning in the river; the buoys were on both the left and the right-
378 hand sides of the channel (Fig. 1), thus the location where the effluent enters the river and the
379 flow patterns would prove to be very important. The major variation shown in Buoy 3 can be
380 explained by human activity affecting the *Fucus* $\delta^{15}N$ values and the distinction between top and
381 bottom samples is reversed (Fig. 5). During weeks 1 and 2 this part of the River Tees was being
382 dredged, which would have thoroughly mixed the water in this region and redistributed bottom-
383 water and sediment nitrogen to the surface water.
384

Conclusions

In this study, we demonstrate that by moving isotopically distinct macroalgae from a site with elevated $\delta^{15}N$ values (i.e. Staithes) to a site that is affected by industrialization (low $\delta^{15}N$ values), the source of the nitrogen can be identified in macroalgae within seven days by analysing the nitrogen isotope ratios of vegetative tips. The rapid incorporation of nitrogen into the cellular structure of *Fucus* vegetative tips opens up the possibility for rapidly identifying pollution trends when using isotopically distinct macroalgae samples. This could be achieved using natural macroalgae samples where isotopically distinct samples can be obtained, as in this study, or by cultivation of macroalgae in isotopically distinct solutions of nitrate/ammonia, which is then deployed in the field. Although this transplantation method is quick and economical, there are several other aspects that require investigation:

(1) how do tidal cycles within rivers and estuaries affect the nitrogen isotope incorporation of nitrogen signals?

(2) are salt wedges and colloid formation, such as flocculation, important in nitrogen metabolism in macroalgae?

(3) is a 7 to 14-day transplantation study long enough to monitor nitrogen isotope inputs through a large section of a river (especially one that crosses a boundary between different nitrogen pollution inputs)?

ACKNOWLEDGMENTS

We are very grateful to OEA Labs Ltd and Durham University for providing funding to BRG and JWM. This research was kindly funded by a Durham University Seedcorn Fund to HCG (26.14.050200) and an MSc scholarship to BRG from OEA Labs Limited. Teeside Nautical

408 Studies provided boats and assistance with the study and collecting the macroalgae. Special
409 thanks to Trevor Forster (Principal & Advanced Powerboat Instructor at Teeside Nautical
410 Studies), and Andrew Ridley (Conservancy Operations Manager at PD Teesport Ltd).

411

412 ## References

413 Allchin CR, Law RJ, Morris S (1999) Polybrominated diphenylethers in sediment and biota
414 downstreams of potential sources in the UK. Environ Pollut 105:197–207

415 Carballeira C, Rey-Asensio A, Carballeira A (2014) Interannual changes in $\delta^{15}N$ values in *Fucus*
416 *vesiculosus* L. Mar Poll Bull 85:141–145

417 Carballeira C, Viana IG, Carballeira A (2013) $\delta^{15}N$ values of macroalgae as an indicator of the
418 potential presence of waste disposal from land-based marine fish farms. J Appl Phycol 25:97–
419 107

420 Carlson L (1991) Seasonal variation in growth, reproduction and nitrogen content of *Fucus*
421 *vesiculosus* in the Öresund, Southern Sweden. Bot Marina 34:447–453

422 Cohen RA, Fong P (2005) Experimental evidence supports the use of $\delta^{15}N$ content of the
423 opportunistic green macroalgae *Enteromorpha intestinalis* (Chlorophyta) to determine
424 nitrogen sources to estuaries. J Phycol 41:287–293

425 Costanzo SD, O'Donohue MJ, Dennison WC, Loneragan NR, Thomas M (2001) A new
426 approach for detecting and mapping sewage impacts. Mar Poll Bull 42:149–156

427 Costanzo SD, Udy J, Longstaff B, Jones A (2005) Using nitrogen stable isotope ratios ($\delta^{15}N$) of
428 macroalgae to determine the effectiveness of sewage upgrades: changes in the extent of
429 sewage plumes over four years in Moreton Bay, Australia. Mar Poll Bull 51:212–217

430 Dailer ML, Smith JE, Smith CM (2012) Responses of bloom forming and non-bloom forming
431 macroalgae to nutrient enrichment in Hawai‘i, USA. Harmful Algae 17:111–125

432 Derse E, Knee KL, Wankel SD, Kendall C, Berg CJ, Paytan A (2007) Identifying sources of
433 nitrogen to Hanalei Bay, Kauai, utilizing the nitrogen isotope signature of macroalgae.
434 Environ Sci Tech 41:5217–5223

435 Deutsch B, Mewes M, Liskow I, Voss M (2006) Quantification of diffuse nitrate inputs to a
436 small river system using stable isotopes of oxygen and nitrogen in nitrate. Org Geochem
437 37:1333–1342

438 Deutsch B, Voss M (2006) Anthropogenic nitrogen input traced by means of $\delta^{15}N$ values in
439 macroalgae: results from in-situ incubation experiments. Sci Total Environ 366:799–808

440 Dudley BD, Barr NG, Shima JS (2010) Influence of light intensity and nutrient source on $\delta^{13}C$
441 and $\delta^{15}N$ signatures in *Ulva pertusa*. Aqua Biol 9:85–93

