
A new set of N isotopic reference values for monitoring *Ulva* green tides in coral reef ecosystems

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Abstract :

Green tides occurrence has increased in coral reefs, yet few reference values have been documented to support bloom management in these ecosystems. Here, we took advantage of recent *Ulva* green tides that occurred in New Caledonia to (i) identify the elements limiting the growth of *Ulva* spp. during these blooms; and (ii) validate the use of isotopic markers for identifying sources of nutrients that generated blooms. N/P ratios highlighted a stronger limitation of algae by phosphorus than by nitrogen on sites under oceanic influence, while the proportions of N and P were optimal for algal growth at sites where green tides occurred. Macroalgae highly exposed to sewage water was characterized by higher $\delta^{15}\text{N}$ than macroalgae collected in areas exposed to synthetic inorganic fertilizers. From these results, we established a new set of threshold values for using $\delta^{15}\text{N}$ in *Ulva* species as an indicator of nitrogen source type in coral reefs.

Highlights

► N/P values were optimal for algal growth at sites where green tides occurred. ► $\delta^{15}\text{N}$ in *Ulva* spp. varied between areas exposed to different nutrient sources. ► Similar trends in $\delta^{15}\text{N}$ at area scales were found for *Ulva* spp. and *Halimeda cylindracea*.

Keywords : Green tides, Eutrophication, Nitrogen, Isotopy, Elemental composition, New Caledonia

1. Introduction

Green tides have spread around the world over the past decades, sometimes leading to significant economic, health and ecologic impacts (Ye *et al.*, 2011; Gladyshev *et al.*, 2019). Green tides of macroalgae result from an excessive growth and proliferation (blooms) of competitive algae, often belonging to the *Ulva* genus. This happens when inorganic nutrients exceed the assimilative capacity of the surrounding ecosystem (Ménèsquen, 2018). Although the largest events have affected temperate areas (Liu *et al.*, 2009; Chávez-Sánchez *et al.*, 2018), their occurrence have increased in coral reefs, and also create nuisances in these socio-ecosystems (Lapointe *et al.*, 2005; Melton III *et al.*, 2016; Brisset *et al.*, 2021).

In temperate regions, the origin of several green tides has been clarified by studying the elemental composition of the algae involved (Valiela *et al.*, 2018; Samanta *et al.*, 2019; Thorsen *et al.*, 2019). On the one hand, measuring the contents of nitrogen, phosphorus, carbon and other trace elements (including metals) necessary for the physiological needs of the algae allows to identify the most limiting element for their growth. In practice, algae utilize the most limiting element more efficiently when other growth elements are present in optimal proportions (Liebig, 1840; Liebscher, 1895; De Baar, 1994; Cullen, 1991; Moore, 2013). In the context of green tides, characterizing the most limiting element is essential, as it has the greatest influence on the algal biomass, and therefore constitute a highly effective management lever (Bermejo *et al.*, 2022).

On the other hand, the use of isotopic markers allows the discrimination of different sources of nutrients (Fry, 2006; Bermejo *et al.*, 2022). Indeed, algae subjected to different inputs, characterized by distinct nitrogen isotope ratios (i.e., heavy isotope-rich septic effluent *versus* light isotope-rich synthetic inorganic fertilizer), are themselves characterized by significantly different nitrogen isotope ratios (Warnakulasooriya *et al.*, 2017; Valiela *et al.*, 2018; Samanta *et al.*, 2019; Thorsen *et al.*, 2019).

Isotopic markers present in benthic macroalgae have demonstrated notable efficacy in evaluating the impact of sewage stress on coral reefs (Risk *et al.*, 2009; Lapointe *et al.*, 2021). Extensive cross-shelf samplings or bioassays performed on specific areas have allowed the detection and modeling of wastewater effluent plumes (Dailer *et al.*, 2012; Mwaura *et al.*, 2017; Abaya *et al.*, 2018). They have been used to assess the role of land-based sources of nitrogen in triggering macroalgal blooms (Lapointe *et al.*, 2005) or to identify the affinity of invasive bloom-forming species for anthropogenic nitrogen (Melton III *et al.*, 2016). However, isotopic coral reef studies that focused specifically on *Ulva* blooms for the purpose of green tide management remain scarce and limited to a handful of fringing reefs (e.g., Mwaura *et al.*, 2017; Abaya *et al.*, 2018). To our knowledge, few studies have yet compared the elemental composition of *Ulva* species during blooms between coral reefs exposed to different types of nitrogen sources (i.e., coral reefs exposed to urban sewage runoff *versus* coral reefs exposed to fertilizers inputs). As a result, isotopic reference values that can be realistically encountered during blooms following exposure to various types of nitrogen sources remain sparse in the coral reef literature; hindering the effective utilization of isotopy for managing green tide in coral reefs. Filling this gap requires dedicated studies performed on reefs exposed to a sufficiently large panel of nutrient sources, and requires characterizing the elemental composition and isotopic ratio of *Ulva* during blooms triggered by different types of sources.

Although using isotopic ratio in *Ulva* species during blooms to identify source of nitrogen input is a promising approach, the operability and representativeness of such an indicator can be questioned, due to the ephemeral nature of the *Ulva* blooms and high turnover rate of nitrogen in *Ulva* tissue, which may hamper sampling (Risk *et al.*, 2009). Linking sampling location and source location may also be complex due to the ability of *Ulva* species to drift with currents during the mobile phase of their life cycle (Liu *et al.*, 2009). In this context, certain calcareous algae that attach to the substrate like *Halimeda cylindracea* (Fig. 1B), may serve as an

interesting proxy for *Ulva*. *Halimeda* is abundant in a wide range of habitats in coral reefs which facilitates its use as a bioindicator (Risk *et al.*, 2009; Briand *et al.*, 2015). Moreover, *Halimeda* is composed of a calcium carbonate skeleton, which enabled some authors to trace back past events using *Halimeda* skeleton in bioherm samples (Erler *et al.*, 2018). Using the elemental composition and isotopic ratio of *Halimeda* as a bioindicator of nutrient source in a green tide management context, however, requires prior validation since the morphology and physiology of *H. cylindracea* is different from *Ulva* (Fig. 1).

