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1 **Macroalgae $\delta^{15}\text{N}$ values in well-mixed estuaries: indicator of**
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4 **anthropogenic nitrogen input or macroalgae metabolism?**
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1 12 **ABSTRACT**

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6 14 Even if nitrogen stable isotope ratio ($\delta^{15}\text{N}$) in macroalgae is widely used as bioindicator of
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8 15 anthropogenic nitrogen inputs to the coastal zone, recent studies suggest the possible role of
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10 16 macroalgae metabolism in $\delta^{15}\text{N}$ variability. Simultaneous determinations of $\delta^{15}\text{N}$ of dissolved
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12 17 inorganic nitrogen (DIN) along the land-sea continuum, inter-species variability of $\delta^{15}\text{N}$ and its
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14 18 sensitivity to environmental factors are necessary to confirm the efficiency of macroalgae $\delta^{15}\text{N}$
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16 19 in monitoring nitrogen origin in mixed-use watersheds. In this study, $\delta^{15}\text{N}$ of annual and
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18 20 perennial macroalgae (*Ulva sp.*, *Enteromorpha sp.*, *Fucus vesiculosus* and *Fucus serratus*) are
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20 21 compared to $\delta^{15}\text{N}$ -DIN along the Charente Estuary, after characterizing $\delta^{15}\text{N}$ of the three main
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22 22 DIN sources (*i.e.* cultivated area, pasture, sewage treatment plant outlet). During late winter
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24 23 and spring, when human activities lead to high DIN inputs, DIN sources exhibit distinct $\delta^{15}\text{N}$
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26 24 signals in nitrate (NO_3^-) and ammonium (NH_4^+): cultivated area ($+6.5 \pm 0.6 \text{‰}$ and $+9 \pm 11$
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28 25 ‰), pasture ($+9.2 \pm 1.8 \text{‰}$ and $+12.4 \text{‰}$) and sewage treatment plant outlet ($+16.9 \pm 8.7 \text{‰}$
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30 26 and $+25.4 \pm 5.9 \text{‰}$). While sources show distinct $\delta^{15}\text{N}$ - NO_3^- in this multiple source watershed,
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32 27 the overall mixture of NO_3^- sources - generally $> 95\%$ DIN - leads to low variations of $\delta^{15}\text{N}$ -
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34 28 NO_3^- at the mouth of the estuary ($+7.7$ to $+8.4 \text{‰}$). Even if estuarine $\delta^{15}\text{N}$ - NO_3^- values are not
35
36 29 significantly different from pristine continental and oceanic site ($+7.3 \text{‰}$ and $+7.4 \text{‰}$),
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38 30 macroalgae $\delta^{15}\text{N}$ values are generally higher at the mouth of the estuary. This highlights high
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40 31 anthropogenic DIN inputs in the estuary, and enhanced contribution of ^{15}N -depleted NH_4^+ in
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42 32 oceanic waters. Although seasonal variations in $\delta^{15}\text{N}$ - NO_3^- are low, the same temporal trends in
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44 33 macroalgae $\delta^{15}\text{N}$ values at estuarine and oceanic sites, and inter-species differences in $\delta^{15}\text{N}$
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46 34 values, suggest that macroalgae $\delta^{15}\text{N}$ values might be modified by the metabolic response of
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48 35 macroalgae to environmental parameters (*e.g.*, temperature, light, DIN concentrations).
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50 36 Differences between annual and perennial macroalgae indicate both a higher integration time
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52 37 of perennial compared to annual macroalgae and the possible role of passive *versus* active
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1 38 uptake mechanisms. Further studies should be carried out to characterize the sensitivity of
2
3 39 macroalgae fractionation to variable environmental conditions and uptake mechanisms.
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8 41 **Keywords:** Nitrogen isotopes; Nitrate; Ammonium; Primary producers; Indicators; Land-sea
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1. INTRODUCTION

The intensification of urbanization, together with agricultural development, have worldwide increased dissolved inorganic nitrogen (DIN) entering estuarine and coastal waters (Nixon, 1995; Middelburg and Nieuwenhuize, 2001). These high DIN supply enhance the total production of ecosystems (Fujita, 1985; Cloern, 2001; Savage et al., 2002), which often lead to environmental disturbances such as ephemeral algal blooms and anoxic events (Valiela et al., 1992; Conley et al., 2009; Howarth et al., 2011). Estuaries are one of the ecosystems most heavily affected by human activities taking place either into estuaries themselves (*e.g.*, fisheries, recreation, introduction of exotic species) or on watersheds (*e.g.*, agriculture, urbanization). They are thus very sensitive to eutrophication (Vitousek et al., 1997; Cloern, 2001; Halpern et al., 2008).

While DIN concentration is commonly used to monitor the spatio-temporal extent of anthropogenic perturbations (*e.g.* eutrophication) in coastal areas, this chemical indicator does not permit identifying DIN origins (*e.g.*, agriculture, sewage, industry). This information may however be important for restricting or eliminating future nitrogen loading. In the last years, nitrogen stable isotope ratios ($\delta^{15}\text{N}$) have been used to identify the origin of anthropogenic nitrogen loads to aquatic ecosystems (Jones et al., 2001; Curt et al., 2004; Costanzo et al., 2005). The success of this tool is based on different isotopic signature of DIN sources. The $\delta^{15}\text{N}$ -DIN of fertilizers - which derive from industrial fixation of atmospheric N_2 - is usually lower (-4 to +4 ‰) than $\delta^{15}\text{N}$ -DIN resulting from soil N mineralization (+4 to +10 ‰; Heaton, 1986; Lindau et al., 1989). Sewage effluents are often more ^{15}N -enriched due to the enzymatic preference of bacteria towards ^{14}N over ^{15}N during ammonia volatilization and denitrification processes (Heaton, 1986; McClelland and Valiela, 1998; Horrigan et al., 1990).

Most studies devoted to trace anthropogenic inputs generally deal with the characterization of only one dominant load from one watershed or sub-watershed – which often

69 concern urban inputs (Costanzo et al., 2005; McClelland et al., 1997; Valiela et al., 1992) or
70 agricultural inputs (Anderson and Cabana, 2005; Howarth, 2008) – or two different N sources
71 (Costanzo et al., 2003; Strauch et al., 2008). Most aquatic systems however receive N from
72 multiple sources: intensive culture, pasture, urban waste, atmospheric and/or natural inputs.
73 Interpretation of the N pools is consequently often difficult and requires first a characterization
74 of each source, including natural ones.

75 The $\delta^{15}\text{N}$ values of ammonium (NH_4^+) and nitrate (NO_3^-) can moreover differ from
76 $\delta^{15}\text{N}$ of DIN sources in estuarine and coastal ecosystems. Estuaries are indeed well known to
77 modify and attenuate DIN transfer from rivers to the coastal sea in response to estuarine
78 processes, such as nitrification, denitrification, volatilization or algal uptake, and mixing of
79 riverine and oceanic waters. It is thus essential to take into account these estuarine processes,
80 which can significantly modify $\delta^{15}\text{N}$ values of NH_4^+ and NO_3^- during the transport of DIN
81 along the land-sea continuum (Cifuentes et al., 1989; Middelburg and Nieuwenhuize, 2001;
82 Sebilo et al., 2006).

83 The concentration and the $\delta^{15}\text{N}$ value of DIN can also vary at small temporal scales
84 (hours, days), which is only resolved by high frequency and heavy surveys. Measuring $\delta^{15}\text{N}$ in
85 benthic sessile species is advantageous as these species integrate spatial and temporal
86 variability of $\delta^{15}\text{N}$ -DIN (Gartner et al., 2002; Savage and Elmgren, 2004). The $\delta^{15}\text{N}$ values in
87 producers are thus broadly used to monitor anthropogenic inputs of DIN to aquatic ecosystems,
88 such as the $\delta^{15}\text{N}$ values in consumers that are commonly used to monitor particulate organic
89 nitrogen (PON) inputs (McClelland et al., 1997; Costanzo et al., 2001; Cole et al., 2004; Cohen
90 and Fong, 2005; Costanzo et al., 2005; Fertig et al., 2010; García-Sanz et al., 2011).

91 While producers and consumers are efficient integrators, $\delta^{15}\text{N}$ values in consumers have
92 been shown to vary with species diet and even more with tissue types (Lorrain et al., 2002).
93 This suggests that the choice of tissues and species is not trivial and must be performed with
94 caution, as recently highlighted for filter feeders along an inshore-offshore gradient on the

1 95 continental shelf of the Bay of Biscay (Nerot et al., 2011). Compared to studies on consumers,
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3 96 few studies have investigated variations between tissues (Savage and Elmgren, 2004) and
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6 97 species of primary producers, and especially macroalgae (*e.g.* Cole et al., 2005; Grall et al.,
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8 98 2006). Savage and Elmgren (2004) showed that decreasing $\delta^{15}\text{N}$ values along the frond of a
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10 99 perennial macroalgae *Fucus vesiculosus* was related to changes in sewage DIN loads, while
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12
13 100 similarity and differences between species is generally related to changes in $\delta^{15}\text{N}$ -DIN and
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16 101 available DIN forms.

17
18 102 Such as for consumers, environmental and/or metabolic factors can also induce
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20 103 variations of $\delta^{15}\text{N}$ values in macroalgae. Several studies have shown the role of environmental
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23 104 factors such as light, temperature or nutrient concentrations in modifying $\delta^{15}\text{N}$ values in
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25 105 macroalgae (Pedersen et al., 2004; Dudley et al., 2010). Even if the role of environmental
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28 106 factors (*e.g.* light, growth rate, nutrient availability) on fractionation has been shown for
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30 107 microalgae (Pennock et al., 1996; Needoba and Harrison, 2004), little is however known about
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33 108 fractionation in macroalgae (Dudley et al., 2010). The main hypotheses to explain fractionation
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35 109 in macroalgae are the control of environmental conditions on uptake and growth rates of
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38 110 macroalgae (Taylor et al., 1998; Cohen and Fong, 2004; Pederson et al., 2004; Dudley et al.,
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40 111 2010) and/or the combination of passive and/or active transport mechanisms of DIN that varies
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42 112 among macroalgae species (Taylor et al., 1998). The comparison between $\delta^{15}\text{N}$ of DIN and
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45 113 macroalgae might permit to verify if $\delta^{15}\text{N}$ values in macroalgae indicate spatial and seasonal
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47 114 variations of $\delta^{15}\text{N}$ -DIN only, or also changes in environmental conditions and/or fractionation.
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50 115 Simultaneous investigations of $\delta^{15}\text{N}$ of sources (DIN or PON) and biological integrators are
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52 116 however not frequent (Deutsch and Voss, 2006).

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54 117 The Charente River and Estuary - that flows into Marennes-Oléron Bay - is a typical
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57 118 example of temperate macrotidal ecosystem impacted by multiple anthropogenic activities.
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59 119 While the watershed is one of the most nitrate (NO_3^-)-polluted watersheds of the South West of
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62 120 France due to intensive agriculture (<http://www.eau-adour-garonne.fr>), the coastal bay is the

1 121 main European shellfish production area. Water management is therefore essential in this area
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3 122 (Bry and Hoflack, 2004; <http://www.eau-adour-garonne.fr>), as human activity changes (*e.g.*
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6 123 DIN loads) control the functioning of downstream ecosystems, and as the good ecological
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8 124 status of surface waters required by the European community may not be reached in 2015
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11 125 (<http://www.observatoire-environnement.org>).

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13 126 The aim of this study is to investigate if $\delta^{15}\text{N}$ values of macroalgae growing at the
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16 127 mouth of a well-mixed estuary receiving high DIN loads from multiple sources are efficient
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18 128 indicators of anthropogenic DIN loads, or if changes in environmental conditions and/or
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21 129 macroalgae metabolism can alter the signal. We especially answer and discuss the following
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23 130 questions: (1) Is $\delta^{15}\text{N}$ -DIN efficient to distinguish the main DIN sources? (2) Is $\delta^{15}\text{N}$ -DIN
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25 131 modified by estuarine processes during the transport of DIN to the coastal zone? (3) Do
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28 132 macroalgae $\delta^{15}\text{N}$ values record $\delta^{15}\text{N}$ values of anthropogenic DIN inputs at the mouth of the
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31 133 estuary? Do species characterized by different uptake rates, *i.e.* annual and perennial species,
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33 134 show similar or different $\delta^{15}\text{N}$? Do differences between $\delta^{15}\text{N}$ values in DIN and macroalgae
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35 135 highlight the possible alteration of $\delta^{15}\text{N}$ values by macroalgae metabolism?

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2. MATERIALS AND METHODS

2.1. Study area

The drainage area of the Charente River extends over 10,000 km² (Fig. 1) and represents the main freshwater input to the Marennes-Oléron Bay (Ravail et al., 1988). Regardless of river flow, the plume of the Charente River is generally limited to the coastal zone (Stanisière et al., 2006). This leads to a mean flushing time of waters at the mouth of the estuary higher than the 11 days calculated in the Bay (Stanisière et al., 2006). Riverine nutrients sustain high primary production in the bay (185 gC m⁻² yr⁻¹; Struski and Bacher, 2006), supporting half of the French shellfish production. NO₃⁻ concentrations are particularly high, often above 400 µmol l⁻¹ at the mouth of the estuary by the end of winter, mainly due to the application of fertilizers within the watershed*. Agriculture activity covers around 79 % of the surface of the watershed, while forests cover only 13 % and are mainly located in its eastern part (Corinne Land Cover 2006, <http://sd1878-2.sivit.org/>). A few small cities, totaling 260,000 inhabitants, equipped with sewage treatment plants, are mainly located along the 360 km of river. The climate is oceanic temperate with episodic and intense rainfall in spring that lead to a rapid increase in stream flow (up to 800 m³ s⁻¹) and dryness during summer (1 m³ s⁻¹) at Saint-Savinien (Bry and Hoflack, 2004).

2.2. Sample collection

Water samples were collected monthly from January to May 2006, a period typically associated with the highest flow conditions of the year (Fig. 2). Three sites were sampled to

* In 2006, the use of fertilizers on spring cultures was allowed from mid-February to the end of June (<http://www.observatoire-environnement.org>) and high spreading activity was observed during our sampling period, even in February.