442 Environment Agency North East Region (1996) River Tees: fact file. Environment Agency,
443 Newcastle-Upon-Tyne

444 Fong P, Fong JJ, Fong CR (2004) Growth, nutrient storage, and release of dissolved organic
445 nitrogen by *Enteromorpha intestinalis* in response to pulses of nitrogen and phosphorus. Aq
446 Bot 78:83–95

447 Gustow L, Rahman MM, Bartl K, Saborowski R, Bartsch I, Wiencke C (2014) Ocean
448 acidification affects growth but not nutritional quality of the seaweed *Fucus vesiculosus*
449 (Phaeophyceae, Fucales). J Exp Mar Biol Ecol 453:84–90

450 Heaton THE (1986) Isotopic studies of nitrogen pollution in the hydrosphere and atmosphere: a
451 review. Chem Geol 59:87–102

452 Hiscock S (1991) *A Field Key to the British Brown Seaweeds (Phaeophyta)*; Field Studies
453 Council: Shrewsbury, UK

454 Hudson-Edwards K, Macklin M, Taylor M (1997) Historic metal mining inputs to Tees river
455 sediment. Sci Total Environ 194:437–445

456 Jona-Lasinio G, Costantini ML, Calizza E, Pollice A, Bentivoglio F, Orlandi L, Careddu G,
457 Rossi L (2015) Stable isotope-based statistical tools as ecological indicator of pollution
458 sources in Mediterranean transitional water ecosystems. Ecol Indicat 55:23–31

459 Kaldy J (2011) Using a macroalgal $\delta^{15}N$ bioassay to detect cruise ship waste water effluent
460 inputs. Mar Poll Bull 62:1762–1771

461 Kim JK, Kraemer GP, Yarish C (2013) Emersion induces nitrogen release and alteration of
462 nitrogen metabolism in the intertidal genus Porphyra. PloS One 8:1–14

463 Korth, F.; Deutsch, B.; Frey, C.; Moros, C.; Voss, M. (2014) Nitrate source identification in the
464 Baltic Sea using its isotopic ratios in combination with a Bayesian isotope mixing model.
465 Biogeosci 11:4913–4924

466 Lapointe BE, Bedford BJ (2007) Drift rhodophyte blooms emerge in Lee County, Florida, USA:
467 Evidence of escalating coastal eutrophication. Harmful Algae 6:421–437

468 Lye CM, Frid CLJ, Gill ME, Cooper DW, Jones DM (1999) Estrogenic alkylphenols in fish
469 tissues sediments, and waters from the UK Tyne and Tees estuaries. Environ Sci Technol
470 33:1009–1014

471 McClelland JW, Valiela I, Michener RH (1997) Nitrogen-stable isotope signatures in estuarine
472 food webs: A record of increasing urbanization in coastal watersheds. Limnol Oceanogr
473 42:930–937

474 McGlathery KJ, Pedersen MF, Borum J (1996) Changes in intracellular nitrogen pools and
475 feedback controls on nitrogen uptake in *Chaetomorpha linum* (chlorophyta). J Phycol 32:393–
476 401

477 Minagawa M, Wada E (1994) Stepwise enrichment of ^{15}N along food chains: further evidence
478 and the relation between $\delta^{15}N$ and animal age. Geochim Cosmochim Acta 48:1135–1140

479 Neori A, Chopin T, Troell M, Buschmann AH, Kraemer GP, Halling C, Shpigel M, Yarish C
480 (2004) Integrated aquaculture: rationale, evolution and state of the art emphasizing seaweed
481 biofiltration in modem mariculture. Aquaculture 231:361–391

482 Ochoa-Izaguirre MJ, Soto-Jiménez MF (2015) Variability in nitrogen stable isotope ratios of
483 macroalgae: consequences for the identification of nitrogen sources. J Phycol 51:46–65

484 Pedersen MF (1994) Transient ammonium uptake in the macroalga *Ulva lactuca* (Chlorophyta):
485 nature, regulation, and the consequences for choice of measuring technique. J Phycol 30:980–
486 986

487 Piñón-Gimate A, Soto-Jiménez M, Ochoa-Izaguirre MJ, García-Pagés E, Páez-Osuna F (2009)
488 Macroalgae blooms and $\delta^{15}N$ in subtropical coastal lagoons from the Southeastern Gulf of

489 California: discrimination among agricultural, shrimp farm and sewage effluents. Mar Poll
490 58:1144–1151

491 Raimonet M, Guillou G, Mornet F, Richard P (2013) Macroalgae $\delta^{15}N$ values in well-mixed
492 estuaries: indicator of anthropogenic nitrogen input or macroalgae metabolism? Estuar Coast
493 Shelf Sci 119:126–138

494 Raven JA (2003) Long-distance transport in non-vascular plants. Plant Cell Env 26:73–85

495 Riera P (1998) $\delta^{15}N$ of organic matter sources and benthic invertebrates along an estuarine
496 gradient in Marennes-Oleron Bay (France): implications for the study of the trophic structure.
497 Mar Ecol Prog Ser 166:143–150

498 Riera P, Stal LJ, Nieuwenhuize J (2000) Heavy $\delta^{15}N$ in intertidal benthic algae and invertebrates
499 in the Scheldt Estuary (The Netherlands): effect of river nitrogen inputs. Estuar Coast Shelf
500 Sci 51:365–372

501 Rosenberg G, Ramus J (1984) Uptake of inorganic nitrogen and seaweed surface-area-volume
502 ratios. Aqua Bot 19:65–72