The present study took advantage of recent blooms of *Ulva batuffolosa*, *U. siganiphyllia*, and *U. ohnoi* that occurred in a subtropical lagoon located in New Caledonia (Fig. 1A) to (i) identify the elements limiting the growth of *Ulva* spp. during these events; (ii) validate the use of isotopic markers to identify the sources of nutrients that generate blooms; and (iii) assess the extent by which *Halimeda cylindracea* can be used in a green tide management context, in coral reefs where *Ulva* and *Halimeda* are conspecific. To achieve these objectives, a comparative approach was implemented, covering different study areas in New Caledonia, exposed to various sources of nutrients.

2. Materials & Methods

2.1 Study area

New Caledonia is a French overseas territory, located in the south-western Pacific Ocean, ~1500 km east of Australia. It comprises one main large island, known as Grande Terre, bordered by a 1600-km long discontinued barrier reef, which delimits a 23,400 km² of lagoons and reefs (Fig. 2; Andréfouët *et al.*, 2009). Due to their good health conditions and high biodiversity, six different areas, or clusters, of New Caledonia reefs and lagoons were listed as UNESCO World Heritage Areas in 2008 (Gairin and Andréfouët, 2020). In the South West coast of Grande Terre, the barrier reef complex is located 10 to 20 km away from the shore,

and is therefore predominantly under oceanic influence, except in the Moindou and Poe-Gouaro-Deva (PGD) areas where it lies directly in front of the shore (Fig. 2).

The sandy terraces of the barrier reef complex faced occasionally or seasonally blooms of *Ulva* spp., generating green tides in areas subject to high nutrient input (Brisset *et al.*, 2021; Lagourgue *et al.*, 2022; Fig. 1A). In the PGD area, two events occurred in 2018 and 2019, which affected the local economy in this hotspot of tourism activity, and raised concern about the possible outcome for the lagoon biodiversity, classified as a UNESCO world heritage site. Analysis of water quality and the use of watershed models revealed that the occurrence of the two green tides was triggered by excessive application of inorganic fertilizers in the region (Brisset *et al.*, 2023). Green tides resulting from an excessive proliferation of *Ulva ohnoi* also occasionally occurred inside an enclosed water body in Noumea (station Hip on Fig. 2A), which is highly exposed to fresh sewage water. This water body is nevertheless connected to the salty lagoon water through a thin channel in its southern part.

2.2 Sampling and identification

Samples of the genus *Ulva* were collected from 2019 to 2020 at 13 stations, of which 6 were located in the PGD lagoon (Table 1). Three stations were sampled in the Moindou lagoon located further East, 15 km away from the source of inorganic fertilizers that affected PGD but nevertheless under terrigenous influence (Fig. 2B), and three stations were located on the sandy terraces of the barrier reef in front of Boulouparis (Fig. 2C), which is under oceanic influence and is assumed to have little exposure to terrigenous inputs. Finally, *Ulva ohnoi* was sampled at Hip station in Noumea (Fig. 2A), highly exposed to sewage water. *Halimeda cylindracea* were sampled at 13 stations, including five similar to *Ulva* spp. sampled. In Noumea, *Halimeda cylindracea* was collected from Ricaudy reef (Fig. 2A) as the species is not present at Hip. For each station, at least 3 replicates were systematically performed (Table 1).

After collection, algae tissues (*Ulva* spp. and *H. cylindracea*) were isolated from all other organisms and sediment and frozen (-80°C) until pre-processing. Potential epiphytes, sometimes numerous on *H. cylindracea* samples, were dissolved by acid treatment (1%) for 1 min, before freezing. The samples of *Ulva* collected were identified to species level using morphological and molecular analyses, following the method described in Lagourgue *et al.* (2022).

The algae were freeze-dried and then finely ground (0.1 mm) using a mechanical grinder or a porcelain mortar. The powder obtained after grinding was stored at room temperature protected from light and moisture pending analysis.

2.3 Carbon and nitrogen content and isotope analyses

None of the shredded material has been decarbonated. A test sample of about 2 mg (precision 0.01 mg) was encapsulated in a tin cup with all the necessary precautions to avoid possible external contamination (i.e., cleaning of the equipment with 70° ethyl alcohol between each sample). Total carbon and nitrogen analysis was performed using an elemental analyzer coupled to an Integra2 Sercon IRMS (Isotope ratio Mass spectrometer) detector. During this process, the samples are oxidized in a combustion furnace at 1000°C, under helium flow, to generate CO₂ and NO_x. The latter is then reduced to N₂ on a copper column at 600°C. No interference with water, sulphur, and halogens biases the analysis because they are trapped as the process proceeds. The separation of N₂ and CO₂ was performed on a gas chromatography column and these molecules were then eluted to the mass detector where their relative abundance was measured, and the isotopic ratios $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ calculated according to equations 1 and 2 (Fry, 2006).

$$\delta^{15}\text{N} = \left(\frac{R_{\text{sample}}}{R_{\text{air}}} - 1 \right) \times 1000 \quad (\text{eq. 1})$$

$$\delta^{13}\text{C} = \left(\frac{R_{\text{sample}}}{R_{\text{PDB}}} - 1 \right) \times 1000 \quad (\text{eq. 2})$$

Where R_{sample} is the ratio of the abundance fraction of the heavy isotope to the abundance fraction of the light isotope measured in the sample, R_{air} this ratio characteristic of air used as a standard for the nitrogen atom ($R_{\text{air}} = 0.0036765$), and R_{PDB} this ratio characteristic of PeeDee Belemnite (PDB) used as a standard for the carbon atom ($R_{\text{PDB}} = 0.011180$). The results were validated through the analysis of certified reference materials: IAEA (IAEA-600 caffeine) and WEPAL, calibrated against the PDB primary standard.