1 161 characterize DIN anthropogenic sources: i) “Culture”, a cultivated area receiving mainly
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3 162 chemical fertilizers at Saint-Coutant, ii) “Pasture”, an extensive pasture fertilized by animal
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6 163 manure at Candé, and iii) “STP”, the outlet of the secondary sewage treatment plant of Saint-
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8 164 Savinien city, characterized by activated muds and extended aeration
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11 165 (<http://assainissement.developpement-durable.gouv.fr>) that enhance nitrification processes
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13 166 (Fig. 1). Water samples were collected in a small stream (at Culture and Pasture stations) or
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16 167 directly in the effluent of the STP to the river. Samples were not available at Culture in May
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18 168 due to the stream dryness induced by culture irrigation. Three other sites were sampled along
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21 169 the Charente Estuary: “St. 1” at Saint-Savinien, “St. 2” at Rochefort, and “St. 4” at the mouth
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23 170 of the estuary. Riverine and oceanic reference sites, more preserved from human activities,
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25 171 were also sampled: i) “ref_R”, a riverine reference site located upstream of the Echelle River, a
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28 172 tributary of the Charente River characterized by a forested sub-watershed, and ii) “ref_O”, an
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30 173 oceanic reference site located on the most oceanic part of Ré island. The latter is considered
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33 174 not to be influenced by the Charente inputs as currents tend to deviate riverine waters to the
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35 175 south of the bay (Dechambenoy et al., 1977; Stanisière et al., 2006). DIN inputs from the
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38 176 Gironde River - which plume extends sometimes to the Marennes-Oléron bay during flood
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40 177 events (Ravail et al., 1988) – and/or from the Sèvre Niortaise River might have been low at the
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42 178 oceanic reference site because of oceanic current influence. No samples were collected at ref_O
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45 179 in January. At all stations, duplicate water samples were collected in acid-washed
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47 180 polypropylene dark 5 l bottles, brought back to the laboratory in a cooler and filtered on GF-C
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50 181 filters (Whatman®). Aliquots were directly analyzed for DIN and the remainder of the samples
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52 182 was frozen at -20°C before DIN extraction for isotope analyses. No replicates were performed
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55 183 for DIN sources, but duplicates were sampled at St. 1, 2 and 4.

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57 184 Two annual species (*Ulva sp.*, *Enteromorpha sp.*^{*}) and two perennial species (*Fucus*

61 * The common denomination *Enteromorpha sp.* was used to distinguish this specimen from *Ulva sp.* although
62 *Enteromorpha sp.* was recently showed to belong to the genera *Ulva* (Hayden et al., 2003).
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1 185 *vesiculosus*, *Fucus serratus*) were sampled. For each species, triplicate fronds of three
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3 186 individuals were collected monthly on intertidal rocky banks at two sites at the mouth of the
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6 187 estuary (St. 3 and 4a), and at ref₀ (Fig. 1). Macroalgae were first washed in filtered seawater,
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8 188 then dipped in HCl 0.1 M for a few minutes and thoroughly rinsed with deionized water to
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11 189 clean macroalgae fronds of calcareous organisms. The whole individual (for annual algae) or
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13 190 the 2 cm of the apex of the longest vegetative fronds, corresponding to the new grown tips (for
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16 191 perennial algae) were stored at -20°C for $\delta^{15}\text{N}$ analyses. Intra-individual variations of $\delta^{15}\text{N}$
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18 192 were investigated along two *Fucus serratus* fronds (1 cm resolution) collected at ref₀ and St.
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21 193 4a on March 1st, 2006. No replicates were taken and the inter-individual variability of $\delta^{15}\text{N}$
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23 194 along the fronds was estimated from the variability measured monthly in the 2 cm of the apex.
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25 195 26 27 196 **2.3. *In situ* measurements of *Fucus* growth rates**

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33 198 In order to evaluate the time period integrated in each cm of perennial species fronds, field
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35 199 growth measurements were performed at St. 4a. Nine individuals of both *Fucus vesiculosus*
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38 200 and *Fucus serratus* were tagged with color plastic collars and with a small notch made at about
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40 201 2 cm from the frond apex. Measurements of the maximum frond length were performed from
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43 202 January to May 2006, along with the measurement of the distance from the notch to the frond
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45 203 tip, in order to check which part of the plant was actively growing.
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47 204 48 49 205 **2.4. Laboratory measurements of NO_3^- uptake rates**

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55 207 As NO_3^- is the dominant DIN form available for macroalgae at the mouth of the estuary
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57 208 (generally > 95 %), NO_3^- uptake experiments were carried out in the concentration range
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60 209 measured at the mouth of the estuary, 50 and 500 $\mu\text{mol l}^{-1}$. In order to compare the uptake rates
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62 210 of annual and perennial species, these experiments were performed on both annual and
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1 211 perennial macroalgae species (*Ulva sp.*, *Enteromorpha sp.*, *Fucus serratus*^{*}) collected at station
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3 212 St. 4a on March 1st, 2006. Macroalgae were thoroughly cleaned and stored in filtered offshore,
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6 213 nutrient-poor seawater during 8 days in order to measure the initial NO₃⁻ uptake rate (Lartigue
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8 214 and Sherman, 2005). Macroalgae were incubated in 250 ml of seawater enriched with NO₃⁻, at
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10 215 12°C and a PAR light level of 200 μmol photons m⁻² s⁻¹. For each species, incubations were
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12 216 performed at 50 and 500 μmol NO₃⁻ l⁻¹, respectively. 10 ml of water was taken in each
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14 217 experimental tank at the start of the experiment and after 1h, 24h and 48h to analyze NO₃⁻
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16 218 concentrations. NO₃⁻ uptake rates were calculated as the decrease of NO₃⁻ with time reported to
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18 219 initial macroalgae biomass (μmol N g DW⁻¹ h⁻¹).
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25 221 2.5. Nutrient and macroalgae sample analyses

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30 223 Nitrate (NO₃⁻, including nitrite) and ammonium (NH₄⁺) concentrations were analyzed using a
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32 224 Skalar continuous flow analyzer SA 40, following the methods developed by Strickland and
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34 225 Parsons (1972), and Koroleff (1969), respectively. DIN is the sum of NO₃⁻ and NH₄⁺
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36 226 concentrations.
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40 227 Analyses of δ¹⁵N-NO₃⁻ and δ¹⁵N-NH₄⁺ were carried out by adapting a two-step
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42 228 ammonia microdiffusion method developed by Sigman et al. (1997) and Holmes et al. (1998).
43
44 229 The microdiffusion method permits the successive and total recovery of NO₃⁻ and NH₄⁺. The
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46 230 method consists in adding diffusion packets (containing acidified glassfiber disks), MgO (first
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48 231 step, transformation of NH₄⁺ to ammonia) and Devarda alloy (second step, transformation of
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50 232 NO₃⁻ to NH₄⁺, transformed then to ammonia by MgO) in sealed bottles. As DIN concentrations
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52 233 and salinity varied among a large range in our study, water samples were first adjusted with (1)
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54 234 deionized water to a final volume of 50 ml in a 100 ml screwcap bottle to get around 30 μmol
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61 * As growth rates of *Fucus vesiculosus* and *Fucus serratus* were similar, uptake rates were only measured on
62 *Fucus serratus*.
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1 235 N I⁻¹ necessary for $\delta^{15}\text{N}$ analyses, and (2) NaCl to obtain a final salinity of 50. Note that only
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3 236 few samples were analyzed for $\delta^{15}\text{N-NH}_4^+$ because of low NH_4^+ concentrations[†]. Two-week
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6 237 incubations and addition of increasing MgO and Devarda's alloy quantities permitted to
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8 238 determine that 4 days and 0.2 mg of MgO and of Devarda's alloy were necessary for the total
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11 239 recovery NO_3^- and NH_4^+ in a shaking water bath at 33°C. Our method allowed the total
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13 240 recovery of NH_4^+ ($97 \pm 3 \%$; first step) and NO_3^- ($98 \pm 3 \%$; second step). All precautions were
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16 241 taken to avoid contaminations: glassware and plasticware were acid-washed, diffusion packets
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18 242 were prepared a few hours before the start of the experiments and stored in a desiccator,
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21 243 diffusion packets were removed from sample bottles after each step and stored in a desiccator
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23 244 over P_2O_5 and concentrated H_2SO_4 beakers to trap any trace of water and ammonia. Glass fiber
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25 245 disks used to trap NO_3^- and NH_4^+ were taken out from diffusion packets, placed in tin capsules
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28 246 and immediately analyzed to determine $\delta^{15}\text{N}$ and avoid any reaction between tin and acidified
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30 247 disks.

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33 248 Frozen macroalgae tissues were freeze-dried, ground using a ball mill and stored in a
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35 249 desiccator chamber before stable isotope analyses. Around 0.750 ± 0.001 mg DW of
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38 250 macroalgae powder were weighed in tin capsules for $\delta^{15}\text{N}$ measurements.

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40 251 For both glass fiber disk and macroalgae powder, N isotope analyses were carried out
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42 252 using an Isoprime IRMS (Micromass, UK) after sample combustion in an elemental analyzer
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45 253 Eurovector EA3024 (Eurovector, Milan, Italy). Results at natural abundance level were
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47 254 expressed in delta notation, using atmospheric nitrogen as a standard, according to the
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49
50 255 following equation:

$$51$$
$$52 \delta^{15}\text{N} \text{ ‰} = [(\text{R}_{\text{sample}}/\text{R}_{\text{standard}}) - 1] \times 1000$$
$$53$$

$$54 \text{ where } \text{R} = {}^{15}\text{N}/{}^{14}\text{N}$$
$$55$$
$$56$$

57 258 The standard deviation of triplicate measurements of $\delta^{15}\text{N}$ was lower than 0.2 ‰ for
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61 [†] A new method recently developed by Zhang et al. (2007), but absent when analyses were performed, allow to
62 measure reliable $\delta^{15}\text{N}$ values over an NH_4^+ concentration range of 0.5-10 μM .
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1 259 macroalgae tissues and lower than 0.3 ‰ for extracted ammonia. The low standard deviation
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3 260 allowed studying seasonal variations of $\delta^{15}\text{N-NO}_3^-$ without replicate analyses.
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6 261 7 8 262 **2.6. Statistics** 9

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13 264 All statistical tests were performed using R software (cran.r-project.org). Before each statistical
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15 265 analysis, Shapiro and Bartlett tests were performed to test dataset normality and
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17
18 266 homoscedasticity, respectively. One-way and two-way ANOVA tests were applied on datasets
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20 267 following normal distributions and showing homogeneous variances. When necessary, inverse
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23 268 or squared transformation of datasets was first performed to obtain normal distributions and
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25 269 homogeneous variances, or non-parametric Kruskal-Wallis test was applied. ANOVA tests
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27
28 270 were used to determine the difference in NO_3^- concentrations depending on DIN source type*
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30 271 (one-way ANOVA) and on DIN source type and station (two-way ANOVA). A one-way
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32
33 272 ANOVA was performed to investigate the station effect on $\delta^{15}\text{N-NO}_3^-$ of sources and on $\delta^{15}\text{N-}$
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35 273 NO_3^- of estuary and reference stations, respectively. A two-way ANOVA permitted to
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37
38 274 determine the effect of DIN source type and station on the $\delta^{15}\text{N-NO}_3^-$ dataset. Statistical tests
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40 275 were not possible on $\delta^{15}\text{N-NH}_4^+$ dataset because of the low number of values. The effect of
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42 276 site, time and species on macroalgae $\delta^{15}\text{N}$ was investigated by a three-way ANOVA, while a
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44
45 277 two-way ANOVA was used to study the effect of site and time on $\delta^{15}\text{N-macroalgae}$ for each
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47 278 species. Variations of $\delta^{15}\text{N}$ along macroalgae fronds were investigated with a Kruskal-Wallis
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50 279 test. Significant differences of growth rates were tested for the factors species and time (two-
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52 280 way ANOVA), and uptake rates for the factor NO_3^- concentration for each species (Kruskal-
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54
55 281 Wallis). Parametric Pearson or non-parametric Spearman tests were performed to identify
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57 282 significant correlations between (1) NO_3^- concentrations, $\delta^{15}\text{N-NO}_3^-$, $\delta^{15}\text{N-macroalgae}$ datasets
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59 283 and Charente River flow, (2) temporal $\delta^{15}\text{N-macroalgae}$ at ref₀, St. 3 and 4a for each species,
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62 * The factor type is either source, estuary or reference.
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1 284 and (3) $\delta^{15}\text{N}$ -macroalgae along the frond of *Fucus serratus* at ref_O and St. 4a. The two latter
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3 285 statistic results were not presented in figures or tables but only in the text. For all statistical
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6 286 tests, a probability (p) of 0.05 was used to determine statistical significance.
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3. RESULTS

3.1. Environmental data

During our study, high river flow occurred from mid-February to mid-April 2006, with a maximum of ca. $400 \text{ m}^3 \text{ s}^{-1}$ by the end of March (Fig. 2A). The river flow in 2006 was higher than 2004-2011 averaged flow, and even more than the river flow of the two previous years of dryness ($< 100 \text{ m}^3 \text{ s}^{-1}$; <http://www.fleuve-charente.net>). At the mouth of the estuary (St. 4), mean temperatures ranged from $5 \text{ }^\circ\text{C}$ in February to $15 \text{ }^\circ\text{C}$ in April and May while mean salinity strongly decreased from 28 in February to 13 in April and reached a maximum of 31 in May (Fig. 2B). The highest mean turbidity ($\sim 350 \text{ NTU}$) was observed in March and the lowest ($0\text{-}10 \text{ NTU}$) in February and May. At the oceanic reference site (ref_O), waters are generally characterized by similar and slightly less variable temperature ($7\text{-}14 \text{ }^\circ\text{C}$) and high salinity (32-35; PREVIMER, <http://www.previmer.org>), but lower turbidity ($< 10 \text{ NTU}$; Gohin, 2010) than at the mouth of the Charente Estuary, from January to June.

NH_4^+ concentrations were generally low and $< 5\%$ of DIN concentrations. NH_4^+ concentrations were $< 6 \text{ } \mu\text{mol l}^{-1}$ at Pasture, St. 1, St. 2, St. 4 and ref_O , except nine values comprised between 11 and $31 \text{ } \mu\text{mol l}^{-1}$ (Fig. 3A, B, C). Conversely, NH_4^+ concentrations reached $47 \text{ } \mu\text{mol l}^{-1}$ in February and $92 \text{ } \mu\text{mol l}^{-1}$ in March (25-50 % of DIN concentrations) at ref_R , and increased from $30 \text{ } \mu\text{mol l}^{-1}$ in January to more than $300 \text{ } \mu\text{mol l}^{-1}$ in May at STP.

NO_3^- concentrations were much higher than NH_4^+ concentrations. NO_3^- concentrations were significantly different between sources (one-way ANOVA, $F=53.4$, $p<0.0001$) and between all stations, *i.e.*, sources, estuary and reference stations (one-way ANOVA, $F=35.2$, $p<0.0001$). NO_3^- concentrations were significantly higher at Culture ($1200\text{-}1300 \text{ } \mu\text{mol l}^{-1}$, Fig. 3D), than at Pasture ($240\text{-}500 \text{ } \mu\text{mol l}^{-1}$, Tukey, $p<0.0001$) and STP ($80\text{-}650 \text{ } \mu\text{mol l}^{-1}$, Tukey, $p<0.0001$). In the Charente Estuary (Fig. 3E), NO_3^- concentrations at St. 4 ($50\text{-}120 \text{ } \mu\text{mol l}^{-1}$,

1 314 with a maximum of 310 $\mu\text{mol l}^{-1}$ in March) were significantly lower than at St. 1 and St. 2 (~
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3 315 400 $\mu\text{mol l}^{-1}$, Tukey, $p<0.001$) but not significantly different from those measured at riverine
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6 316 and oceanic reference sites (Tukey, $p=0.999$ and $p=0.935$). NO_3^- concentrations were close to
7
8 317 75-140 $\mu\text{mol l}^{-1}$ at ref_R and 3-20 $\mu\text{mol l}^{-1}$ (with a maximum of 110 $\mu\text{mol l}^{-1}$ in February) at ref_O
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11 318 (Fig. 3F). Temporal variations of NO_3^- concentrations of each source were generally low.