503 Savage C (2005) Tracing the influence of sewage nitrogen in a coastal ecosystem using stable
504 nitrogen isotopes. Ambio 34:145–150

505 Savage C, Elmgren R (2004) Macroalgal (*Fucus vesiculosus*) $\delta^{15}N$ values trace decrease in
506 sewage influence. Ecol Appl 14:517–526

507 Strömgren T (1977) Short-term effect of temperature upon the growth of intertidal fucales. J Exp
508 Mar Biol Ecol 29:181–195

509 Swart PK, Evans S, Capo T, Altabet MA (2014) The fractionation of nitrogen and oxygen
510 isotopes in macroalgae during the assimilation of nitrate. Biogeosci 11:6147–6157

511 Valiela I, McClelland J, Hauxwell J, Behr PJ, Hersh D, Foreman K (1997) Macroalgal blooms in
512 shallow estuaries: controls and ecophysiological and ecosystem consequences. Limnol
513 Oceanogr 42:1105–1118

514 Viana IG, Bode A (2013) Stable nitrogen isotopes in coastal macroalgae: geographic and
515 anthropogenic variability. Sci Total Env 443:887–895

516 Viana IG, Bode A (2015) Variability in $\delta^{15}N$ of intertidal brown algae along a salinity gradient:
517 Differential impact of nitrogen sources. Sci Total Env 512:167–176

518 Viana IG, Bode A, Bartholomew M, Valiela I (2015) Experimental assessment of the macroalgae
519 *Ascophyllum nodosum* and *Fucus vesiculosus* for monitoring N sources at different time-
520 scales using stable isotope composition. J Exp Mar Biol Ecol 466:24–33

521 Viana IG, Fernandez JA, Aboal JR, Carballeira A (2011) Measurement of $\delta^{15}N$ in macroalgae
522 stored in an environmental specimen bank for regional scale monitoring of eutrophication in
523 coastal areas. Ecol Indic 11:888–895

524 Vizzini S, Mazzola A (2004) Stable isotope evidence for the environmental impact of a land-
525 based fish farm in the western Mediterranean. Mar Poll 49:61–70

526 Wang Y, Liu D, Richard P, Di B (2016) Selection of effective macroalgal species and tracing
527 nitrogen sources on the different part of Yantai coast, China indicated by macroalgal $\delta^{15}N$
528 values. Sci Total Env 542:306–314

529 Warwick RM, Ashman CM, Brown AR, Clarke KR, Dowell B, Hart B, Lewis RE, Shillabeer N,
530 Somerfield PJ, Tapp JF (2002) Inter-annual changes in the biodiversity and community
531 structure of the macrobenthos in Tees Bay and the Tees estuary, UK, associated with local
532 and regional environmental events. Mar Ecol Prog Ser 234:1–13

533 Williams A, Milner N, O'Keeffe N, Clarke A, Webb H (2009) Environment Agency: River Tees
534 Salmon Action Plan Review

Figure captions

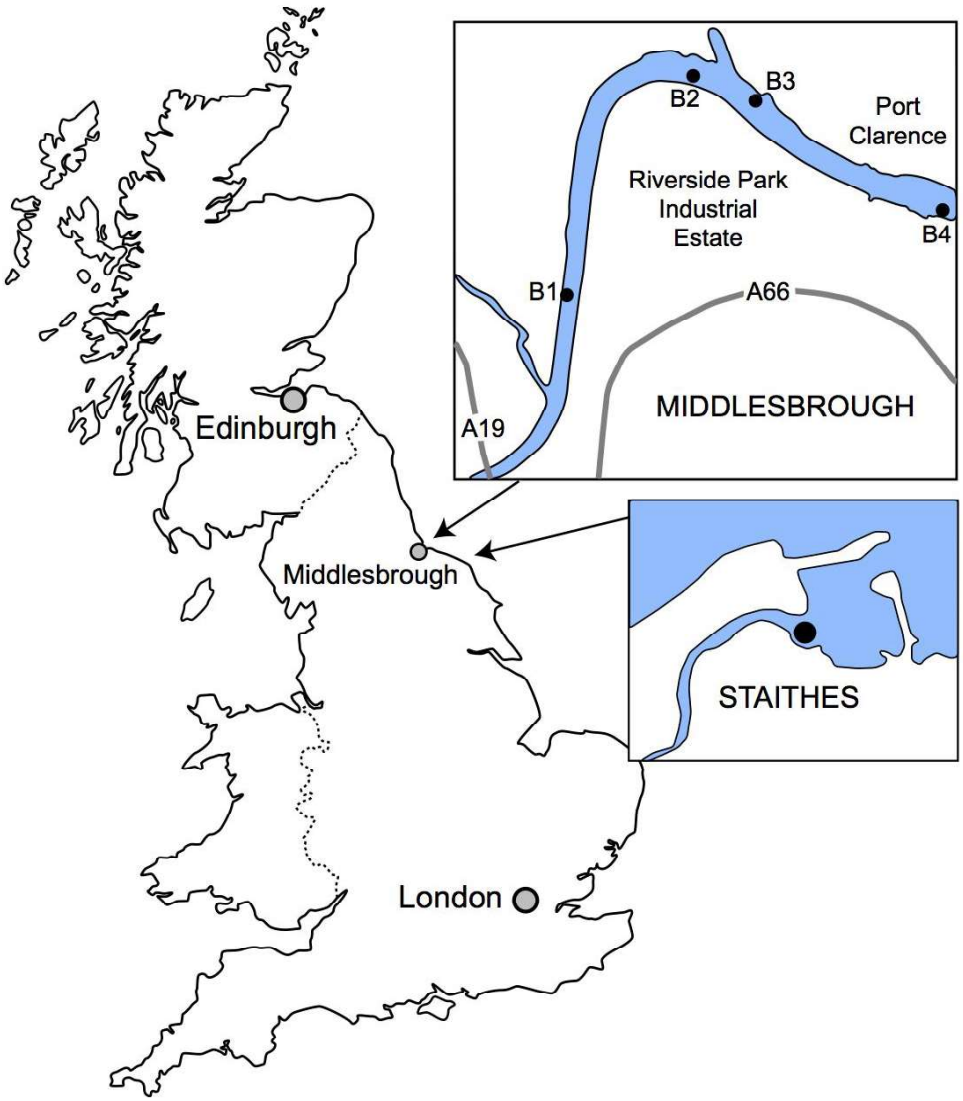
Figure 1 Part map of the United Kingdom showing the two locations discussed in this study, River Tees, Middlesbrough (top insert), and Staithes, North Yorkshire (bottom insert). Buoy locations are labelled; buoy 1 (B1), buoy 2 (B2), buoy 3 (B3) and buoy 4 (B4). Sample collection of macoralgae from Staithes are represented by the large dot.