2.4 Total phosphorus and trace elements analysis

In order to quantify the mass concentrations of total phosphorus in the samples, a mineralization reaction of the organic matter under acidic conditions was carried out on each of the grindings. A sample of 0.5 g (precision 0.001 g) of algae was placed in a mixture of concentrated nitric acid and oxygen peroxide (suprapur grade). Mineralization was catalysed at high temperature under hyperbaric conditions in a microwave oven (Milestone Ethos 1). The product of each mineralization was analysed, after cooling, with an ICP-EOS VARIAN 730 ES. The procedure consists in bringing the samples to a torch as a very fine aerosol thanks to a constant flow of pure argon. Argon is ionized by an electric arc and maintained under the effect of a magnetic field allowing it to reach a high temperature (plasma $\sim 10\,000$ °K). The energy thus available allows the elements present in the plasma to enter an excited state and to emit photons at the characteristic wavelengths of phosphorus. The same protocol although provided content of iron, manganese, cobalt, copper, zinc, boron, sulphur and molybdenum in algae samples. The results of this method were validated using certified reference materials (WEPAL) that underwent the same treatment as the samples.

2.5 Statistical analyses

For all the parameters measured, the replicates from each station were averaged, and all the results are expressed as mean \pm standard deviation in this paper. Statistical differences between study areas (i.e., PGD, Moindou, Boulouparis, and Noumea) were performed at the genus level for *Ulva* spp. and were tested using a non-parametric Kruskal-Wallis test, performed with the `Kruskal.test` function of package “stats” in R.3.5.1 (R Development Core Team, 2023). When differences were significant, pairwise comparisons tests between areas were performed using Dunn's test via the `dunnTest` function of the FSA package. Differences between stations within each area were tested at species level and using the same statistical tests as for inter-areas comparisons.

3. Results

3.1 Elemental composition of *Ulva* spp.

Ulva ohnoi collected at Hip were characterized by higher mass concentrations than *Ulva* spp. collected in other areas, for nitrogen (43.49 ± 3.48 g kg⁻¹ DW *versus* < 19.36 g kg⁻¹ DW for other areas, $4.3 \times 10^{-6} < p < 0.13$), for phosphorus (2.11 ± 0.38 g kg⁻¹ DW *versus* < 0.71 g kg⁻¹ DW, $0.02 < p < 0.11$), and to a lesser extent for carbon (389.0 ± 22.2 g kg⁻¹ DW *versus* < 349.75 g kg⁻¹ DW, $4.6 \times 10^{-6} < p < 0.14$). This resulted in the lowest values of C/N (9.0 ± 0.4 *versus* $> 16.1 \pm 4.0$), and C/P (188.3 ± 32.1 *versus* $> 247.9 \pm 106.1$) ratios for the Noumea samples, but N/P ratios intermediate compared to the other areas (Fig. 3). The N/P ratios measured in this study ranged from 12.5 to 34.3, with higher mean values for the *U. siganiphyllia* samples collected at Boulouparis barrier reef (28.9 ± 5.7 ; $3.3 \times 10^{-4} < p < 0.35$), and lower mean values for the *U. batuffolosa* samples collected in the Moindou area (14.5 ± 2.2 ; $3.3 \times 10^{-4} < p < 0.1$). The values obtained for the C/N ratio were variable within the PGD and Moindou areas and ranged from 13.10 ± 0.21 to 25.75 ± 0.36 (Fig. 3C). For the *U. batuffolosa* samples collected

in the PGD area, the lowest values were measured at station P13 (14.4 ± 3.1) related to a higher nitrogen content at this station ($23.80 \pm 5.67 \text{ g kg}^{-1} \text{ DW}$ versus $< 14.24 \text{ g kg}^{-1} \text{ DW}$ at the other stations in the PGD area, Fig. 3A). In contrast, the highest C/N values were measured at stations P4 and P9 (25.32 ± 1.64 and 25.75 ± 0.36 , respectively).

Mass concentrations of trace elements were highly variable between areas (Table 2), whether for cobalt (from $0.42 \pm 0.19 \text{ mg kg}^{-1} \text{ DW}$ to $3.89 \pm 8.15 \text{ mg kg}^{-1} \text{ DW}$), iron (from $302 \pm 59.4 \text{ mg kg}^{-1} \text{ DW}$ to $1972 \pm 4035 \text{ mg kg}^{-1} \text{ DW}$), manganese (from $8.24 \pm 2.12 \text{ mg kg}^{-1} \text{ DW}$ to $59.2 \pm 41.2 \text{ mg kg}^{-1} \text{ DW}$), and zinc (from $12.5 \pm 23.2 \text{ mg kg}^{-1} \text{ DW}$ to $165.8 \pm 108.2 \text{ mg kg}^{-1} \text{ DW}$). Despite a high variability between samples and within areas, significant differences were found between Noumea and PGD for cobalt ($p = 0.013$), between Boulouparis and Noumea as well as between Boulouparis and PGD for iron ($p = 0.022$ and $p = 0.039$, respectively), between PGD and Moindou as well as between PGD and Noumea for manganese ($p = 0.011$ and $p = 0.006$, respectively), and between Boulouparis and PGD for zinc ($p = 0.0001$).