13 319 14 15 320 **3.2. $\delta^{15}\text{N}$ -DIN of anthropogenic sources**

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18 321
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20 322 The few $\delta^{15}\text{N-NH}_4^+$ values available were generally higher than $\delta^{15}\text{N-NO}_3^-$ for the three DIN
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23 323 sources (Fig. 3G). $\delta^{15}\text{N-NH}_4^+$ was +1.1 ‰ in January and +16.8 ‰ in May at Culture, +12.4 ‰
24
25 324 in January at Pasture, and varied from +18.8 to +31.2 ‰ from January to March at STP.

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27
28 325 $\delta^{15}\text{N-NO}_3^-$ values were significantly different depending on sources (one-way ANOVA,
29
30 326 $F=4.72$, $p<0.05$). $\delta^{15}\text{N-NO}_3^-$ at Culture (5.7-7 ‰) were significantly lower than at STP (10.1-
31
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33 327 31.8 ‰, Tukey, $p<0.05$) but not significantly different from Pasture (7.3-11.2 ‰, Tukey,
34
35 328 $p=0.755$). Temporal variation of $\delta^{15}\text{N-NO}_3^-$ of each source was generally low, excepted at STP
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37
38 329 where $\delta^{15}\text{N-NO}_3^-$ increased from 10.1 ‰ in February to 31.8 ‰ in May.

39 40 330 41 42 331 **3.3. $\delta^{15}\text{N}$ -DIN along the estuary**

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46
47 333 The few $\delta^{15}\text{N-NH}_4^+$ values available varied from 1.7-1.8 ‰ at St. 1 to 6.6 ‰ at St. 2 in March,
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50 334 and 2.5 ‰ at St. 4 in January (Fig. 3H). Even if only few data were available and prevented
51
52 335 statistical analyses, these estuarine $\delta^{15}\text{N-NH}_4^+$ values were similar or higher than those
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55 336 measured at reference sites (1.8 ‰ at ref_O and 0.3-1.2 ‰ at ref_R; Fig. 3I).

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57 337 $\delta^{15}\text{N-NO}_3^-$ were not significantly different along the Charente Estuary and at reference
58
59
60 338 stations (one-way ANOVA, $F=0.88$, $p=0.486$). The values measured along the estuary (6.4-7.8
61
62 339 ‰ at St. 1, 7.0-7.9 ‰ at St. 2, 7.2-9 ‰ at St. 4) were in the range of values measured at

1 340 riverine and oceanic reference sites (6.9-7.6 ‰ at ref_R and 6.8-7.9 ‰ at ref_O). $\delta^{15}\text{N-NO}_3^-$ of
2
3 341 each estuarine station were not significantly different from $\delta^{15}\text{N-NO}_3^-$ measured at Culture and
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6 342 Pasture, but significantly lower than at STP (Tukey, $p<0.05$).
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10 344 **3.4. $\delta^{15}\text{N}$ of annual and perennial macroalgae**

13 345 Significant differences of $\delta^{15}\text{N}$ were observed between species, time, site and combinations of
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15 346 these factors (Table 1, three-way ANOVA). The factors time and site explained > 50 % of the
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18 347 variability. Tukey post-hoc tests showed that each macroalgae species had significantly
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21 348 different $\delta^{15}\text{N}$ ($p<0.01$), excepted E and FV ($p=0.985$). For each species, $\delta^{15}\text{N}$ were
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23 349 significantly different depending on factors time and site, excepted FS (Table 2, two-way
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25 350 ANOVA). All $\delta^{15}\text{N}$ values were represented in Fig. 4.
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30 352 Whereas $\delta^{15}\text{N}$ values were not significantly different between St. 3 and St. 4a for *Ulva*
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33 353 *sp.* (10.1 +/- 1.2 ‰ versus 9.8 +/- 1.8 ‰; Tukey, $p=0.215$), *Enteromorpha sp.* (8.5 +/- 1.9 ‰
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35 354 versus 7.1 +/- 2.2 ‰; Tukey, $p=0.283$) and *Fucus vesiculosus* (9.5 +/- 1.0 ‰ versus 9.8 +/- 1.3
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37 355 ‰; Tukey, $p=0.994$), $\delta^{15}\text{N}$ of *Fucus serratus* were higher at St. 3 than at St. 4a (10.5 +/- 0.3 ‰
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40 356 versus 8.0 +/- 2.1 ‰; Tukey, $p<0.001$). $\delta^{15}\text{N}$ values were significantly lower at ref_O than at St.
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42 357 4a for *Ulva sp.* (6.2 +/- 1.8 ‰; Tukey, $p<0.0001$) and *Fucus serratus* (2.1 +/- 3.3 ‰; Tukey,
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44
45 358 $p<0.0001$), higher for *Enteromorpha sp.* (7.7 +/- 0.5 ‰; Tukey, $p<0.05$) and similar for *Fucus*
46
47 359 *vesiculosus* (6.4 +/- 2.3 ‰; Tukey, $p=0.128$).
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50 360 As similar trends were observed at St. 4a and St. 3 (see above), trends at ref_O were only
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52 361 compared to those of St. 4a. Similar trends were observed at ref_O compared to St. 4a, especially
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54
55 362 for *Ulva sp.* (Pearson, $\text{cor}=0.70$, $p<0.01$) and *Fucus serratus* (Pearson, $\text{cor}=0.58$, $p<0.05$).
56

57 363 $\delta^{15}\text{N}$ significantly decreased from January to April for *Ulva sp.*, were similar from
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59 364 January to April and higher in May for *Enteromorpha sp.*, were similar over the overall period
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62 365 for *Fucus vesiculosus*, and significantly increased from February to May for *Fucus serratus*.
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1 366 As no differences were observed between the two *Fucus* species and between St. 3 and
2
3 367 St. 4a, $\delta^{15}\text{N}$ along the frond were only analyzed for *Fucus serratus* at St. 4a and compared to
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5
6 368 ref_0 . As observed during the overall study (Fig. 4), $\delta^{15}\text{N}$ values of *Fucus serratus* apex were
7
8 369 significantly higher at St. 4a than at ref_0 (+7.1 ‰ versus -0.2 ‰; Tukey, $p < 0.01$) in March
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10 370 (Fig. 5). The difference observed at the apex disappeared quickly after 4 cm. $\delta^{15}\text{N}$ of *Fucus*
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13 371 *serratus* fronds were correlated (Spearman, $\text{cor} = 0.76$, $p < 0.01$) and not significantly different at
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16 372 St. 4a and ref_0 (Kruskal-Wallis, $K^2 = 0.936$, $p = 0.11$).

18 373 19 20 374 **3.5. Growth and uptake rates of annual versus perennial macroalgae**

21 375
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23 376 Growth rates were not significantly different between the two perennial species *Fucus*
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25 377 *vesiculosus* and *Fucus serratus* (Fig. 6; two-way ANOVA, $F = 0.103$, $p = 0.750$), but
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28 378 significantly different with time (two-way ANOVA, $F = 0.33$, $p < 0.001$). Tukey post-hoc tests
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31 379 showed lower growth rates in February than March and May ($p < 0.01$). Growth rates of *Fucus*
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33 380 *serratus* and *Fucus vesiculosus* increased from 1.8 +/- 1.3 and 1.2 +/- 0.9 cm month⁻¹ in
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35 381 February to 3.9 +/- 2.0 and 4.6 +/- 1.6 cm month⁻¹ in May.

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37 382 As growth rates were identical for both *Fucus* species (Fig. 6), uptake rates were only
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40 383 measured for *Fucus serratus*. Mean hourly uptake rates (at NO_3^- concentrations of 500 $\mu\text{mol l}^{-1}$
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42 384 and 50 $\mu\text{mol l}^{-1}$, respectively) were 6.5 +/- 0.2 and 3.9 +/- 1.0 $\mu\text{mol N g DW}^{-1} \text{ h}^{-1}$ for *Ulva sp.*,
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45 385 4.1 +/- 1.1 and 2.3 +/- 0.4 $\mu\text{mol N g DW}^{-1} \text{ h}^{-1}$ for *Enteromorpha sp.* and 1.6 +/- 0.4 and 0.5 +/-
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47 386 0.3 $\mu\text{mol N g DW}^{-1} \text{ h}^{-1}$ for *Fucus sp.* (Fig. 7). Uptake rates were significantly higher for annual
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50 387 species (*Ulva sp.* and *Enteromorpha sp.*) than for perennial species *Fucus serratus* (Kruskal-
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52 388 Wallis, $K^2 = 9.72$, $p < 0.01$). Uptake rates were significantly higher at NO_3^- concentrations of 500
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55 389 $\mu\text{mol l}^{-1}$ than 50 $\mu\text{mol l}^{-1}$ for *Enteromorpha sp.* (Kruskal-Wallis, $K^2 = 3.86$, $p < 0.05$), but no
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57 390 statistical tests were performed for *Ulva sp.* and *Fucus serratus* because only duplicates were
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60 391 available at 500 $\mu\text{mol l}^{-1}$ and 50 $\mu\text{mol l}^{-1}$, respectively.
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4. DISCUSSION

4.1. Characterization of DIN sources in a multiple source watershed

Studying the natural isotopic signature of DIN ($\delta^{15}\text{N-NO}_3^-$ and $\delta^{15}\text{N-NH}_4^+$, respectively) in streams flowing into the Charente Estuary proves successful in discriminating the three main DIN sources in a multiple source watershed (Table 3): Culture ($+6.5 \pm 0.6 \text{ ‰}$ and $+9 \pm 11 \text{ ‰}$), Pasture ($+9.2 \pm 1.8 \text{ ‰}$ and $+12.4 \text{ ‰}$) and STP ($+16.9 \pm 8.7 \text{ ‰}$ and $+25.4 \pm 5.9 \text{ ‰}$).

The lower $\delta^{15}\text{N-NO}_3^-$ associated to high NO_3^- concentrations at the cultivated site indicates the anthropogenic delivery of fertilizers. Synthetic fertilizers have usually low $\delta^{15}\text{N-NO}_3^-$ (-7.5 to $+6.6 \text{ ‰}$; Vitoria et al., 2004), as they are made by industrial fixation of atmospheric N_2 (Heaton, 1986; Lindau et al., 1989). In this study, $\delta^{15}\text{N-NO}_3^-$ measured at the cultivated site is higher than the median $\delta^{15}\text{N-NO}_3^-$ of synthetic fertilizers ($+1.8 \text{ ‰}$; Vitoria et al., 2004), and only slightly lower than at riverine and oceanic reference sites. This confirms that fields receiving synthetic fertilizers do not always show low $\delta^{15}\text{N-NO}_3^-$ (Vitoria et al., 2004) and that measurement must be performed at the local scale. The high $\delta^{15}\text{N-NO}_3^-$ at the cultivated site compared to the lower median value of fertilizers ($+1.8 \text{ ‰}$) might be explained by (1) synthetic fertilizers characterized by the upper range of $\delta^{15}\text{N-NO}_3^-$ values, possibly reaching $+6.6 \text{ ‰}$ (Vitoria et al., 2004), (2) inputs of generally $\delta^{15}\text{N}$ -enriched NO_3^- originated from manure ($> +5.9 \text{ ‰}$; Curt et al., 2004), and/or (3) inputs of N stored in lands during the two previous years of dryness and characterized by higher $\delta^{15}\text{N-NO}_3^-$ due to denitrification - which process increases $\delta^{15}\text{N-NO}_3^-$ values ($+15$ to $+30 \text{ ‰}$; Kendall, 1998).

While the cultivated site shows distinct $\delta^{15}\text{N-DIN}$ values compared to riverine and oceanic reference sites, $\delta^{15}\text{N-NO}_3^-$ measured at the pasture site is not significantly different from riverine and oceanic reference sites and from the cultivated site, which prevented the

1 418 distinction between pasture and ambient NO_3^- . $\delta^{15}\text{N}$ -DIN at the pasture site ($> +9\%$) is in the
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3 419 highly variable range of values found in manure (+5.9 to +36.7‰; Curt et al., 2004), generally
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6 420 ^{15}N -enriched by ammonia volatilization (Mantilla Morales, 1995). In addition to denitrification
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8 421 indicated by slightly higher $\delta^{15}\text{N}$ - NO_3^- compared to reference sites, the higher $\delta^{15}\text{N}$ - NH_4^+
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11 422 compared to $\delta^{15}\text{N}$ - NO_3^- at cultivated and pasture sites indicates that nitrification probably
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13 423 happens in these small streams as observed in the Scheldt Estuary (Middelburg and
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16 424 Nieuwenhuize, 2001). We assume however that denitrification and nitrification are limited by
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18 425 the low temperatures in winter, which explain the low differences between the cultivated,
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21 426 pasture and reference sites. Discrimination of the different sources may however be much
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23 427 higher in summer when bacterial processes are enhanced by higher temperatures.
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25 428 Contrary to cultivated and pasture areas, the high $\delta^{15}\text{N}$ - NO_3^- and $\delta^{15}\text{N}$ - NH_4^+ values
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28 429 observed at STP outlets agree with values commonly $> +10\%$ in used and sewage waters or in
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30 430 highly urbanized watersheds (Curt et al., 2004; McClelland and Valiela, 1998). Such high
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33 431 $\delta^{15}\text{N}$ -DIN are mostly explained by sewage treatments (*e.g.*, water aeration and bacterial
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35 432 enhancement), which enhance PON degradation and bacterial processes (*e.g.*, ammonification,
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38 433 nitrification, denitrification). Bacteria metabolize preferentially light isotopes ^{14}N and leave
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40 434 heavy isotopes ^{15}N in the environment (Cifuentes et al., 1989; Cline and Kaplan, 1975; Owens,
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42 435 1987), which leads to a ^{15}N -enrichment of DIN in the system (Kellman and Hillaire-Marcel,
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45 436 1998). The increase of $\delta^{15}\text{N}$ - NO_3^- during spring in the STP outlet results from increasing
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47 437 temperature and decreasing river flow conditions, which enhances bacterial activity and water
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50 438 residence time, and decreases sludge dilution. The concurrent increase of $\delta^{15}\text{N}$ - NO_3^- with
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52 439 temperature and the decrease of river discharge, precipitation and soil leaching in summer
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55 440 suggest that the contribution of STP outlets to estuarine waters may be more significant in
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57 441 summer in temperate ecosystems, and show even more visible $\delta^{15}\text{N}$ -DIN increase.
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59 442 The main anthropogenic inputs of DIN to the Charente Estuary, *i.e.*, fertilizers
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62 443 (Culture), animal manure (Pasture) and sewage treatment plant outlets (STP), are mainly
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1 444 constituted of NO_3^- (> 95 %). Additionally to the high quantitative NO_3^- delivery associated to
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3 445 human activities, NO_3^- is also the dominant DIN form because of its higher turnover time
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6 446 compared to NH_4^+ and NO_2^- (Middelburg and Nieuwenhuize, 2000). In the Charente Estuary,
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8 447 such as in any system receiving high riverine DIN fluxes (high flow and DIN concentrations),
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11 448 the atmospheric contribution to DIN - whose $\delta^{15}\text{N}\text{-NO}_3^-$ and $\delta^{15}\text{N}\text{-NH}_4^+$ generally vary in a
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13 449 wide range of -15 to +15 ‰ (Heaton, 1986; Kendall, 1998; Paerl and Fogel, 1994) - is
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15
16 450 negligible. The main NO_3^- sources to the Charente River and Estuary originate from the use of
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18 451 fertilizers on agricultural fields that cover ~ 79 % of the watershed area. The strong
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21 452 contribution of fertilizers is highlighted by high NO_3^- concentrations in small streams of
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23 453 cultivated sub-watersheds (1200-1300 $\mu\text{mol l}^{-1}$, this study), and along the Charente River and
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25 454 at the mouth of the estuary (50-500 $\mu\text{mol l}^{-1}$, this study).
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30 456 **4.2. Mixing of DIN sources in a well-mixed estuary**