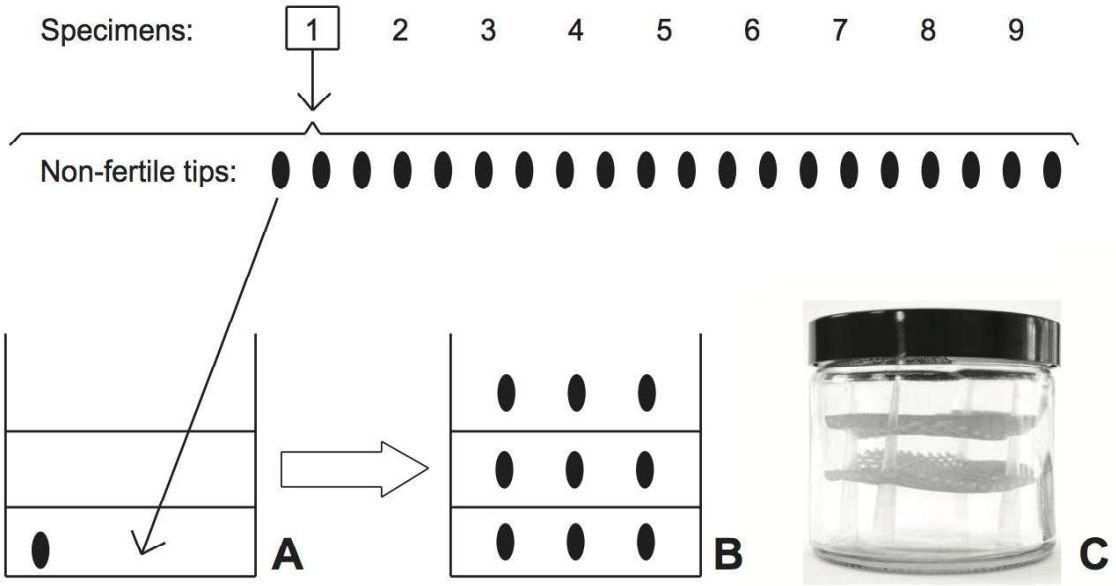
Figure 2 Experimental set up for the *in vitro* cultures used for nitrate and ammonia solutions and vegetative macroalgae tips from Staithes, UK. (**A**). Each jar was filled with two meshes ending up with three levels, which store three vegetative tips each (**B**). (**C**) Picture of the culture jar set up used in this experiment (jar height = 10cm).

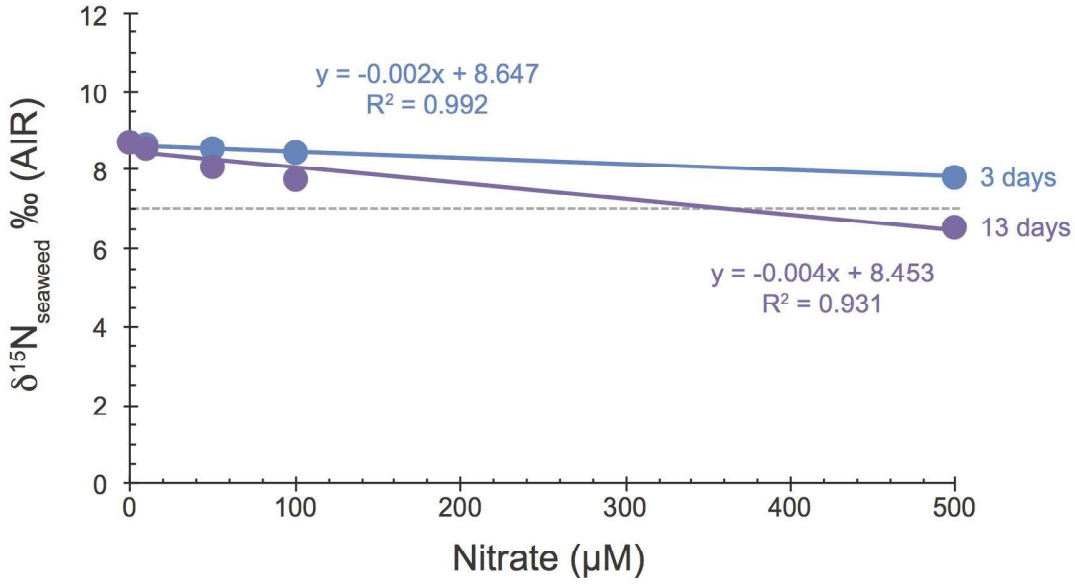
Figure 3 $\delta^{15}N_{seaweed}$ results from the *in vitro* cultures of nitrate (top) and ammonia (bottom). Dashed grey line represents the $\delta^{15}N$ value of the nitrate and ammonia solutions used.