3.2 Isotopic ratios of *Ulva* spp.

$\delta^{15}\text{N}$ values were an order of magnitude higher for *Ulva ohnoi* collected at Noumea ($\delta^{15}\text{N} = 21.30 \pm 2.56 \text{ ‰}$) than for *Ulva* spp. collected at the other area ($< \delta^{15}\text{N} = 3.09 \pm 0.02 \text{ ‰}$). In contrast, *U. batuffolosa* samples collected in the PGD area were characterized by lower $\delta^{15}\text{N}$ values ($\delta^{15}\text{N}$ from $0.68 \pm 0.79 \text{ ‰}$ to $1.75 \pm 0.25 \text{ ‰}$) than samples of the same species collected at Moindou ($\delta^{15}\text{N}$ from $2.06 \pm 0.17 \text{ ‰}$ to $3.09 \pm 0.02 \text{ ‰}$) and samples of *Ulva siganiphyllia* collected on the Boulouparis reef ($\delta^{15}\text{N}$ from $2.23 \pm 0.11 \text{ ‰}$ to $2.71 \pm 0.19 \text{ ‰}$). Although not significant, a coast-wide gradient seems to affect the nitrogen isotope ratio within PGD and Moindou lagoons, with the lowest $\delta^{15}\text{N}$ values measured near the littoral fringe (notably at station P4 for PGD and stations M5 at Moindou, Fig. 4A).

The $\delta^{13}\text{C}$ values measured for *U. ohnoi* samples collected at Noumea were also higher ($\delta^{13}\text{C} = -10.19 \pm 1.29 \text{ ‰}$) than at the other area ($< \delta^{13}\text{C} = -19.39 \text{ ‰}$). Notably, all the $\delta^{13}\text{C}$ values measured at PGD, Moindou, and Boulouparis were around -20‰.

3.3 Comparison between *Ulva* spp. and *Halimeda cylindracea*.

In a same station, samples of *H. cylindracea* showed $\delta^{15}\text{N}$ of a lower order of magnitude ($\delta^{15}\text{N}$ ranging from $-2.16 \pm 0.19 \text{ ‰}$ to $-0.27 \pm 0.25 \text{ ‰}$) than *Ulva* spp. samples ($\delta^{15}\text{N}$ ranging from $0.68 \pm 0.79 \text{ ‰}$ to $2.71 \pm 0.19 \text{ ‰}$). However, in both cases, the $\delta^{15}\text{N}$ values of algae present in the PGD lagoon ($\delta^{15}\text{N}$ between $0.68 \pm 0.79 \text{ ‰}$ and $1.29 \pm 0.66 \text{ ‰}$ for *Ulva batuffolosa* and between $-2.16 \pm 0.19 \text{ ‰}$ and $-0.85 \pm 0.76 \text{ ‰}$ for *H. cylindracea*) were significantly lower than those of algae collected from the barrier reef in front of Boulouparis ($\delta^{15}\text{N}$ between $2.33 \pm 0.20 \text{ ‰}$ and $2.71 \pm 0.19 \text{ ‰}$ for *Ulva siganiphyllia*. and between $-0.61 \pm 0.20 \text{ ‰}$ and $-0.27 \pm 0.25 \text{ ‰}$ for *H. cylindracea*, $p = 2.7 \times 10^{-5}$ and $p = 2.2 \times 10^{-4}$ respectively; Fig. 5).

Within areas, the correspondence between *Ulva* spp. and *H. cylindracea* isotopic ratio was not systematically verified, notably with the highest $\delta^{15}\text{N}$ values obtained at station P3 in the case of *Ulva batuffolosa*, versus at P10 for *H. cylindracea* (Fig. 5). However, these differences between stations were not significant ($p > 0.11$ for *H. cylindracea* and $p > 0.88$ for *Ulva batuffolosa*).

Halimeda cylindracea samples collected at Ricaudy reef in the urban area of Noumea were characterized by a significant proportion of heavy nitrogen isotopes ($\delta^{15}\text{N} = 0.17 \pm 0.88 \text{ ‰}$ at Ric) compared to samples collected in other areas ($\delta^{15}\text{N} < -0.27 \pm 0.25 \text{ ‰}$ at PGD and Boulouparis; Fig. 6). However, the difference between Noumea and other areas was less pronounced for *H. cylindracea* than for *Ulva* spp. (Fig. 4 and Fig. 6). Finally, at the PGD area, the lowest values of $\delta^{15}\text{N}$ were observed for some stations close to the coast (P4, P5 and P8 in particular, with $\delta^{15}\text{N} = -2.16 \pm 0.19 \text{ ‰}$, $-2.91 \pm 0.22 \text{ ‰}$ and $-2, 20 \pm 1.54 \text{ ‰}$, respectively),

while the highest values were obtained for stations farther from the coast (P10 and P14 with $\delta^{15}\text{N} = -0.85 \pm 0.76 \text{ ‰}$ and $-0.36 \pm 0.74 \text{ ‰}$, respectively).