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33 458 As the Charente watershed is dominated by agriculture activities (*e.g.*, fertilizer use) and as we
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35 459 found that $\delta^{15}\text{N}\text{-NO}_3^-$ was lower at the cultivated site compared to the riverine and oceanic
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38 460 reference sites, we expected that estuarine $\delta^{15}\text{N}\text{-NO}_3^-$ decreased in response to enhanced
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40 461 synthetic fertilizer use or soil leaching during high precipitation and river discharge. The
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42 462 significant correlations between NO_3^- concentrations and the river flow at the upper and mid
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45 463 estuary (St. 1 and 2; Fig. 8A) confirm the increase of NO_3^- concentrations due to soil leaching
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47 464 under high precipitation regime. The lack of correlation at the mouth of the estuary (St. 4; Fig.
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50 465 8A) emphasizes however the dilution and mixing of NO_3^- -enriched riverine waters with
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52 466 oceanic waters, which phenomenon is commonly observed downward of estuaries (Fry, 2002).
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55 467 Correlations between NO_3^- concentrations (indicated by macroalgae N content) and $\delta^{15}\text{N}\text{-NO}_3^-$
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57 468 have already been observed in agriculture watersheds dominated by ^{15}N -depleted fertilizers or
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60 469 by ^{15}N -enriched sewage (Costanzo et al., 2003). In our study, the absence of significant
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1 470 correlation between NO_3^- concentrations and $\delta^{15}\text{N-NO}_3^-$ along the estuary (St. 1, 2 and 4; Fig.
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3 471 8B) highlights that increases of river flow and NO_3^- concentrations are not always associated
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6 472 with a decrease of $\delta^{15}\text{N-NO}_3^-$, which has already been linked to the complexity and
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8 473 fragmentation of land uses in the drainage basin of the Mississippi River (Chang et al., 2002).
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11 474 The absence of seasonal variations of $\delta^{15}\text{N-NO}_3^-$ in the Charente Estuary highlights that
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13 475 temporal variations are not always observed in a well-mixed system submitted to multiple DIN
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15 476 sources. This might be explained by (1) the absence of drastic $\delta^{15}\text{N-NO}_3^-$ differences between
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18 477 the dominant NO_3^- source (*e.g.* fertilizers) and reference sites, and (2) a strong signature of
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21 478 residual terrestrial DIN stored during the previous years of dryness.

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23 479 The temporal variability of $\delta^{15}\text{N-DIN}$ at shorter time scales (Cifuentes et al., 1989;
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25 480 Horrigan et al., 1990) may have been low in our study because of large NO_3^- stocks and low
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27
28 481 NO_3^- turnover compared to NH_4^+ . We compared then monthly $\delta^{15}\text{N-DIN}$ and $\delta^{15}\text{N}$ of biological
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30 482 DIN integrators - *i.e.*, macroalgae - at the mouth of the Charente Estuary and at an oceanic site.
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33 483 34 35 484 **4.3. Macroalgae, efficient integrator of $\delta^{15}\text{N-DIN}$ in a well-mixed estuary?**

36 37 485 38 39 40 486 4.3.1. Signature of anthropogenic DIN loads from the estuary

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42 487 If we only consider $\delta^{15}\text{N}$ of macroalgae but not $\delta^{15}\text{N-DIN}$, the generally higher $\delta^{15}\text{N}$ at the
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45 488 mouth of the Charente Estuary compared to the oceanic site in the tissues of all macroalgae
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47 489 species (excepted *Enteromorpha sp.*, Fig. 4) could be first attributed to higher anthropogenic
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50 490 DIN inputs flowing from the drainage basin. The $\delta^{15}\text{N}$ enrichment in macroalgae tissues have
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52 491 often been observed along urbanized estuaries receiving enriched $\delta^{15}\text{N-NO}_3^-$ (Cole et al., 2004,
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55 492 2005; Costanzo et al., 2005; Fertig et al., 2009). In such human-impacted ecosystems,
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57 493 macroalgae are broadly used to trace spatio-temporal distribution of anthropogenic inputs
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60 494 (Costanzo et al., 2001), such as marine dilution or improvement of sewage treatment plant
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62 495 efficiency (Costanzo et al., 2005). The absence of $\delta^{15}\text{N}$ differences between macroalgae
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1 496 collected at the two stations of the Charente Estuary mouth highlights that both stations receive
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3 497 similar $\delta^{15}\text{N}$ -DIN inputs. For *Fucus serratus*, the lower $\delta^{15}\text{N}$ at the outer than at the inner
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6 498 station of the estuary mouth however suggests a small decreasing influence of anthropogenic
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8 499 DIN sources from the Charente Estuary to the ocean due to dilution, as already observed for
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10
11 500 benthic filter feeders in this ecosystem (Fry, 2002). Nevertheless, these arguments are not
12
13 501 enough to explain ^{15}N -enriched macroalgae tissues at the estuary mouth compared to the
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15
16 502 oceanic site, as similar $\delta^{15}\text{N}$ of NO_3^- - the dominant DIN form - is observed at both sites.
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18 503 Similar absence of evident relationship between $\delta^{15}\text{N}$ of estuarine primary producers and
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21 504 riverine nutrient loads has already been highlighted in Australian estuarine lagoons (Scanes et
22
23 505 al., 2007). Our results highlight thus that studying $\delta^{15}\text{N}$ -DIN is essential to confirm the
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25 506 observed trends of macroalgae $\delta^{15}\text{N}$. The physiological response of annual and perennial
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27
28 507 macroalgae to environmental conditions (*e.g.*, other N forms, abiotic parameters) is thus
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30 508 discussed below.

31 32 33 509 34 35 510 4.3.2. Physiological response of macroalgae to environmental conditions

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37 511 The lower $\delta^{15}\text{N}$ of macroalgae (excepted *Enteromorpha sp.*) at the oceanic site is expected to
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40 512 reflect the lower $\delta^{15}\text{N}$ -DIN generally observed in marine waters (+4 to +7‰; Altabet et al.,
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42 513 1999; Minagawa and Wada, 1986; Owens, 1987). In this study, $\delta^{15}\text{N}$ - NO_3^- is however similar
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45 514 at the oceanic and estuarine sites and cannot explain these differences. On the contrary, $\delta^{15}\text{N}$ -
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47 515 NH_4^+ is lower at the oceanic site (+1.8 ‰) and might contribute to the lower $\delta^{15}\text{N}$ of
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49
50 516 macroalgae. NH_4^+ can indeed constitute a significant source of DIN as (1) NH_4^+ is
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52 517 preferentially assimilated by macroalgae compared to NO_3^- (Cohen and Fong, 2004), and (2)
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54 518 the absence of visible variations of NH_4^+ concentrations can be hidden by balanced sources and
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57 519 sinks of NH_4^+ (Middelburg and Nieuwenhuize, 2001; Sebilo et al., 2006). The uptake of NH_4^+
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60 520 is even expected to be higher at oceanic sites where NO_3^- and NH_4^+ concentrations are
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62 521 respectively lower and slightly higher than at the estuarine mouth (this study).
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1 522 At the mouth of the Charente Estuary, the higher $\delta^{15}\text{N}$ of macroalgae compared to the
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3 523 oceanic reference site rather reflects $\delta^{15}\text{N}\text{-NO}_3^-$ and not $\delta^{15}\text{N}\text{-NH}_4^+$. NH_4^+ concentrations were
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6 524 always low at the estuary mouth (1-7 % DIN) and its contribution to macroalgae uptake might
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8 525 have been insignificant compared to NO_3^- concentrations (50-350 $\mu\text{mol l}^{-1}$). As for the oceanic
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11 526 site, the low concentrations and small temporal variations of NH_4^+ can have hidden significant
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13 527 NH_4^+ uptake due to a balance between NH_4^+ uptake and production, as already observed in the
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16 528 Loire and Seine estuaries (Middelburg and Nieuwenhuize, 2001; Sebilo et al., 2006). The
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18 529 absence of spatial variations of $\delta^{15}\text{N}\text{-NO}_3^-$ along the estuary suggests however that
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21 530 mineralization and nitrification processes might have been low along the estuary, reinforcing
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23 531 the low uptake of NH_4^+ by macroalgae.

25 532 Two other processes can increase $\delta^{15}\text{N}$ values in macroalgae tissues. The bacterial
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28 533 processes at the surface of macroalgae frond, *e.g.*, organic N mineralization and N fixation
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30 534 (Goecke et al., 2010), might increase $\delta^{15}\text{N}\text{-DIN}$ at the vicinity of the frond. Benthic NH_4^+
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33 535 fluxes - which might be ^{15}N -enriched due to sedimentary nitrification (Brandes and Devol,
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35 536 1997) - are also hypothesized to explain the higher $\delta^{15}\text{N}$ in macroalgae compared to $\delta^{15}\text{N}\text{-DIN}$,
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38 537 as extended intertidal mudflats are present at the mouth of the estuary (Fig. 1). Both bacterial
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40 538 processes on the frond and benthic NH_4^+ fluxes are however expected to be low in this study
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43 539 because of the low winter temperatures (Feuillet-Girard et al., 1997). As explained above, the
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45 540 absence of variations along the estuary is rather explained by the intense and rapid mixing of
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48 541 riverine and marine waters by tidal currents and waves in the Charente Estuary, which was also
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50 542 highlighted for POM in this estuary (Modéran et al., 2012). The role of benthic fluxes and
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52 543 bacterial processes might significantly modify macroalgae $\delta^{15}\text{N}$ at higher summer
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55 544 temperatures, especially in shallow and productive ecosystems characterized by high residence
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57 545 time.

59 546 Another factor potentially controlling $\delta^{15}\text{N}$ values in macroalgae tissues is the possible
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62 547 uptake of dissolved organic N (DON) by macroalgae. Macroalgae uptake of urea and amino
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1 548 acid was recently emphasized by Tyler et al. (2005). Van Engeland et al. (2011) even showed
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3 549 that DON uptake could be equivalent to DIN uptake for macrophytes. Even if urea is
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6 550 increasingly spread on the Charente watershed, concentrations in urea and amino acids were
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8 551 still low in estuarine waters in 2002-2005 ($<8 \mu\text{mol l}^{-1}$ and $<4 \mu\text{mol l}^{-1}$, respectively;
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10 552 Bechemin, 2008). These low DON concentrations compared to NO_3^- concentrations emphasize
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13 553 the negligible contribution of DON to macroalgae $\delta^{15}\text{N}$ values. In spite of low concentrations,
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16 554 the increasing trend in the Charente Estuary suggests that this source of N could become
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18 555 significant in the next years and should be monitored. DON is rarely measured in estuarine
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21 556 studies, as it is quickly mineralized during its transport along estuaries, and as DIN is often the
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23 557 dominant N form in highly fertilized watersheds. Quantitative DON loads can however account
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25 558 for 20 to 90 % of estuarine N loads (Seitzinger and Sanders, 1997). The role of DON on
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28 559 macroalgae growth should be more investigated, especially in estuaries receiving urea-
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30 560 fertilized waters. The few available values of estuarine $\delta^{15}\text{N}$ -DON generally range between +3
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33 561 and +10.8 ‰ (review in Guo et al., 2003). Further quantifications of spatio-temporal variations
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35 562 of $\delta^{15}\text{N}$ -DON, in addition to DON concentrations, would help in estimating the role of DON in
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38 563 modifying macroalgae $\delta^{15}\text{N}$ values, and especially its temporal variations in estuarine and
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40 564 coastal ecosystems.

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42 565 The same monthly trends of macroalgae $\delta^{15}\text{N}$ at estuarine and oceanic sites suggests
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44
45 566 either (1) anthropogenic NO_3^- inputs to the oceanic site, (2) similar temporal variations of $\delta^{15}\text{N}$ -
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47 567 NO_3^- in estuarine and oceanic waters, or (3) physiological processes. The input of
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49
50 568 anthropogenic NO_3^- to the oceanic site is discarded due to water currents in the Marennes-
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52 569 Oléron Bay (Stanisière et al., 2006), and the temporal variations of estuarine and oceanic $\delta^{15}\text{N}$ -
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55 570 NO_3^- are dismissed due to constant monthly $\delta^{15}\text{N}$ - NO_3^- at both sites (this study). The
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57 571 physiological response of macroalgae to local and regional changes of environmental
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60 572 parameters, *e.g.*, temperature and light, might more reliably explain the similar seasonal
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62 573 variations of macroalgae $\delta^{15}\text{N}$ at both estuarine and oceanic sites. Although fractionation
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1 574 studies are numerous on phytoplankton species (*e.g.* Pennock et al., 1996; Needoba and
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3 575 Harrison, 2004), much fewer exist on macroalgae (Cohen and Fong, 2004; Dudley et al.,
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6 576 2010). It is reliable from these studies that variations of environmental parameters could
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8 577 modify algae uptake and growth rates, which might influence fractionation. Temperature and
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11 578 light similarly increased from winter to summer at both sites, and especially after March,
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13 579 leading to the enhancement of macroalgae growth rates (this study). The enhancement of
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15 580 macroalgae metabolism might have increased fractionation, as already observed for plants
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18 581 (McKee et al., 2002) and macroalgae (Dudley et al., 2010). This might have led to a lower
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20 582 selection of light isotopes, explaining the increase of $\delta^{15}\text{N}$ values from April (for annual
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23 583 species) or March (for perennial species) to May (this study). Seasonal variations of
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25 584 metabolism have also been reported to lead to higher temporal variations of $\delta^{15}\text{N}$ values in
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28 585 consumers than diet changes (*i.e.* pectinidae, Lorrain et al., 2002). As reported for consumers,
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30 586 our study suggests that the physiological response of primary producers (*i.e.* macroalgae) to
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33 587 changing environmental conditions might alter macroalgae fractionation and, thus, $\delta^{15}\text{N}$ values.
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35 588 More studies on macroalgae are however needed to confirm the hypothesis that increases of
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38 589 light and temperature as well as nutrient concentrations could enhance macroalgae metabolism
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40 590 and led to increasing $\delta^{15}\text{N}$ in both estuarine and oceanic waters.