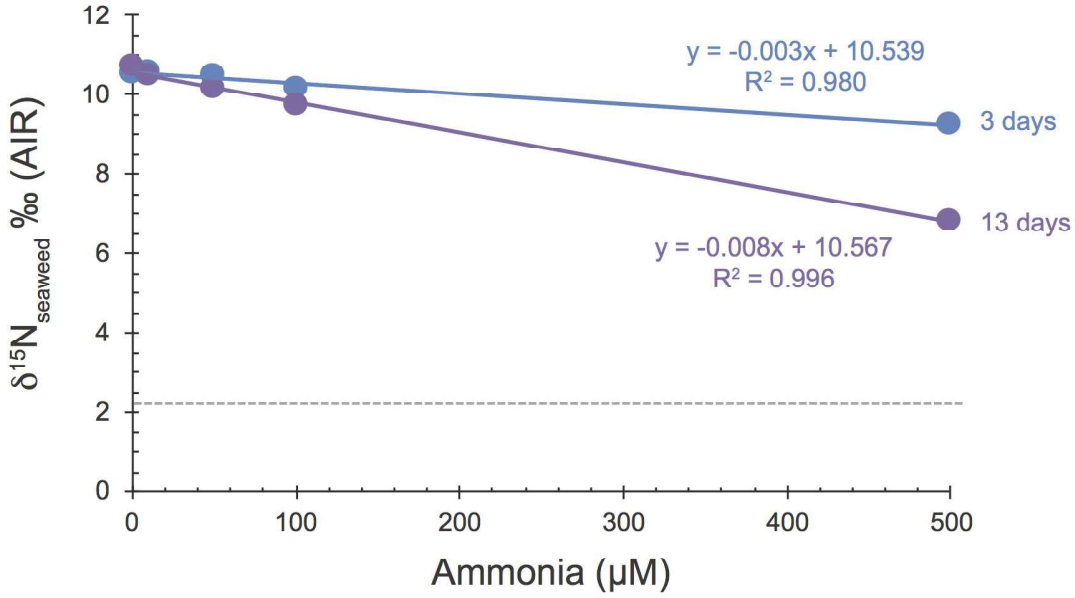
Figure 4 $\delta^{15}N_{seaweed}$ results from the *in situ* Experiment 2 (short-term) done in the River Tees, Middlesbrough, UK. For Buoy number locations refer back to Fig. 1. Bottom and top refer to the position on the rope at each buoy. The dashed grey line represents the 50:50 mass balance value between the $\delta^{15}N$ background value of *Fucus* non-fertile tips from Staithes and all components from the River Tees.