4. Discussion

4.1 Main elements limiting the growth of *Ulva* spp. during the 2018-2019 blooms in New Caledonia

The cellular nitrogen quotas required by *Ulva* reported in the literature are from cold and temperate waters and are variable within the studied species. Critical cell quotas (i.e., N-mass concentration below which the growth of algae is limited) of 20, 25, and 32 g N kg⁻¹ DW have been reported for *U. rigida*, *U. intestinalis*, and *U. fenestrata*, respectively (Björnsäter & Wheeler, 1990; Lavery *et al.*, 1991). Björnsäter & Wheeler (1990) also reported a subsistence quota (i.e., N-mass concentration below which the growth of the algae is cancelled) of 18 g N kg⁻¹ DW for *U. fenestrata*. Although the values obtained in our subtropical study are difficult to compare with those in the (temperate) literature, the mass concentrations of nitrogen measured in this study for *U. batuffolosa* at PGD (14.47 ± 5.01 g N kg⁻¹ DW), and Moindou (9.46 ± 2.06 g N kg⁻¹ DW), and for *Ulva siganiphyllia* at Boulouparis (19.36 ± 1.61 g N kg⁻¹ DW; Table 2) were below the critical quotas and below or near the subsistence quotas published. This result suggests that the growth of *Ulva* collected on these barrier reef complexes has been limited by the amount of nitrogen available, regardless of the proximity of the barrier reef to the coast, and level of exposure to terrigenous inputs. The high renewal with oligotrophic water from the ocean at PGD may explain the N-limitation despite its proximity to N sources (Lalau *et al.*, 2022), and higher nitrogen content may be expected in N-exposed coral reefs characterized by poor oligotrophic water renewal. Notably within the enclosed water body highly exposed to sewage water in Noumea, nitrogen in *U. ohnoi* was above critical concentrations (cell quotas of 43.49 ± 3.48 g N kg⁻¹ DW), which confirms that, under high

nitrogen load in poorly flushed water bodies, *Ulva* may be predominantly limited by other nutrients.

Concerning phosphorus requirements, the mass concentrations of phosphorus measured in the PGD, Moindou, and Boulouparis samples (Table 2) were between the subsistence (0.5; 0.27 g P kg⁻¹ DW) and critical quotas (1.0; 0.81; 1.0 g P kg⁻¹ DW) reported for *Ulva* spp. from laboratory experiments (Le Bozec, 1996; Villares & Carbelleira, 2004). These results suggest that algal growth in these three areas was also limited by phosphorus. As for nitrogen, no trend between phosphorus content and proximity to terrestrial inputs could be highlighted in this study. Inter-area comparisons are difficult to interpret, because we collected different species at Noumea (*U. ohnoi*), Boulouparis (*U. siganiphyllia*), and PGD and Moindou (*U. batuffolosa*). Indeed, the reference values documented for phosphorus requirements by *Ulva* are highly variable in the literature, possibly due to species and environmental differences, since Björnsäter & Wheeler (1990) reported a subsistence quota of 2.0 g P kg⁻¹ DW for *U. fenestrata*, while Lavery *et al.* (1991) reported a critical quota of 0.4 g P kg⁻¹ DW for *U. rigida*. However, the cell quotas measured in *U. ohnoi* samples collected at Noumea within the enclosed water body highly exposed to sewage water (2.11 ± 0.384 g P kg⁻¹ DW) were much higher compared to the barrier reef sites, which suggests that *Ulva* has the capacity to uptake and store high phosphorus concentrations under P-replete condition. Low salinity may in some extent increase nutrient content in *U. ohnoi* (Angell *et al.*, 2015), but the influence of salinity on nutrient content remains weak compared to nutrient availability (Bews *et al.*, 2021), thus salinity alone cannot explain the high differences in nutrient content reported here between *Ulva ohnoi* collected at Hip and other *Ulva* species collected in PGD, Boulouparis, and Moindou.

For all samples collected, carbon did not appear to be a limiting element, with C:N and C:P ratios systematically higher than the values predicted by Redfield's ratio (C:N = 6.625 and C:P = 106; Redfield, 1934). These results suggest that across all studied sites, the available light

conditions (depth, turbidity) were adequate to enable algae to perform photosynthesis and generate the required carbon for their growth. The N:P ratios calculated in our study were mostly higher than the value predicted by the Redfield ratio (N:P = 16; Redfield, 1934), except for some samples collected at Moindou. Although the relevance of the N:P ratio predicted by Redfield may be questioned for algae of the genus *Ulva*, Björnsäter & Wheeler (1990) report, on the basis of experimental cultures of *U. fenestrata*, N:P ratios of 16 to 24 when conditions were optimal for growth, while phosphorus limitation induced N:P ratios > 24 in their experiments. These results confirm a stronger limitation of *U. siganiphyllia* by phosphorus than by nitrogen on the barrier reef in front of Boulouparis (N:P = 28.86 ± 5.72), while the proportions of N and P in *U. ohnoi* collected at Noumea and in *U. batuffolosa* collected in PGD were optimal for algal growth (N:P = 20.96 ± 2.33 and 19.36 ± 4.51 respectively). However, these differences between areas may also reflect differences in meteorological conditions, differences in *Ulva* species collected, or in bloom phase, since areas were opportunistically sampled following the occurrence of blooms that were reported, which did not happen at the same time nor involved the same species for all areas (Björnsäter & Wheeler, 1990).