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43 44 45 592 4.3.3. Annual *versus* perennial species

46
47 593 The decrease of $\delta^{15}\text{N}$ values from January to April followed by an increase in May in
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50 594 annual species is not observed in perennial species, which might be explained by differences in
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52 595 growth and uptake rates and/or uptake mechanisms between annual and perennial species.
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55 596 These variations of macroalgae $\delta^{15}\text{N}$ values might have resulted from the faster metabolic
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57 597 response of annual species to environmental changes compared to perennial species. The
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60 598 annual and r-selected species *Ulva sp.* and *Enteromorpha sp.* (Raven and Taylor, 2002) are
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62 599 indeed characterized by high N demand and uptake rates (Raven and Taylor, 2003; Pedersen
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1 600 and Borum, 1997; Teichberg et al., 2008). The high uptake rates - even faster than those of
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3 601 perennial species (this study) - often lead to higher productivity compared to perennial *Fucus*
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5
6 602 *sp.* (North America shores; Littler, 1980). Transplantation experiments showed that this rapid
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8 603 turnover of *Ulva sp.* tissues explains that $\delta^{15}\text{N}$ values quickly reflects external DIN loads and
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10
11 604 not the internal initial content in *Ulva sp.* (Aguiar et al., 2003; Teichberg et al., 2008). The $\delta^{15}\text{N}$
12
13 605 values in annual species tissues consequently integrate shorter time periods and more transient
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15 606 $\delta^{15}\text{N}$ -DIN variations than perennial species (Aguiar et al., 2003; Teichberg et al., 2008).
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17
18 607 Finally, the low fractionation during DIN uptake in annual species can be related to the
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20 608 dominance of diffusive DIN transport, which process has already been shown to prevail in
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23 609 *Ulva sp.*, in particular in DIN-enriched environments (Taylor et al., 1998).
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25 610 The two perennial macroalgae *Fucus serratus* and *Fucus vesiculosus* growing at the
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27 611 mouth of the Charente Estuary do not show large monthly variations of $\delta^{15}\text{N}$. These results
28
29 612 agree with the constant $\delta^{15}\text{N}$ - NO_3^- in estuarine waters (this study) and confirm that monthly
30
31 613 measurements of $\delta^{15}\text{N}$ - NO_3^- are representative of monthly trends in systems submitted to low
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33 614 $\delta^{15}\text{N}$ - NO_3^- variations. Even not significant, the slightly positive correlation between $\delta^{15}\text{N}$ of
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35 615 *Fucus* species and $\delta^{15}\text{N}$ - NO_3^- in estuarine waters (Fig. 8C) suggested that perennial macroalgae
36
37 616 tissues have integrated the slight increase in estuarine $\delta^{15}\text{N}$ - NO_3^- . The similar $\delta^{15}\text{N}$ of *Fucus*
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39 617 *serratus* and *Fucus vesiculosus* is consistent with the similar *in situ* growth rates of both
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41 618 species (2-4 cm month⁻¹, this study). These growth rates are in the range of the maximal
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43 619 growth rates of *Fucus serratus* measured in the Great Bay Estuary in northeastern America
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45 620 (2.5-3.6 cm month⁻¹; Mathieson et al., 1976), and confirm that these two species integrate the
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47 621 same time period. Sampling of the first 2 cm (this study) integrate thus a time period ranging
48
49 622 from 2 weeks (in March and May) to 4 weeks (in February and April). Even if this study
50
51 623 confirms that perennial species integrate longer temporal changes in DIN loads, due to the
52
53 624 slower uptake and tissue turnover compared to annual macroalgae, the difference in uptake
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55 625 rates might also impact fractionation which potentially explains the differences in seasonal
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1 626 changes of $\delta^{15}\text{N}$ in annual *versus* perennial macroalgae.

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3 627 As passive and active transport mechanisms might have an impact on macroalgae
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5
6 628 fractionation (Taylor et al., 1998; Dudley et al., 2010), we also expect the role of uptake
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8 629 mechanisms, as well as the loss of ^{14}N subsequent to uptake (Dudley et al., 2010), to explain
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11 630 $\delta^{15}\text{N}$ enrichments that are not linked to increases in $\delta^{15}\text{N}$ -DIN. Further work is however
12
13 631 required to elucidate the role of metabolic processes in controlling macroalgae $\delta^{15}\text{N}$ values, and
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15
16 632 especially to quantify the net fractionation in macroalgae, its control by environmental
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18 633 parameters, and the relative contribution of transport mechanisms in macroalgae $\delta^{15}\text{N}$ values.
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20 634 21 22 23 635 4.3.4. Perennial macroalgae, retroactive indicators of DIN inputs?

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25 636 As growing apex of perennial macroalgae could integrate weekly-monthly changes of $\delta^{15}\text{N}$ -
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27
28 637 DIN, we have investigated the integration of annual and pluri-annual $\delta^{15}\text{N}$ -DIN variations
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30 638 along macroalgae fronds at estuarine and oceanic sites. In this study, $\delta^{15}\text{N}$ values increases
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32
33 639 from the apex to the basis of *Fucus serratus* at both sites (Fig. 5). This trend has already been
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35 640 observed in *Fucus vesiculosus* and attributed to a decrease of sewage loads (Savage and
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38 641 Elmgren, 2004). In the Charente Estuary, anthropogenic DIN inputs, mostly due to fertilizers,
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40 642 has increased over the last 25 years (<http://www.eau-adour-garonne.fr>) and might not have
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42 643 drastically changed over the previous years, while at the oceanic site, the low DIN inputs are
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44
45 644 little influenced by anthropogenic activities and characterized by low DIN concentrations (Fig.
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47 645 3C, F). At both sites, the role of drastic $\delta^{15}\text{N}$ -DIN changes over the past years might probably
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49
50 646 not explain the observed increase of $\delta^{15}\text{N}$ along the frond. Additionally, $\delta^{15}\text{N}$ differences
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52 647 between *Fucus serratus* fronds at estuarine and oceanic sites quickly disappear after the 4th cm
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54
55 648 from the apex (corresponding to 1 to 2 months from measured *in situ* growth rates). The
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57 649 similarity of $\delta^{15}\text{N}$ values in the oldest parts of the frond was not expected because
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60 650 environmental conditions (*e.g.*, DIN concentrations, turbidity and salinity) are usually different
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62 651 at these sites, and different $\delta^{15}\text{N}$ values are observed in macroalgae apex over the sampled
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1 652 period (Fig. 4). The variations of $\delta^{15}\text{N}$ values along the frond might more likely be due to
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3 653 physiological processes, *e.g.*, growth rate variations, as suggested above for seasonal variations
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6 654 of $\delta^{15}\text{N}$ at the apex. The similar $\delta^{15}\text{N}$ values along the frond (except the apex) at both sites are
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8 655 rather explained by the biochemical processes involved in the formation of the frond than by
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11 656 variations of DIN inputs, *e.g.* the loss of ^{14}N subsequent to uptake (Dudley et al., 2010). The
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13 657 biochemical processes and the growth rate of *Fucus* fronds may be more important than $\delta^{15}\text{N}$ -
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15
16 658 DIN in determining $\delta^{15}\text{N}$ of perennial macroalgae tissues (Deutsch and Voss, 2006). From our
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18 659 results, the use of macroalgae as indicators of N input variations over a long time should be
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21 660 completed by studies of the variability of macroalgae fractionation in response to
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23 661 environmental parameters and growth rates, and by comparison with reference sites.
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1 662 **5. CONCLUSIONS**

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6 664 This study in the Charente watershed confirms that the main DIN sources (*i.e.*, cultivated area,
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8 665 pasture, STP outlet) are characterized by distinct $\delta^{15}\text{N}$ values. Although the main sources of
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10
11 666 anthropogenic DIN, mostly NO_3^- , have distinct $\delta^{15}\text{N}$, their mixture lead to estuarine $\delta^{15}\text{N-NO}_3^-$
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13 667 close to riverine and oceanic reference sites. The high NO_3^- concentrations measured in streams
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16 668 of cultivated areas associated to relatively high $\delta^{15}\text{N}$ values compared to synthetic fertilizers
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18 669 suggest that heavy rains do not only flush out freshly spread fertilizers, but also DIN formed by
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21 670 the degradation of organic matter stored during the previous dry years. The integration of $\delta^{15}\text{N-}$
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23 671 DIN in macroalgae tissues leads to generally higher $\delta^{15}\text{N}$ in macroalgae growing at the mouth
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25 672 of the estuary than at the oceanic site. Our results suggest that, even if macroalgae (partially or
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27
28 673 totally) integrate $\delta^{15}\text{N-DIN}$ over time, the metabolic response of macroalgae to environmental
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30 674 parameters (*e.g.*, turbidity, temperature and DIN concentrations) might strongly modify
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33 675 macroalgae $\delta^{15}\text{N}$ values. A better understanding of the control of environmental parameters on
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35 676 macroalgae fractionation is thus needed, which have strong implications for the use of
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38 677 macroalgae $\delta^{15}\text{N}$ to monitor anthropogenic loads. Coupling these results with quantitative and
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40 678 qualitative database of nutrient runoffs for each landuse would help to move from a
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42 679 bioindicative to a more quantitative and predictive tool.
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1 686 **LITERATURE CITED**

- 2
3 687
4
5
6 688 Aguiar, A.B., Morgan, J.A., Teichberg, M., Fox, S., Valiela, I., 2003. Transplantation and
7
8 689 isotopic evidence of the relative effects of ambient and internal nutrient supply on the
9
10
11 690 growth of *Ulva lactuca*. *Biological Bulletin* 205, 250-251.
12
13 691 Altabet, M.A., Pilskaln, C., Thunell, R., Pride, C., Sigman, D., Chavez, F., Francois, R., 1999.
14
15 692 The nitrogen isotope biogeochemistry of sinking particles from the margin of the Eastern
16
17
18 693 North Pacific. *Deep-Sea Research Part I: Oceanographic Research Papers* 46, 655–679.
19
20 694 Anderson, C., Cabana, G., 2005. $\delta^{15}\text{N}$ in riverine food webs: effects of N inputs from
21
22
23 695 agricultural watersheds. *Canadian Journal of Fisheries and Aquatic Sciences* 62, 333-340.
24
25 696 Bechemin, C., 2008. Chimiodiversité des substances dissoutes et biodiversité
26
27 697 phytoplanctonique dans le bassin de Marennes Oléron. Bilan d'action CPER/DDPC 2008,
28
29
30 698 Ifremer, 19 pp.
31
32 699 Brandes, J.A., Devol, A.H., 1997. Isotopic fractionation of oxygen and nitrogen in coastal
33
34
35 700 marine sediments. *Geochimica et Cosmochimica Acta* 61, 1793-1801.
36
37 701 Bry, C., Hoflack, P., 2004. Le bassin versant de la Charente : une illustration des problèmes
38
39
40 702 posés par la gestion quantitative de l'eau. *Courrier de l'environnement de l'INRA* 52, 81-96.
41
42 703 Cifuentes, L.A., Fogel, M.L., Pennock, J.R., Sharp, J.H., 1989. Biogeochemical factors that
43
44
45 704 influence the stable nitrogen isotope ratio of dissolved ammonium in the Delaware Estuary.
46
47 705 *Geochimica et Cosmochimica Acta* 53, 2713-2721.
48
49 706 Chang, C.C.Y., Kendall, C., Silva, S.R., Battaglin, W.A., Campbell, D.H., 2002. Nitrate stable
50
51
52 707 isotope: tools for determining nitrate sources among different land uses in the Mississippi
53
54
55 708 River Basin. *Canadian Journal of Fishery and Aquatic Science* 59, 1874-1885.
56
57 709 Cline, J.D., Kaplan, I.R., 1975. Isotopic fractionation of dissolved nitrate during denitrification
58
59
60 710 in the eastern tropical north Pacific ocean. *Marine Chemistry* 3, 271-299.
61
62 711 Cloern, J.E., 2001. Our evolving conceptual model the coastal eutrophication problem. *Marine*
63
64
65

- 1 712 Ecological Progress Series 210, 223-253.
2
- 3 713 Cohen, R.A., Fong, P., 2004. Nitrogen uptake and assimilation in *Enteromorpha intestinalis*
4
5
6 714 (L.) Link (Chlorophyta): using ^{15}N to determine preference during simultaneous pulses of
7
8 715 nitrate and ammonium. *Journal of Experimental Marine Biology and Ecology* 309, 67-77.
9
- 10 716 Cohen, R.A., Fong, P., 2005. Experimental evidence supports the use of delta ^{15}N content of
11
12
13 717 the opportunistic green macroalga *Enteromorpha intestinalis* (Chlorophyta) to determine
14
15
16 718 nitrogen sources to estuaries. *Phycological Society of America* 41, 287-293.
17
- 18 719 Cole, M.L., Valiela, I., Kroeger, K.D., Tomasky, G.L., Cebrian, J., Wigand, C., McKinney,
19
20
21 720 R.A., Grady, S.P., Carvalho da Silva, M.H., 2004. Assessment of a $\delta^{15}\text{N}$ isotopic method to
22
23 721 indicate anthropogenic eutrophication in aquatic ecosystems. *Journal of Environmental*
24
25 722 *Quality* 33, 124-132.
26
27
- 28 723 Cole, M.L., Kroeger, K.D., McClelland, J.W., Valiela, I., 2005. Macrophytes as indicators of
29
30 724 land-derived wastewater: application of a $\delta^{15}\text{N}$ method in aquatic systems. *Water*
31
32
33 725 *Ressources Res* 41, W01014, doi:10.1029/2004WR003269.
34
- 35 726 Conley, D.J., Paerl, H.W., Howarth, R.W., Boesch, D.F., Seitzinger, S.P., Havens, K.E.,
36
37
38 727 Lancelot, C., Likens, G.E., 2009. Controlling Eutrophication: Nitrogen and Phosphorus.
39
40 728 *Science* 323, 1014-1015.
41
- 42 729 Costanzo, S.D., O'Donohue, M.J., Dennison, W.C., Loneragan, N.R., Thomas, M., 2001. A
43
44
45 730 new approach for detecting and mapping sewage impacts. *Marine Pollution Bulletin* 42,
46
47 731 149-156.
48
- 49 732 Costanzo, S.D., O'Donohue, M.J., Dennison, W.C., 2003. Assessing the seasonal influence of
50
51
52 733 sewage and agricultural nutrient inputs in a subtropical river estuary. *Estuaries* 26, 857-865.
53
- 54 734 Costanzo, S.D., Udy, J., Longstaff, B., Adrian, J., 2005. Using nitrogen stable isotope ratios
55
56
57 735 ($\delta^{15}\text{N}$) of macroalgae to determine the effectiveness of sewage upgrades: changes in
58
59
60 736 the extent of sewage plumes over four years in Moreton Bay, Australia. *Marine Pollution*
61
62 737 *Bulletin* 51, 212-217.
63
64
65