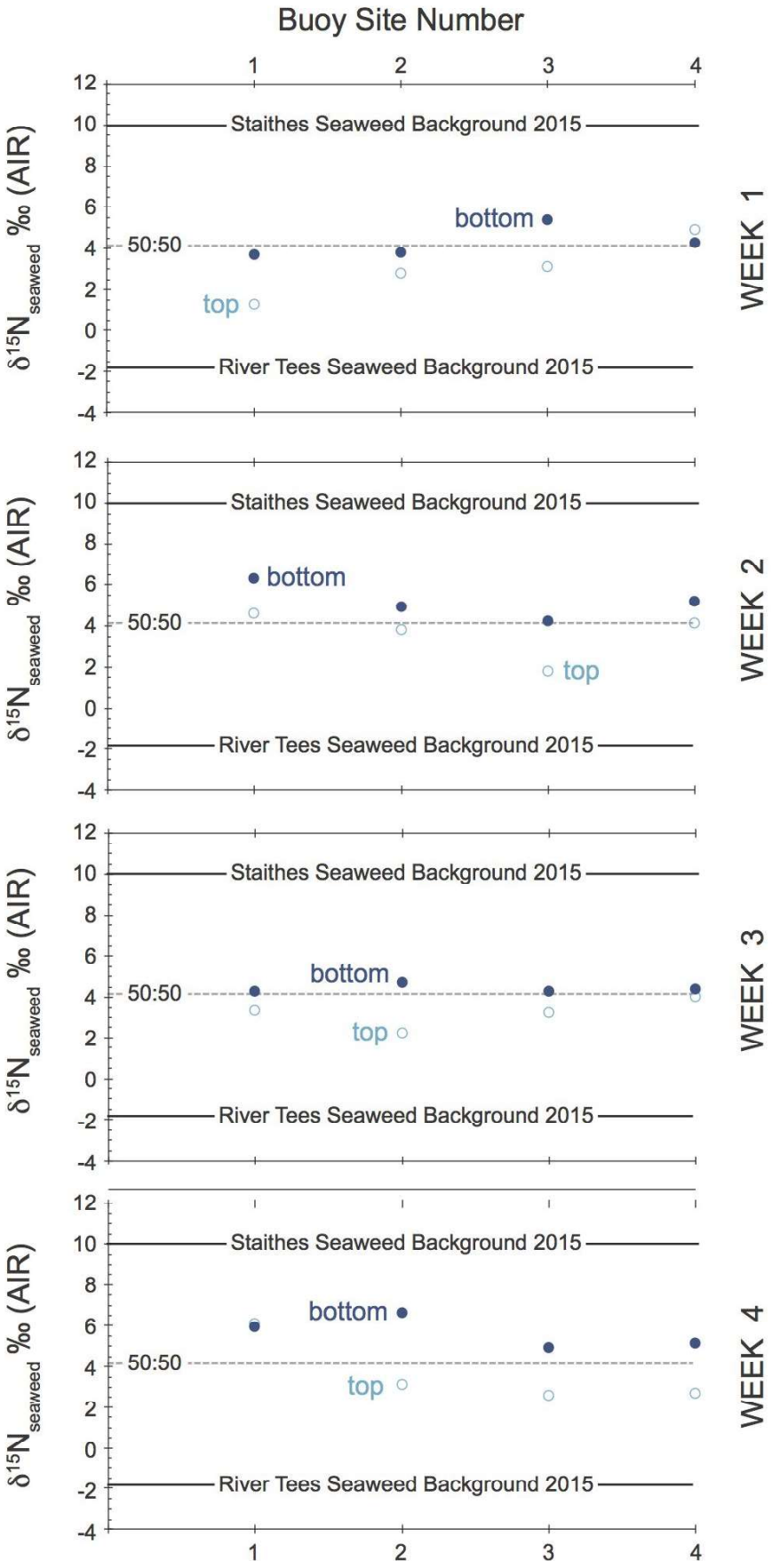
Figure 5 $\delta^{15}N_{seaweed}$ results from the *in situ* Experiment 1 (long-term) done in the River Tees, Middlesbrough, UK. For Buoy number locations refer back to Fig. 1. Bottom and top refer to the position on the rope at each buoy. The dashed grey line represents the 50:50 mass balance value between the $\delta^{15}N$ background value of *Fucus* non-fertile tips from Staithes and all components from the River Tees.


B2
B3
Port
Clarence
Riverside Park
Industrial
Estate
B4
B1
A66
MIDDLESBROUGH
A19
Edinburgh
Middlesbrough
STAITHES
London


Specimens:
1
2
3
4
5
6
7
8
9
Non-fertile tips:
A
B
C


y = -0.002x + 8.647
R² = 0.992
y = -0.004x + 8.453
R² = 0.931
3 days
13 days
δ15Nseaweed ‰ (AIR)
Nitrate (μM)
0
2
4
6
8
10
12
0
100
200
300
400
500


y = -0.003x + 10.539
R² = 0.980
y = -0.008x + 10.567
R² = 0.996
3 days
13 days
δ15Nseaweed ‰ (AIR)
Ammonia (μM)
0
2
4
6
8
10
12
0
100
200
300
400
500


Buoy Site Number
1
2
3
4
12
10
8
6
4
2
0
-2
-4
Staithes Seaweed Background 2015
River Tees Seaweed Background 2015
50:50
bottom
top
δ15Nseaweed ‰ (AIR)
WEEK 1
WEEK 2
WEEK 3
WEEK 4