The mass concentrations of trace elements measured in this study are comparable to those reported in the literature for other cultured and naturally collected *Ulva*, whether for calcium (from 10 to 122 g kg⁻¹; Kumar *et al.*, 2010; Rodriguez-Castañeda *et al.*, 2006; Peña-Rodriguez *et al.*, 2011), cobalt (from 0.18 to 11 mg kg⁻¹; Haritonidis & Malea, 1995; Favero *et al.*, 1996), iron (from 74 to 14,000 mg kg⁻¹, Peña-Rodriguez *et al.*, 2011; Paez-Osuna *et al.*, 2000; Rodriguez-Castañeda *et al.*, 2006; Favero *et al.*, 1996; Mohamed and Khaled, 2005), manganese (11-1721 mg kg⁻¹; Favero *et al.*, 1996), and zinc (2.8-206 mg kg⁻¹; Peña-Rodriguez *et al.*, 2011; Favero *et al.*, 1996). Note that the range of metal mass concentrations reported in the literature is wide, due to the ability of *Ulva* species to accumulate metals (Valdés *et al.*, 2018). The samples collected at Hip, however, do not meet the standard for calcium, with low

calcium levels compared to those reported in the literature ($0.96 \pm 0.6 \text{ mg kg}^{-1}$). The low calcium levels in algae collected in this enclosed water body at Noumea is congruent with the lower salinity at this site. Samples collected at PGD and Boulouparis were characterized by manganese levels slightly below the values reported in the literature ($5.41 \pm 3.06 \text{ mg kg}^{-1}$ and $7.91 \pm 2.13 \text{ mg kg}^{-1}$, respectively), which might have also contributed in limiting algal growth, in a punctual manner on these barrier reefs.

4.2 *The origin of elements*

Ulva ohnoi highly exposed to urban discharges and septic effluents in the enclosed water body at Noumea were enriched in heavy isotopes ($\delta^{15}\text{N} = 21.30 \pm 2.56 \text{ ‰}$) compared to other areas. Similar trends have been documented in the literature for other *Ulva* species. In South Korea, *Ulva* sp. exposed to septic effluent, sewage, or aquaculture farm waste exhibited greater enrichment in heavy isotopes ($\delta^{15}\text{N} > 5 \text{ ‰}$) than those exposed to other nitrogen sources ($\delta^{15}\text{N} < 5 \text{ ‰}$; Samanta *et al.*, 2019). Using laboratory experiments, Thorsen *et al.* (2019) measured higher $\delta^{15}\text{N}$ values ($\delta^{15}\text{N} \approx 11 \text{ ‰}$) for *Ulva* species fed with organic waste than for *Ulva* species fed with synthetic inorganic fertilizer ($\delta^{15}\text{N} \approx 3 \text{ ‰}$). Valiela *et al.* (2018) obtained similar results for *U. prolifera* in China, with $\delta^{15}\text{N}$ values approaching 11 ‰ for specimen collected from areas exposed to urban and livestock discharges, compared to $\delta^{15}\text{N}$ values approaching 4 ‰ in areas exposed to other source types. In coral reef ecosystems, Abaya *et al.* (2018) found $\delta^{15}\text{N}$ values approaching 8 ‰ near the shoreline of a fringing reef exposed to sewage water, and Mwaura *et al.* (2017) found $\delta^{15}\text{N}$ values of 10 – 12 ‰ in *Ulva* sp. located nearshore (< 100 m) and exposed to sewage water *versus* $\delta^{15}\text{N}$ of 5.5 ‰ in areas less exposed. Melton III *et al.* (2016) measured $\delta^{15}\text{N}$ of 13.15 in *U. ohnoi* sampled at Deering Estate, Florida, an area exposed to sewage effluent. Interestingly in an intertidal coral reef ecosystem, Lapointe *et al.*, (2021) found opposite results for *U. chaetomorhoides*, with $\delta^{15}\text{N} = -7.0 \text{ ‰}$ measured in a nutrient polluted and intertidal zone. This discrepancy has been attributed to high rates of bacterial nitrification

that convert ammonium to nitrite and nitrate under aerobic conditions and result in isotopic fractionation and depletion of macroalgae $\delta^{15}\text{N}$ values by - 6 to - 9 ‰ (Ochoa-Izaguirre and Soto-Jimenez, 2015). Anyway, our results highlight that $\delta^{15}\text{N}$ values associated to *Ulva* blooms fed quasi-exclusively with sewage water can reach up to 21.30 ± 2.56 ‰, which is, to our knowledge, the highest $\delta^{15}\text{N}$ value ever reported for wild *Ulva* samples (but see Dailer *et al.*, 2012, which reported $\delta^{15}\text{N}$ value of 30-35 ‰ using bioassays located over freshwater seeps). Low salinity can, in some extent, affect $\delta^{15}\text{N}$ values in *Ulva* sp. (Bews *et al.*, 2021), but the influence of salinity remains negligible compared to the influence of nutrient concentrations, and this factor is therefore unlikely to have biased $\delta^{15}\text{N}$ reported in this study at Hip. Notably in their experimental study using *U. lactuca*, Bews *et al.* (2021) have shown that variation among different nutrient levels explained approximately 94% of the total variation in $\delta^{15}\text{N}$ ‰ values, while differences in salinity treatment (35 *versus* 15 psu) explained only about 3% of this variation. The value of $\delta^{15}\text{N}$ reported here for *Ulva ohnoi* exclusively fed with sewage water allow us to establish a new set of threshold values for using $\delta^{15}\text{N}$ in *Ulva* species as an indicator of nitrogen source type in coral reefs (Table 3).

The present study also provides the range of values that can be expected on a barrier reef, more or less exposed to inorganic fertilizers. The synthetic inorganic fertilizers used in the PGD area from 2015 to 2018 are characterized by low $\delta^{15}\text{N}$ values (-0.91 ± 1.65 ‰; Supplementary Material SM1), which explain the lower $\delta^{15}\text{N}$ in algae collected at PGD. Interestingly, although not addressed in this study, the organic fertilizers used from 2018 onwards are characterized by $\delta^{15}\text{N}$ values lower than those recorded in algae exposed to sewage waters ($\delta^{15}\text{N} = 5.15 \pm 0.62$ ‰ *versus* 21.30 ± 2.56 ‰), suggesting that nitrogen isotopy might also be used, in case of heavy sewage pollution, to discriminate blooms of algae triggered by sewage waters from those triggered by organic fertilizers.