- 1 738 Curt, M.D., Aguado, P., Sanchez, G., Bigeriedo, M., Fernandez, J., 2004. Nitrogen isotope
2
3 739 ratios of synthetic and organic sources of nitrate water contamination in Spain. *Water, Air,*
4
5
6 740 & *Soil Pollution* 151, 135-142.
7
- 8 741 Dechambenoy, C.L., Pontier, F., Sirou, F., Vouvé, J., 1977. Apport de la thermographie
9
10 742 infrarouge aéroportée à la connaissance de la dynamique superficielle des estuaires (système
11
12
13 743 Charente-Seudre-Anse de l'Aiguillon). *Comptes Rendus de l'Académie des Sciences, Paris*
14
15 744 284, 1269–1272.
16
- 17
18 745 Deutsch, B., Voss, M., 2006. Anthropogenic nitrogen input traced by means of $\delta^{15}\text{N}$ values in
19
20 746 macroalgae: Results from in-situ incubation experiments. *Science of the Total Environment*
21
22 747 366, 799-808.
23
- 24
25 748 Dudley, B.D., Barr, N.G., Shima, J.S., 2010. Influence of light intensity and nutrient source on
26
27 749 $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ signatures in *Ulva pertusa*. *Aquatic Biology* 9, 85-93.
28
29
- 30 750 Fertig, B., Carruthers, T.J.B., Dennison, W.C., Jones, A.B., Pantus, F., Longstaff, B., 2009.
31
32 751 Oyster and macroalgae bioindicators detect elevated $\delta^{15}\text{N}$ in Maryland's coastal bays.
33
34 752 *Estuaries and Coasts* 32, 773-786.
35
36
- 37 753 Fertig, B., Carruthers, T.J.B., Dennison, W.C., Fertig, E.J., Altabet, M.A., 2010. Eastern oyster
38
39 754 (*Crassostrea virginica*) $\delta^{15}\text{N}$ as a bioindicator of nitrogen sources: Observations and
40
41 755 modelling. *Marine Pollution Bulletin* 60, 1288-1298.
42
43
- 44 756 Feuillet-Girard, M., Gouleau, D., Blanchard, G., Joassard, L., 1997. Nutrient fluxes on an
45
46 757 intertidal mudflat in Marennes-Oléron Bay, and influence of the emersion period. *Aquatic*
47
48 758 *Living Resources* 10, 49-58.
49
50
- 51
52 759 Fry, B., 2002. Conservative mixing of stable isotopes across estuarine salinity gradients: A
53
54 760 conceptual framework for monitoring watershed influences on downstream fisheries
55
56 761 production. *Estuaries* 25, 264-271.
57
58
- 59 762 Fujita, R.M., 1985. The role of nitrogen status in regulating transient ammonium uptake and
60
61 763 nitrogen storage by macroalgae. *Journal of Experimental Marine Biology and Ecology* 92,
62
63
64
65

- 1 764 283-301.
2
- 3 765 García-Sanz, T., Ruiz, J.M., Pérez, M., Ruiz, M., 2011. Assessment of dissolved nutrients
4
5
6 766 dispersal derived from offshore fish-farm using nitrogen stable isotope ratios ($\delta^{15}\text{N}$) in
7
8 767 macroalgal bioassays. *Estuarine, Coastal and Shelf Science* 91, 361-370.
9
- 10
11 768 Gartner, A., Lavery, P., Smith, A.J., 2002. Use of delta ^{15}N signatures of different functional
12
13 769 forms of macroalgae and filter-feeders to reveal temporal and spatial patterns in sewage
14
15 770 dispersal. *Marine Ecology Progress Series* 235, 63-73.
16
17
- 18 771 Goecke, F., Labes, A., Wiese, J., Imhoff, J.F., 2010. Chemical interactions between marine
19
20 772 macroalgae and bacteria. *Marine Ecology Progress Series* 409, 267-300.
21
22
- 23 773 Gohin, F., 2010. Atlas de la température, de la concentration en chlorophylle et de la turbidité
24
25 774 de surface du plateau continental français et de ses abords de l'Ouest européen. DCSMM
26
27 775 report, Laboratoire Dynamiques de l'Environnement Côtier DYNECO/PELAGOS, Ifremer,
28
29 776 Brest, 53 pp.
30
31
- 32
33 777 Grall, J., Le Loc'h, F., Guyonnet, B., Riera, P., 2006. Community structure and food web
34
35 778 based on stable isotopes ($\delta^{15}\text{N}$ and $\delta^{13}\text{C}$) analysis of a North Eastern Atlantic maerl bed.
36
37 779 *Journal of Experimental Marine Biology and Ecology* 338, 1-15.
38
39
- 40 780 Guo, L., Tanaka, N., Schell, D.M., Santschi, P.H., 2003. Nitrogen and carbon isotopic
41
42 781 composition of high-molecular-weight dissolved organic matter in marine environments.
43
44 782 *Marine Ecology Progress Series* 252, 51-60.
45
46
- 47 783 Halpern, B.S., Walbridge, S., Selkoe, K.A., Kappel, C.V., Micheli, F., D'Agrosa, C., Bruno,
48
49 784 J.F., Casey, K.S., Ebert, C., Fox, H.E., Fujita, R., Heinemann, D., Lenihan, H.S., Madin,
50
51
52 785 E.M.P., Perry, M.T., Selig, E.R., Spalding, M., Steneck, R., Watson, R., 2008. A global map
53
54 786 of human impact on marine ecosystems. *Science* 319, 948-952.
55
56
- 57 787 Heaton, T.H.E., 1986. Isotopic studies of nitrogen pollution in the hydrosphere and
58
59 788 atmosphere: a review. *Chemical Geology* 59, 87-102.
60
61
- 62 789 Howarth, R.W., 2008. Coastal nitrogen pollution: A review of sources and trends globally and
63
64
65

1 790 regionally. Harmful Algae 8, 14-20.
2
3 791 Howarth, R., Chan, F., Conley, D.J., Garnier, J., Doney, S.C., Marino, R., Billen, G., 2011.
4
5
6 792 Coupled biogeochemical cycles: eutrophication and hypoxia in temperate estuaries and
7
8 793 coastal marine ecosystems. Frontiers in Ecology and the Environment 9, 18-26.
9
10 794 Holmes, R.M., McClelland, J.W., Sigman, D.M., Fry, B., Peterson, B.J., 1998. Measuring ^{15}N -
11
12
13 795 NH_4^+ in marine, estuarine and fresh waters: An adaptation of ammonia diffusion method for
14
15 796 samples with low ammonium concentrations. Marine Chemistry 60, 235-243.
16
17
18 797 Horrigan, S.G., Montoya, J.P., Nevins, J.L., McCarthy, J.J., 1990. Natural isotopic composition
19
20 798 of dissolved inorganic nitrogen in the Chesapeake Bay. Estuarine, Coastal and Shelf Science
21
22
23 799 30, 393-410.
24
25 800 Jones, A., O'Donohue, M.J., Udy, J., Dennison, W.C., 2001. Assessing ecological impacts of
26
27 801 shrimp and sewage effluent: biological indicators with standard water quality analyses.
28
29
30 802 Estuarine, Coastal and Shelf Science 52, 91-109.
31
32
33 803 Kellman, L.M., Hillaire-Marcel, C., 1998. Nitrate cycling in streams: using natural abundances
34
35 804 of $\text{NO}_3\text{-d}^{15}\text{N}$ to measure denitrification. Biogeochemistry 43, 273-292.
36
37
38 805 Kendall, C., 1998. Tracing nitrogen sources and cycling in catchments. Kendall C., McDonnell
39
40 806 J.J. (Eds.). Isotope tracers in catchment hydrology. Elsevier Science B.V., Amsterdam, pp.
41
42 807 519-576.
43
44
45 808 Koroleff, F., 1969. Direct determination of ammonia in natural water as indophenol blue.
46
47 809 International Council for the Exploration of the Sea Meeting C9, 1-6.
48
49
50 810 Lartigue, J., Sherman, T.D., 2005. Response of *Enteromorpha sp.* (Chlorophyceae) to a nitrate
51
52 811 pulse: nitrate uptake, inorganic nitrogen storage and nitrate reductase activity. Marine
53
54 812 Ecology Progress Series 292, 147-157.
55
56
57 813 Lindau, C.W., Delaune, R.D., Patrick, W.H. Jr, Lambremont, E.N., 1989. Assessment of stable
58
59 814 nitrogen isotopes in fingerprinting surface water inorganic nitrogen sources. Water, Air, &
60
61 815 Soil Pollution 48, 489-496.
62
63
64
65

- 1 816 Littler, M.M., 1980. Morphological form and photosynthetic performances of marine
2
3 817 macroalgae: Tests of a functional/form hypothesis. *Botanica Marina* 21, 161-165.
4
5
6 818 Lorrain, A., Paulet, Y.-M., Chauvaud, L., Savoye, N., Donval, A., Saout, C., 2002. Differential
7
8 819 $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ signatures among scallop tissues: Implications for ecology and physiology.
9
10
11 820 *Journal of Experimental Marine Biology and Ecology* 275, 47-61.
12
13 821 Mantilla Morales, G., 1995. Modélisation des transferts de nitrates, confrontation des concepts,
14
15 822 des données et des informations : application au bassin de la Charente. Ph.D. Thesis,
16
17
18 823 Cemagref, Antony, France, 179 pp.
19
20
21 824 Mathieson, A.C., Shipman, J.W., O'shea, J.R., Hasevlat, R.C., 1976. Seasonal growth and
22
23 825 reproduction of estuarine fucoid algae in New England. *Journal of Experimental Marine*
24
25 826 *Biology and Ecology* 25, 273-284.
26
27
28 827 McClelland, J.W., Valiela, I., Michener, R.H., 1997. Nitrogen-stable isotope signatures in
29
30 828 estuarine food webs: A record of increasing urbanization in coastal watersheds. *Limnology*
31
32
33 829 *and Oceanography* 42, 930-937.
34
35 830 McClelland, J.W., Valiela, I., 1998. Linking nitrogen in estuarine producers to land-derived
36
37 831 sources. *Limnology and Oceanography* 43, 577-585.
38
39
40 832 McKee, K.L., Feller, I.C., Popp, M., Wanek, W., 2002. Mangrove isotopic ($\delta^{15}\text{N}$ and $\delta^{13}\text{C}$)
41
42 833 fractionation across a nitrogen vs. phosphorus limitation gradient. *Ecology* 83, 1065-1075.
43
44
45 834 Middelburg, J.J., Nieuwenhuize, J., 2000. Uptake of dissolved inorganic nitrogen in turbid ,
46
47 835 tidal estuaries. *Marine Ecology Progress Series* 192, 79:88.
48
49
50 836 Middelburg, J.J., Nieuwenhuize, J., 2001. Nitrogen isotope tracing of dissolved inorganic
51
52 837 nitrogen behavior in tidal estuaries. *Estuarine, Coastal and Shelf Science* 53, 385-391.
53
54
55 838 Minagawa, M., Wada, E., 1986. Stepwise enrichment of ^{15}N along food chains: further
56
57 839 evidence and the relation between ^{15}N and animal age. *Geochimica et Cosmochimica Acta*
58
59 840 48, 1135-1340.
60
61
62 841 Modéran, J., David, V., Bouvais, P., Richard, P., Fichet, D., 2012. Organic matter exploitation
63
64
65

1 842 in a highly turbid environment: Planktonic food web in the Charente estuary, France.
2
3 843 Estuarine, Coastal and Shelf Science 98, 126-137.
4
5
6 844 Needoba, J.A., Harrison, P.J., 2004. Influence of low light and a light:dark cycle on NO₃⁻
7
8 845 uptake, intracellular NO₃⁻, and nitrogen isotope fractionation by marine phytoplankton.
9
10
11 846 Journal of Phycology 40, 505-516.
12
13 847 Nerot, C., Lorrain, A., Grall, J., Gillikin, D.P., Munaron, J.-M., Le Bris, H., Paulet, Y.-M.,
14
15
16 848 2011. Stable isotope variations in benthic filter feeders across a large depth gradient on the
17
18 849 continental shelf. Estuarine, Coastal and Shelf Science 96, 228-235.
19
20
21 850 Nixon, S.W., 1995. Coastal marine eutrophication: a definition, social causes and future
22
23 851 concerns. Ophelia 41, 199-219.
24
25 852 Owens, N.J.P., 1987. Natural variations in ¹⁵N in the marine environment. Advances in Marine
26
27
28 853 Biology 24, 389-443.
29
30 854 Paerl, H.W., Fogel, M.L., 1994. Isotopic characterization of atmospheric nitrogen inputs as
31
32
33 855 sources of enhanced primary production in coastal Atlantic Ocean waters. Marine Biology
34
35 856 119, 635-645.
36
37
38 857 Pedersen, M.F., Borum, J., 1997. Nutrient control of algal growth in estuarine waters. Nutrient
39
40 858 limitation and the importance of nitrogen requirements and nitrogen storage among
41
42 859 phytoplankton and species of macroalgae. Marine Ecology Progress Series 142, 261-272.
43
44
45 860 Pedersen, A., Kraemer, G., Yarish, C., 2004. The effects of temperature and nutrient
46
47 861 concentrations on nitrate and phosphate uptake in the different species of Porphyra from
48
49 862 Long Island Sound (USA). Journal of Experimental Marine Biology and Ecology 312: 235-
50
51
52 863 252.
53
54
55 864 Pennock, J.R., Velinsky, D.J., Ludlan, J.M., Sharp, J.H., 1996. Isotopic fractionation of
56
57 865 ammonium and nitrate during uptake by *Skeletonema costatum*: implications for δ¹⁵N
58
59 866 dynamics under bloom conditions. Limnology and Oceanography 41, 451-459.
60
61
62 867 Ravail, B., Héral, M., Maestrini, S.Y., Robert, J.-M., 1988. Incidence du débit de la Charente
63
64
65