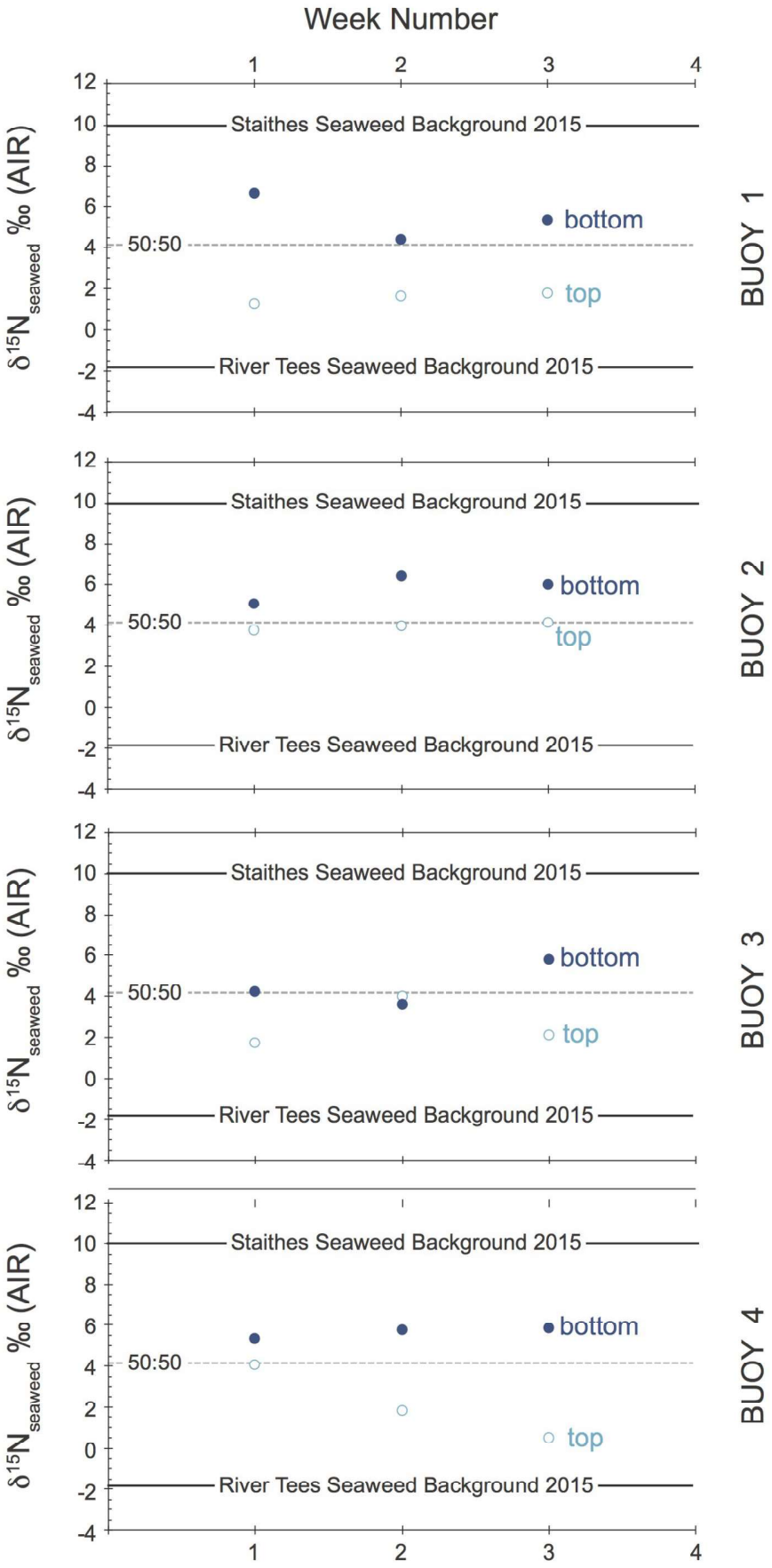

Week Number
1
2
3
4
12
10
8
6
4
2
0
-2
-4
Staithes Seaweed Background 2015
River Tees Seaweed Background 2015
50:50
bottom
top
δ15Nseaweed ‰ (AIR)
BUOY 1
BUOY 2
BUOY 3
BUOY 4

Table 1 $\delta^{15}N$ data, average and standard deviation from the *in vitro* nitrate lab experiment of the three replicates per treatment ($n = 3$).

Sample IDs	$\delta^{15}N$ ‰	Sample IDs	$\delta^{15}N$ ‰
3 days		**13 days**	
0 µM		0 µM	
1	8.6	*1*	8.4
2	8.7	*2*	8.8
3	8.7	*3*	8.9
Average (3 days)	8.7	Average (13 days)	8.7
Std dev (3 days)	0.0	Std dev (13 days)	0.2
-		-	
10 µM		10 µM	
1	8.7	*1*	8.6
2	8.5	*2*	8.5
3	8.7	*3*	8.5
Average (3 days)	8.6	Average (13 days)	8.6
Std dev (3 days)	0.1	Std dev (13 days)	0.1
-		-	
50 µM		50 µM	
1	8.5	*1*	8.1
2	8.2	*2*	8.2
3	8.9	*3*	8.0
Average (3 days)	8.5	Average (13 days)	8.1
Std dev (3 days)	0.3	Std dev (13 days)	0.1
-		-	
100 µM		100 µM	
1	8.5	*1*	8.1
2	8.4	*2*	7.5
3	8.5	*3*	7.6
Average (3 days)	8.5	Average (13 days)	7.8
Std dev (3 days)	0.1	Std dev (13 days)	0.2
-		-	
500 µM		500 µM	
1	8.0	*1*	6.3
2	7.6	*2*	6.7
3	7.9	*3*	6.5
Average (3 days)	7.8	Average (13 days)	6.5
Std dev (3 days)	0.1	Std dev (13 days)	0.2

Table 2 $\delta^{15}N$ data, average and standard deviation from the *in vitro* ammonia lab experiment of the three replicates per treatment ($n = 3$).

Sample IDs	$\delta^{15}N$ ‰	Sample IDs	$\delta^{15}N$ ‰
3 days		**13 days**	
0 µM		0 µM	
1	10.5	*1*	10.3
2	10.5	*2*	10.5
3	10.5	*3*	11.4
Average (3 days)	10.5	Average (13 days)	10.7
Std dev (3 days)	0.0	Std dev (13 days)	0.5
-		-	
10 µM		10 µM	
1	10.8	*1*	10.5
2	10.3	*2*	10.0
3	10.6	*3*	10.9
Average (3 days)	10.6	Average (13 days)	10.5
Std dev (3 days)	0.2	Std dev (13 days)	0.4
-		-	
50 µM		50 µM	
1	10.6	*1*	9.6
2	11.0	*2*	10.9
3	9.8	*3*	9.9
Average (3 days)	10.5	Average (13 days)	10.1
Std dev (3 days)	0.5	Std dev (13 days)	0.6
-		-	
110 µM		110 µM	
1	10.2	*1*	9.7
2	10.2	*2*	9.3
3	10.1	*3*	10.1
Average (3 days)	10.2	Average (13 days)	9.7
Std dev (3 days)	0.0	Std dev (13 days)	0.3
-		-	
500 µM		500 µM	
1	9.3	*1*	7.3
2	9.2	*2*	7.0
3	9.3	*3*	6.1
Average (3 days)	9.3	Average (13 days)	6.8
Std dev (3 days)	0.1	Std dev (13 days)	0.5