Phosphorous is a monotopic element (P has only one stable isotope), which precludes using P isotopy in a similar way than we used N isotopy in this study. In ecosystems where P is mostly found in phosphates (PO_4^{3-}), the isotopes of oxygen in phosphates ($\delta^{18}\text{O}_\text{P}$) can be used (Davies *et al.*, 2014; Gooddy *et al.*, 2018). However, such approaches have two strong limitations that precluded their use in our case study. First, analytical methods required for measuring $\delta^{18}\text{O}_\text{P}$ are not as accessible as isotopy of nitrogen. Notably, no infrastructure currently exists in New Caledonia to perform $\delta^{18}\text{O}_\text{P}$ analyses. Using $\delta^{18}\text{O}_\text{P}$ therefore require to export the algal material for analysis, which is a long and costly process. Such constraints are inadequate in the light of our objective, which is to design a cost-effective indicator that can be easily performed by managers in New Caledonia to quickly tackle the source of nutrient discharge in case of algal blooms. Second, the isotopic approach to phosphates is still in its infancy and its potential applications in distinguishing sources have not yet been clearly established (Desaulty & Petelet-Giraud, 2018; Tamburini *et al.*, 2014; Davies *et al.*, 2014). Some authors have obtained promising results (Gooddy *et al.*, 2018), but more research is still required to use $\delta^{18}\text{O}_\text{P}$ for discriminating phosphate sources. Meanwhile, and because N is the major nutrient element that determine the occurrence of *Ulva* blooms, focusing effort on N isotopy to discriminate nutrient sources is justified in the New Caledonian context.

Since the carbon that makes up *Ulva* comes primarily from photosynthesis, the values of carbon isotope ratios ($\delta^{13}\text{C}$) in their tissues provide information on the type of metabolic path used by the algae. C3-type metabolism results in $\delta^{13}\text{C}$ values in the range of -35 to -22‰, whereas C4-type metabolism results in $\delta^{13}\text{C}$ values in the range of -17 to -11 ‰ (Valiela *et al.*, 2018). The $\delta^{13}\text{C}$ values obtained at PGD, Moindou, and Boulouparis fall between these two ranges of values ($\delta^{13}\text{C} \approx 20$ ‰). Similar results have also been reported in the literature, most notably for *Ulva prolifera* in China ($\delta^{13}\text{C}$ from -22 to 15 ‰; Valiela *et al.*, 2018). Such intermediate $\delta^{13}\text{C}$ values can be explained by a co-occurrence of C3 and C4 metabolism in *Ulva*

and other opportunistic green algae (Xu *et al.*, 2012). Their ability to switch between the two types of metabolism depending on environmental conditions could give them an advantage over other species, fostering rapid and large blooms (Valiela *et al.*, 2018). According to this hypothesis, it should be noted that the specimen of *U. ohnoi* collected at the Noumea enclosed water body essentially used a C4-type metabolism ($\delta^{13}\text{C} = -10.19 \pm 1.29 \text{ ‰}$; Fig. 4B). Differences in salinity is unlikely to explain the higher $\delta^{13}\text{C}$ in algae collected in the enclosed water body in Noumea compared to PGD, Moindou and Boulouparis zones, since this factor has been shown to hold very little influence on $\delta^{13}\text{C}$ in *Ulva* species (Bews *et al.*, 2021). Rather, the higher $\delta^{13}\text{C}$ values found for Noumea samples may result from species-specific trait of *U. ohnoi*, as high $\delta^{13}\text{C}$ values have also been reported by other studies focussing on this species (Cañavate *et al.*, 2021).

4.3 Using *Halimeda cylindracea* as surrogate of *Ulva* sp.

The use of *Halimeda cylindracea* as a surrogate for algae of the genus *Ulva* to discriminate different nitrogen sources is attractive for several reasons. Firstly, although *Ulva* species are ubiquitous and can reach significant biomasses locally at some sites, their distribution is less homogeneous than *H. cylindracea*, the latter being very frequent on most of the reef flats and terraces of New Caledonia. Second, *Halimeda* species are firmly anchored to the sediments, and are therefore more representative of their growing site than *Ulva* species, which can be detached from their substrate and often present a mobile phase during their life cycle. Finally, the longer lifespan, the calcareous skeleton, and the time-integrating segment morphology of *Halimeda cylindracea* provide an opportunity to trace the temporal dynamics of $\delta^{15}\text{N}$ (Erler *et al.*, 2018), whereas *Ulva* individuals provide only a near-instantaneous picture of environmental conditions. Indeed, in contrast to the fast (weekly) turnover rates of macroalgae tissues (Vroom *et al.*, 2003), the calcareous skeleton of *H. cylindracea* can provide a chemical picture of older events (Erler *et al.*, 2018; Thibault *et al.*, 2022).