- 1 868 sur la capacité biotique du bassin ostréicole de Marennes-Oléron. Journal de Recherche
2
3 869 Océanographique (Union des Océanographes de France, Institut Océanographique Paris) 13,
4
5
6 870 48-52.
7
8 871 Raven, J.A., Taylor, R., 2003. Macroalgal growth in nutrient-enriched estuaries: A
9
10 872 biogeochemical and evolutionary perspective. Water, Air, & Soil pollution: Focus 3, 7-26.
11
12
13 873 Savage, C., Elmgren, R., Larsson, U., 2002. Effects of sewage-derived nutrients on an
14
15 874 estuarine macrobenthic community. Marine Ecology Progress Series 243, 67-82.
16
17
18 875 Savage, C., Elmgren, R., 2004. Macroalgal (*Fucus vesiculosus*) delta¹⁵N values trace decrease
19
20 876 in sewage influence. Ecological Applications 14, 517-526.
21
22
23 877 Scanes, P., Coade, G., Doherty, M., Hill, R., 2007. Evaluation of the utility of water quality
24
25 878 based indicators of estuarine lagoon condition in NSW, Australia. Estuarine, Coastal and
26
27
28 879 Shelf Science 74, 306-319.
29
30 880 Sebilo, M., Billen, G., Mayer, B., Billiou, D., Grably, M., Garnier, J., Mariotti, A., 2006.
31
32 881 Assessing nitrification and denitrification in the Seine River and Estuary using chemical and
33
34
35 882 isotopic techniques. Ecosystems 9, 564-577.
36
37
38 883 Seitzinger, S.P., Sanders, R.W., 1997. Contribution of dissolved organic nitrogen from rivers to
39
40 884 estuarine eutrophication. Marine Ecology Progress Series 159, 1-12.
41
42 885 Sigman, D.M., Altabet, M.A., Michener, R., McCorkle, D.C., Fry, B., Holmes, R.M., 1997.
43
44 886 Natural abundance-level measurement of the nitrogen isotopic composition of oceanic
45
46
47 887 nitrate: an adaptation of the ammonia diffusion method. Marine Chemistry 57, 227-242.
48
49
50 888 Stanisière, J.Y., Dumas, F., Plus, M., Maurer, M., Robert, S., 2006. Caractérisation des
51
52 889 composantes hydrodynamiques d'un système côtier semi-fermé : le bassin de Marennes-
53
54 890 Oléron. DOP/LER.LER/PC/06.1059 <http://archimer.ifremer.fr/doc/00000/2353/>
55
56
57 891 Strauch, G., Möder, M., Wennrich, R., Osenbrück, K., Gläser, H.R., Schladitz, T., Müller, C.,
58
59 892 Schirmer, K., Reinstorf, F., Schirmer, M., 2008. Indicators for assessing anthropogenic
60
61
62 893 impact on urban surface and groundwater. Journal of Soils and Sediments 8, 23-33.
63
64
65

- 1 894 Strickland, J.D., Parsons, T.R., 1972. A practical handbook of seawater analyses. 2nd edition.
2
3 895 Bulletin/Fisheries Research Board of Canada, 167, 310 pp.
4
5
6 896 Struski, C., Bacher, C., 2006. Preliminary estimate of primary production by phytoplankton in
7
8 897 Marennes-Oléron Bay, France. Estuarine, Coastal and Shelf Science 66, 323-334.
9
10
11 898 Taylor, R., Peek, J.T.A., Rees, T.A.V., 1998. Scaling of ammonium uptake by seaweeds to
12
13 899 surface area:volume ratio: geographical variations and role of uptake by passive diffusion.
14
15
16 900 Marine Ecology Progress Series 169, 143-148.
17
18 901 Teichberg, M., Fox, S.E., Aguila, C., Olsen, Y.S., Valiela, I., 2008. Macroalgal responses to
19
20 902 experimental nutrient enrichment in shallow coastal waters: growth, internal nutrient pools,
21
22
23 903 and isotopic signatures. Marine Ecology Progress Series 368, 117-126.
24
25 904 Tyler, A.C., McGlathery, K.J., Macko, S.A., 2005. Uptake of urea and amino acids by the
26
27 905 macroalgae *Ulva lactuca* (Chlorophyta) and *Gracilaria vermiculophylla* (Rhodophyta).
28
29
30 906 Marine Ecology Progress Series 294, 161-172.
31
32
33 907 Valiela, I., Foreman, K., LaMontagne, M., Hersh, D., Peckol, P., DeMeo-Andreson, B.,
34
35 908 D'Avanzo, C., Babione, M., Sham, C.H., Brawley, J., Lajtha, K., 1992. Couplings of
36
37 909 watersheds and coastal waters: Sources and consequences of nutrient enrichment in Waquoit
38
39
40 910 Bay, Massachusetts. Estuaries 15, 443-457.
41
42 911 Van Engeland, T., Bouma, T.J., Morris, E.P., Brun, F.G., Peralta, G., Lara, M., Hendriks, I.E.,
43
44 912 Soetaert, K., Middelburg, J.J., 2011. Potential uptake of dissolved organic matter by
45
46
47 913 seagrasses and macroalgae. Marine Ecology Progress Series 427, 71-81.
48
49
50 914 Vitoria, L., Otero, N., Soler, A., Canals, A., 2004. Fertilizer characterization: isotopic data (N,
51
52 915 S, O, C, and Sr). Environmental Science and Technology 38, 3254-3262.
53
54
55 916 Vitousek, P.M., Aber, J.D., Howarth, R.W., Likens, G.E., Matson, P.A., Schindler, D.W.,
56
57 917 Schlesinger, W.H., Tilman, D.G., 1997. Human alteration of the global nitrogen cycle:
58
59 918 sources and consequences. Ecological Applications 7, 737-750.
60
61
62 919 Zhang, L., Altabet, M.A., Wu, T., Hadas, O., 2007. Sensitive measurement of NH_4^+ $^{15}\text{N}/^{14}\text{N}$
63
64
65

1 920 ($\delta^{15}\text{NH}_4^+$) at natural abundance levels in fresh and saltwaters. Analytical Chemistry 79,
2
3 921 5297-5303.
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
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1 922 Figure captions

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Fig. 1: Study area and location of the stations sampled along the Charente River and Estuary:

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DIN sources (Culture: cultivated area, Pasture: pasture zone, STP: Sewage Treatment Plant

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of Saint-Savinien Palles), estuarine stations (St. 1, St. 2, St. 3, St. 4), and reference stations

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(ref_R: riverine reference, ref_O: oceanic reference). Water samples were collected at all

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stations, and macroalgae were sampled at St. 3, 4 and ref_O. Are also indicated the major

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cities (black circles), the limit of dynamic tidal influence (grey line), the river flow

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measurement station at Beillant (black star).

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Fig. 2: (A) Charente River flow measured at Beillant in 2006 compared to the 2004-2011 and

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2004-2005 averaged river flows (source RPDE, <http://info.eau-poitou-charente.org>), and

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sampling dates of water (black arrows) and macroalgae (grey arrows); (B) Temperature

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(black points), salinity (grey points) and turbidity (white points) measured at St. 4b located

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at the mouth of the Charente Estuary (source Ifremer,

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<http://archimer.ifremer.fr/doc/00041/15210/12538.pdf>).

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Fig. 3: Temporal evolution of NH₄⁺ concentrations (A, B, C), NO₃⁻ concentrations (D, E, F),

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δ¹⁵N-NO₃⁻ and δ¹⁵N-NH₄⁺ (G, H, I) from January to May 2006 for DIN sources (left panels),

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estuarine stations (middle panels) and reference stations (right panels). Open and full

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symbols represent NH₄⁺ and NO₃⁻, respectively. Post-hoc statistical differences between

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temporal trends at each station are indicated by letters (a, b) on the right of each plot.

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Fig. 4: Temporal evolution of δ¹⁵N in macroalgae tissues of *Ulva sp.* (A), *Enteromorpha sp.*

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(B), *Fucus serratus* (C) and *Fucus vesiculosus* (D) at St. 3 (inner estuary mouth, white

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circles), St. 4a (outer estuary mouth, black circles) and ref_O (oceanic reference, grey circles).

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Post-hoc statistical differences between δ¹⁵N values at each station and at each season are

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indicated by letters (a, b, c) on the right and on the top of each plot, respectively.

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Fig. 5: Evolution of δ¹⁵N along *Fucus serratus* fronds sampled at St. 4a (estuary, black circles)

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1 948 and ref₀ (ocean, grey circles). The grey area corresponds to the winter 2005-2006.

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3 949 Fig. 6: *In situ* growth rate of *Fucus serratus* (black circles) and *Fucus vesiculosus* (white
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6 950 circles) at St. 4a. n=9. Post-hoc statistical differences between growth rates for the different
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8 951 seasons are indicated by letters (a, b).

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10 952 Fig. 7: NO₃⁻ uptake rates determined in controlled conditions for *Ulva sp.* (U), *Enteromorpha*
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13 953 *sp.* (E) and *Fucus serratus* (FS) as a function of NO₃⁻ concentrations: 500 μmol l⁻¹ (black
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15 954 bar) and 50 μmol l⁻¹ (grey bar). n=3. Post-hoc statistical differences between uptake rates for
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18 955 the different species are indicated by letters (a, b).

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20 956 Fig. 8: (A) NO₃⁻ concentrations as a function of river flow, (B) δ¹⁵N-NO₃⁻ as a function of NO₃⁻
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23 957 concentrations, at St. 1, St. 2 and St. 4 over the sampled period, and (C) δ¹⁵N of *Ulva sp.*
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25 958 (U), *Enteromorpha sp.* (E), *Fucus serratus* (FS) and *Fucus vesiculosus* (FV) as a function of
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28 959 δ¹⁵N-NO₃⁻ at St. 4a over the sampled period. The correlation coefficient (r) is indicated for
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30 960 each station, and a black star highlights the significant correlations.

1 961 Tables

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3 962

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6 963 Table 1: Results of three-way ANOVA on macroalgae $\delta^{15}\text{N}$ depending on interacting factors

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8 964 time, site and species. df=degree of freedom; F=statistical values; $p=p$ value.

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10
11 965 Table 2: Results of repeated two-way ANOVA for each macroalgae species depending on

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13 966 interacting factors time and site. df=degree of freedom; F=statistical values; $p=p$ value.

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16 967 Table 3: Mean $\delta^{15}\text{N-NH}_4^+$ and $\delta^{15}\text{N-NO}_3^-$ values of DIN sources, estuarine and reference

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18 968 stations for the sampled period (January-May 2006). The number of values (n) depended on

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20 969 stations.

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Table 1[Click here to download Table\(s\): table1_R1.ps](#)

Factors	df	%SS	F	<i>p</i>
time	4	20.6	92.54	<0.0001
site	4	32.2	154.74	<0.0001
species	4	2.0	22.75	<0.0001
time:site	13	11.1	12.56	<0.0001
time:species	12	6.6	12.23	<0.0001
site:species	8	17.1	49.08	<0.0001
time:site:species	25	10.4	3.04	<0.0001

Table 2

[Click here to download Table\(s\): table2_R1.ps](#)

Species	Factors	df	F	p
<i>Ulva sp.</i>	time	4	28.36	<0.0001
	site	2	104.37	<0.0001
	time:site	7	6.78	<0.001
<i>Enteromorpha sp.</i>	time	4	9.97	<0.001
	site	2	5.19	<0.05
	time:site	7	1.94	0.130
<i>Fucus serratus</i>	time	4	0.76	0.560
	site	2	2.74	0.080
	time:site	7	0.916	0.508
<i>Fucus vesiculosus</i>	time	4	23.51	<0.0001
	site	2	90.82	<0.0001
	time:site	7	25.26	<0.0001

Table 3

[Click here to download Table\(s\): table3_R1.ps](#)

Type	Site	$\delta^{15}\text{N-NH}_4^+$ (‰)			$\delta^{15}\text{N-NO}_3^-$ (‰)		
		Mean	SD	n	Mean	SD	n
Source	Culture	9	11	2	6.5	0.6	4
	Pasture	12.4	-	1	9.2	1.8	4
	STP	25.4	5.9	4	16.9	8.7	5
Estuary	St. 1	1.8	1.8	2	7.5	1.2	10
	St. 2	6.6	-	1	7.5	0.3	8
	St. 4	2.5	-	1	8.5	3.9	10
Reference	ref _O	1.8	-	1	7.4	0.8	2
	ref _R	0.8	0.6	2	7.3	0.3	8