Table 3 $\delta^{15}N$ data from Staithes *Fucus* collected between 27/05/2015 to 25/08/2015.

Sample IDs	$\delta^{15}N$ ‰
July vegetative tips	11.1
July vegetative tips	9.3
July vegetative tips	10.3
July vegetative tips	9.9
July vegetative tips	9.6
July vegetative tips	9.6
July vegetative tips	9.6
July vegetative tips	9.1
July vegetative tips	10.1
July vegetative tips	9.9
July vegetative tips	9.5
July vegetative tips	9.6
July vegetative tips	9.7
July vegetative tips	10.2
July vegetative tips	10.6
July vegetative tips	10.1
August vegetative tips	10.5
August vegetative tips	10.7
August vegetative tips	10.5
August vegetative tips	10.2
August vegetative tips	10.1
August vegetative tips	10.1
August vegetative tips	10.6
August vegetative tips	10.0
August vegetative tips	10.1
August vegetative tips	9.8
August vegetative tips	10.4
Average Staithes ‘15	10.0
Std dev Staithes ‘15	0.5

Table 4 $\delta^{15}N$ data from River Tees *Fucus* collected between 27/05/2015 to 01/07/2015.

Sample IDs	$\delta^{15}N$ ‰	Sample IDs	$\delta^{15}N$ ‰	Sample IDs	$\delta^{15}N$ ‰
***Fucus sp.* blades**		***Fucus sp.* fertile tips**		***Fucus sp.* non-fertile tips**	
July	-3.8	*July*	-3.9	*July*	-5.2
July	-5.3	*July*	-4.7	*July*	-4.2
July	-1.7	*July*	-1.3	*July*	-1.1
July	8.6	*July*	-3.2	*July*	10.8
July	3.8	*July*	-5.8	*July*	-6.3
July	-8.5	*July*	-6.5	*July*	-9.1
July	-5.0	*July*	-4.4	*July*	-5.0
July	-6.2	*July*	-4.9	*July*	-5.4
July	-8.1	*July*	-6.7	*July*	-7.8
July	-2.7	*July*	-7.4	*July*	-2.6
June	-3.6	*June*	-2.8	*June*	-2.4
June	-8.1	*June*	-5.9	*June*	-5.5
June	-4.6	*June*	-3.3	*June*	-1.9
June	-1.1	*June*	3.1	*June*	3.2
June	-4.5	*June*	-6.1	*June*	-8.4
June	-7.5	*June*	-4.8	*June*	-7.5
June	-3	*June*	-2.6	*June*	-2.7
June	-6.4	*June*	-5.3	*June*	-4.4
June	-4.3	*June*	-2.1	*June*	-2.8
June	-5.8	*June*	-5.9	*June*	-3.9
May	2.5	*May*	3.0	*May*	4.3
May	-0.8	*May*	4.8	*May*	0.6
May	-1.6	*May*	2.4	*May*	3.4
May	3.5	*May*	-2.3	*May*	3.9

May	3.8
May	0.6
May	-2.8
May	1.8
Average blades	-2.5
Std dev blades	4.2

ALL average	-1.7
ALL std dev	4.3

May	1.5
May	3.8
May	2.4
May	4.2
May	4.1
May	2.3
May	-0.5
May	-0.6
May	3.7
May	3.8
May	3.1
May	1.3
May	1.7
Average fertile	-1.2
Std dev fertile	3.9

May	4.5
May	0.4
May	4.0
May	2.9
May	2.3
Average fertile	-1.6
Std dev fertile	4.8

Table 5 Average $\delta^{15}N$ data from River Tees Experiment 2 (short-term).

	Week 1		Week 2		Week 3		Week 4	
Position	top	bottom	top	bottom	top	bottom	top	bottom
Buoy 1	1.3	3.7	4.6	6.3	3.3	4.3	6.1	5.9
Buoy 2	2.8	3.8	3.8	4.9	2.2	4.7	3.1	6.6
Buoy 3	3.1	5.4	1.8	4.2	3.2	4.3	2.6	4.9
Buoy 4	4.9	4.3	4.1	5.2	4.0	4.4	2.7	5.1

Table 6 Average $\delta^{15}N$ data from River Tees Experiment 1 (long-term).

	Buoy 1		Buoy 2		Buoy 3		Buoy 4	
Position	top	bottom	top	bottom	top	bottom	top	bottom
Week 1	1.1	6.3	3.8	4.9	1.7	4.2	4.1	5.1
Week 2	1.5	4.5	4.0	6.1	4.0	3.6	1.9	5.6
Week 3	1.7	5.3	4.1	6.0	2.2	5.8	0.4	5.7