Figure 1

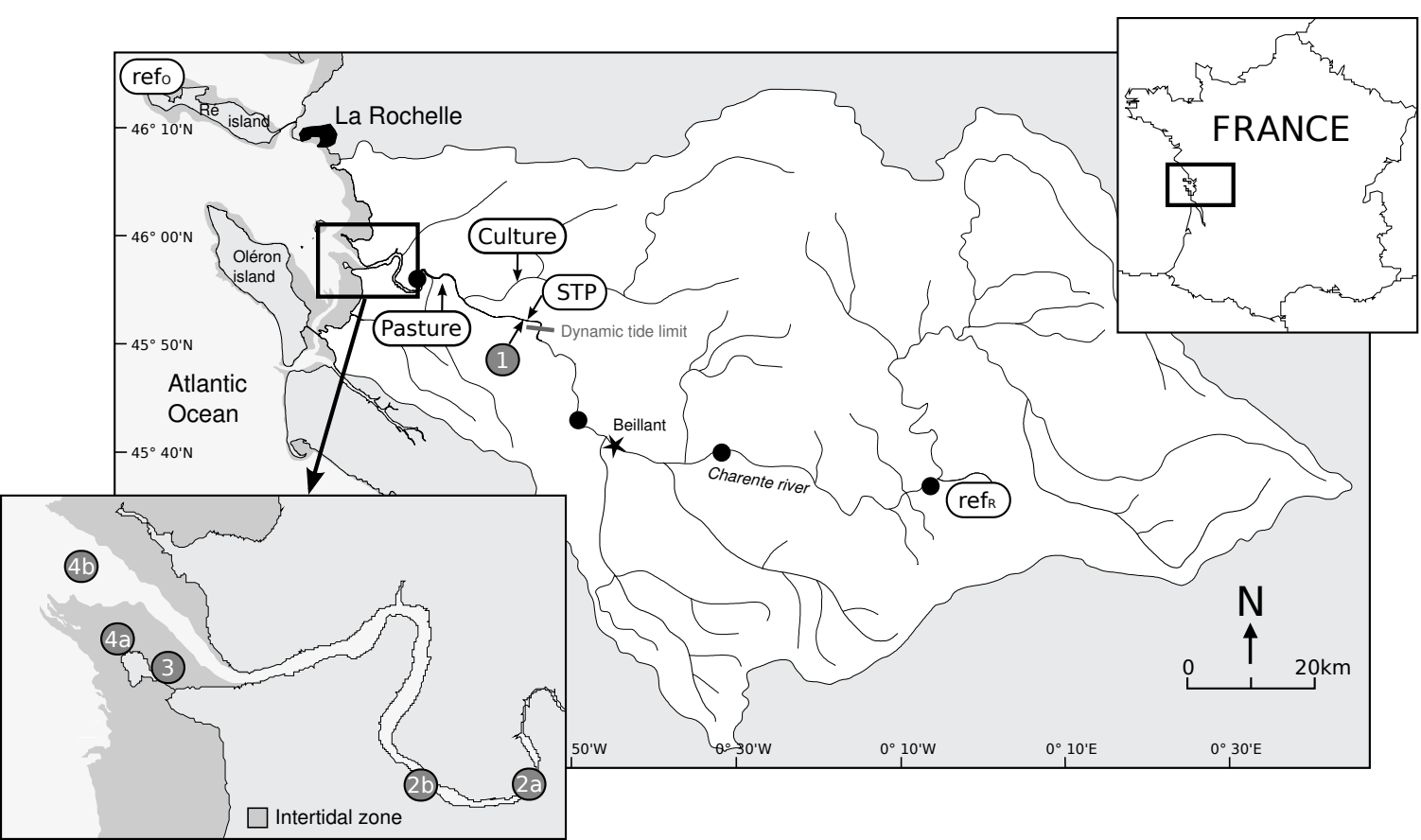


Figure 2

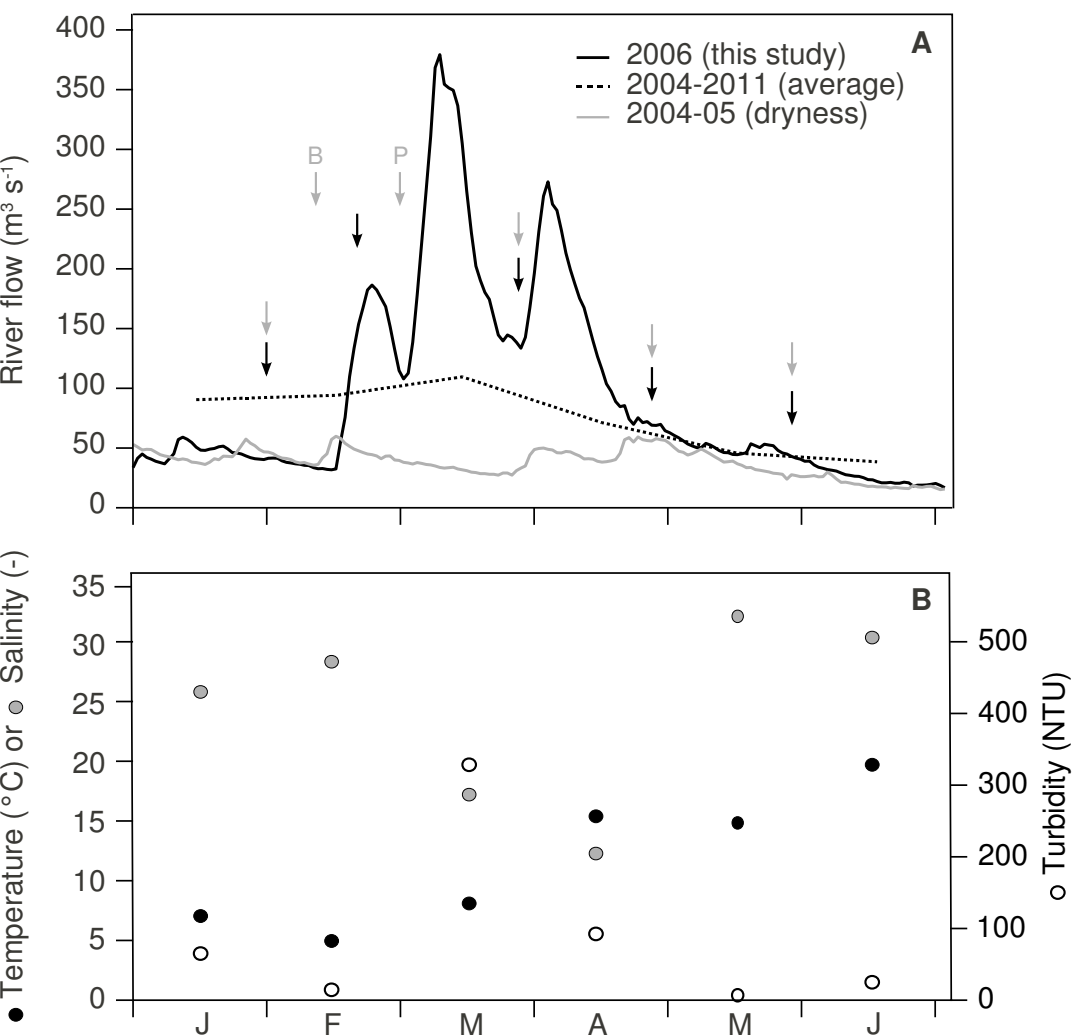


Figure 3

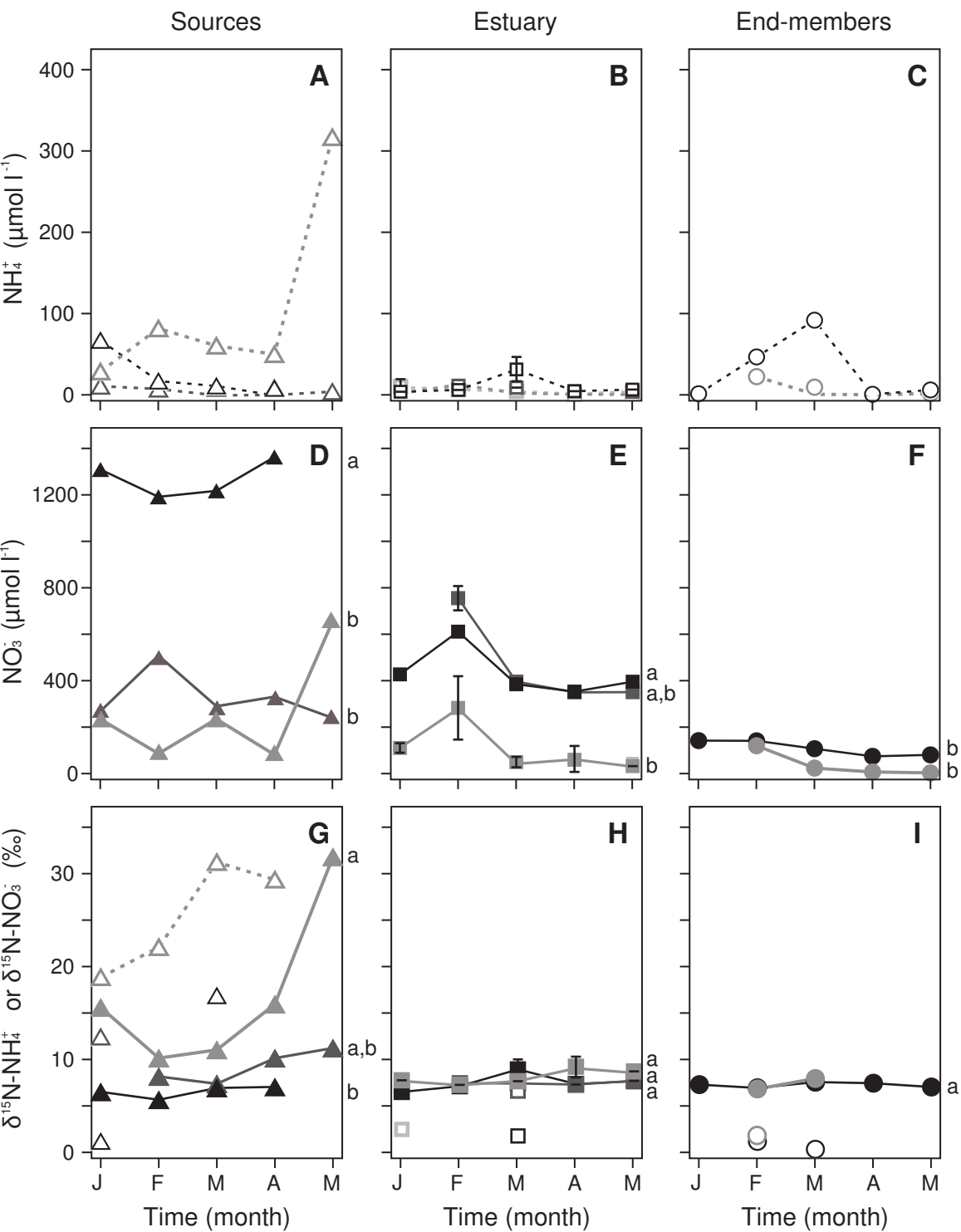


Figure 4

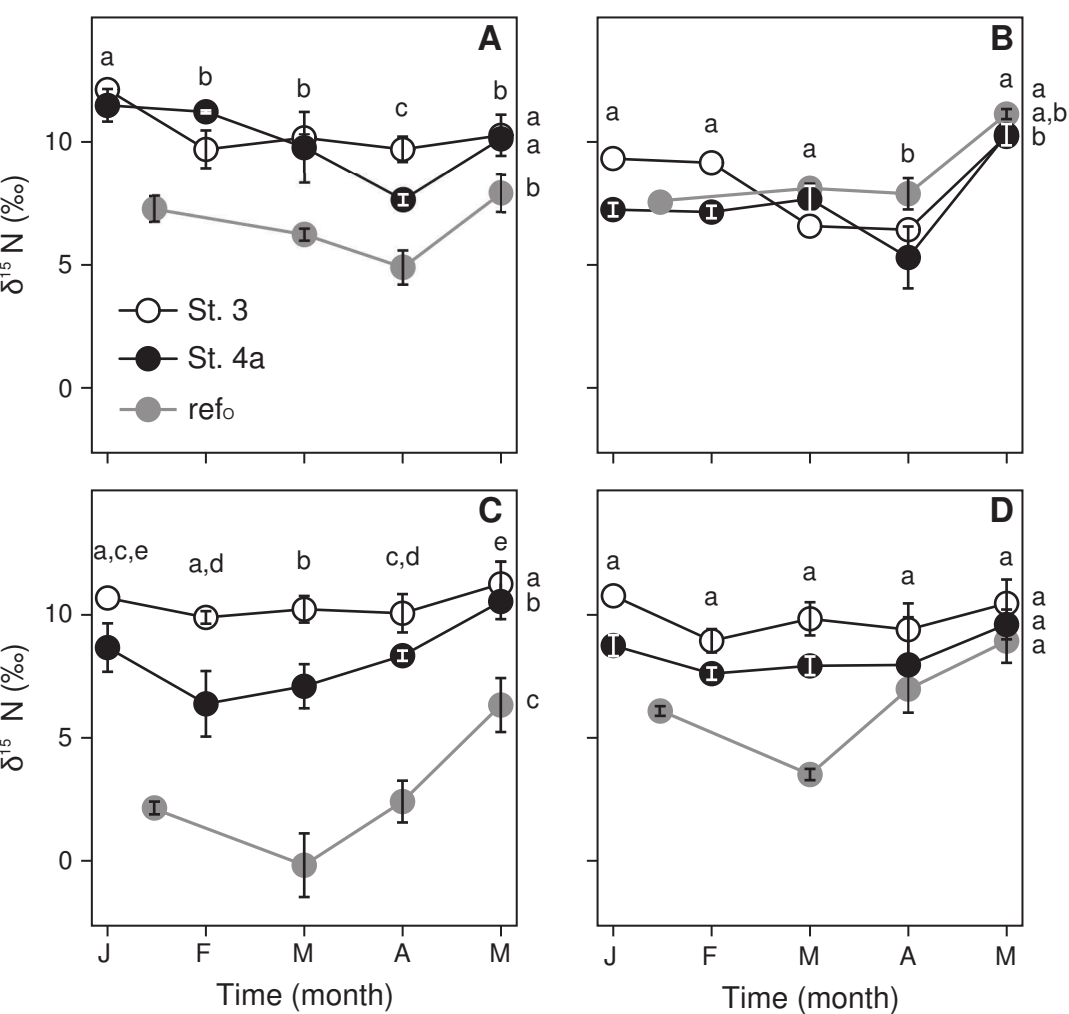


Figure 5

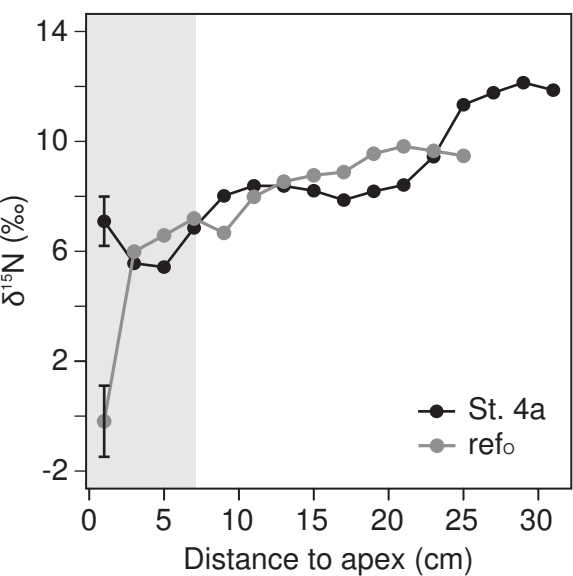
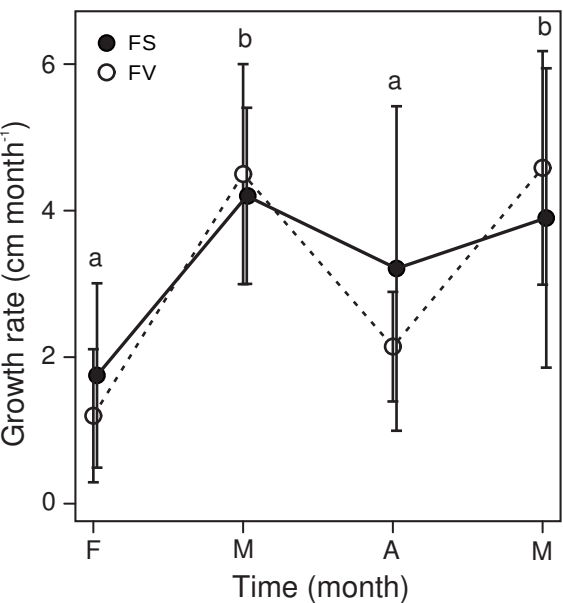


Figure 6



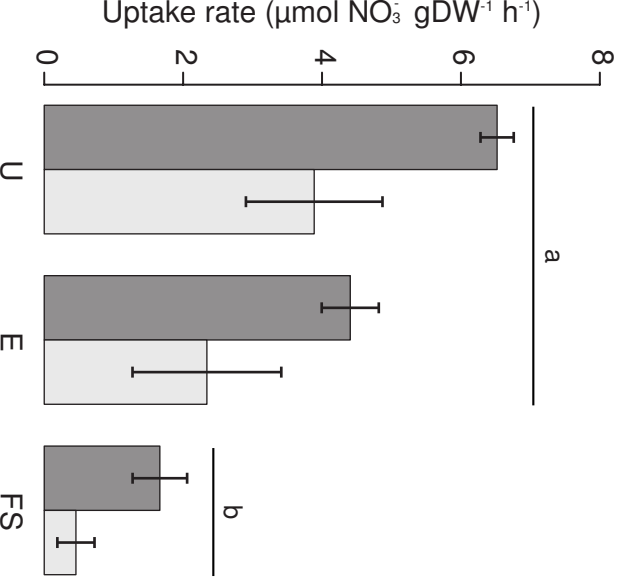


Figure 8