The $\delta^{15}\text{N}$ values measured for *H. cylindracea* samples collected at PGD (from -2.91 ± 0.22 ‰ to -0.36 ± 0.74 ‰) are lower than those reported by Briand *et al.* (2015) for the same species in the southwestern New Caledonia lagoon (from 0.52 ± 1.59 ‰ to 0.91 ± 0.17 ‰). At Entrecasteaux reefs, Northern part of New Caledonia, Thibault *et al.* (2022) also found higher $\delta^{15}\text{N}$ in *Halimeda* spp. than reported here, even at reference sites located far away from nutrient sources ($\delta^{15}\text{N} = 2.1 \pm 0.2$ for algae collected at Huon islets, and $\delta^{15}\text{N} = 2.1 \pm 0.6$ for algae collected at Surprise islets). These results, as well as the trends between areas (lower $\delta^{15}\text{N}$ values obtained for the PGD lagoon than for Boulouparis and Noumea) confirm those obtained from *Ulva* samples, with lower $\delta^{15}\text{N}$ values obtained for areas exposed to inorganic fertilizers, and higher $\delta^{15}\text{N}$ values for samples collected in areas exposed to sewage and/or septic effluents. Interestingly, *H. cylindracea* exhibited lower values of $\delta^{15}\text{N}$ than *Ulva* sp. at PGD, which may be the result of its calcareous skeleton that allows for capturing past discharges of fertilizers rather than the recent event only (see Erler *et al.*, 2018 for an example using *Halimeda* in bioherms samples). To our knowledge, studies that used lived individuals of *Halimeda* as bioindicator of nutrient discharge in coral reefs focused only on the apical, most recent, segment, or on all segments confounded (Briand *et al.*, 2015; Thibault *et al.*, 2022), but a segment-by-segment approach is worth to consider in the future for tracing back specific past (1-5 years) events. In this respect, for a given area, this study confirms the interest of using *H. cylindracea* to discriminate the different sources of nutrient enrichment in coral reefs, beyond the green tide management context.

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Tables

Table 1: List of algae samples analysed for the elemental estimation. n refers to the number of samples. The location of the stations is shown in Fig. 2. PGD: Poe-Gouaro-Deva. Dates are in the format dd/mm/yyyy. PGD was predominantly exposed to inorganic nutrients; Moindou exposed to a diversity of sources; Boulouparis was under oceanic influence; Noumea area exposed to sewage water. *U.* refers to *Ulva*, and *H.* to *Halimeda*.

Area	Station	Longitude	Latitude	Species	n	Sampling date
PGD	P1	165.3483	-21.5996	<i>H. cylindracea</i>	5	04/07/2019
	P2	165.3572	-21.6021	<i>H. cylindracea</i>	5	06/07/2019
	P3	165.3626	-21.6046	<i>H. cylindracea</i>	5	06/02/2019
				<i>U. batuffolosa</i>	5	06/02/2019
				<i>U. batuffolosa</i>	2	19/02/2020
	P4	165.3673	-21.6069	<i>H. cylindracea</i>	5	04/02/2019
				<i>U. batuffolosa</i>	4	04/02/2019
	P5	165.3738	-21.6084	<i>H. cylindracea</i>	5	05/02/2019
	P6	165.3862	-21.6102	<i>H. cylindracea</i>	5	06/02/2019
	P7	165.3858	-21.6120	<i>H. cylindracea</i>	10	05/02/2019
	P8	165.4035	-21.6139	<i>H. cylindracea</i>	5	06/02/2019
	P9	165.4097	-21.6182	<i>U. batuffolosa</i>	3	04/07/2019
	P10	165.3958	-21.6236	<i>H. cylindracea</i>	5	04/02/2019
				<i>U. batuffolosa</i>	8	04/02/2019
	P11	165.3778	-21.6153	<i>U. batuffolosa</i>	4	04/02/2019
	P13	165.3715	-21.6209	<i>U. batuffolosa</i>	4	05/02/2019
	P14	165.3519	-21.6154	<i>H. cylindracea</i>	5	05/02/2019
Moindou	M1	165.4673	-21.6817	<i>U. batuffolosa</i>	3	08/09/2020
	M2	165.4765	-21.6940	<i>U. batuffolosa</i>	3	08/09/2020
	M5	165.4885	-21.6880	<i>U. batuffolosa</i>	3	08/09/2020
Boulouparis	SV1	165.9682	-22.03468	<i>U. siganiphyllia</i>	5	07/07/2020
	SV2	165.9683	-22.03722	<i>U. siganiphyllia</i>	5	07/07/2020

				<i>H. cylindracea</i>	5	07/07/2020
	SV4	165.9738	-22.0484	<i>U. siganiphyllia</i>	5	07/07/2020
				<i>H. cylindracea</i>	5	07/07/2020
Noumea	Ric	166.4576	-22.3111	<i>H. cylindracea</i>	5	07/07/2020
	Hip	166.4503	-22.3005	<i>U. ohnoi</i>	5	07/07/2020

Table 2: Mean \pm standard deviation of mass concentration values of nitrogen (N), phosphorus (P), carbon (C), calcium (Ca), cobalt (Co), iron (Fe), manganese (Mn), and zinc (Zn) measured at the different study sites in *Ulva* spp. tissues. N, P, C, and Ca values are expressed in g kg⁻¹, while Co, Fe, Mn, and Zn values are expressed in mg kg⁻¹. Note that species collected were *Ulva batuffolosa* at PGD and Moindou, *Ulva siganiphyllia* at Boulouparis, and *Ulva ohnoi* at Noumea. The number of samples analysed and used for computing mean at the scale of areas is provided in brackets.

	PGD	Moindou	Boulouparis	Noumea
N	14.47 \pm 5.01 (30)	9.46 \pm 2.06 (9)	19.36 \pm 1.61 (15)	43.49 \pm 3.48 (5)
P	0.709 \pm 0.242 (14)	0.625 \pm 0.140 (6)	0.680 \pm 0.130 (6)	2.11 \pm 0.384 (3)
C	304.33 \pm 39.37 (30)	154.65 \pm 62.91 (9)	349.75 \pm 22.53 (15)	389.0 \pm 22.2 (5)
Ca	21.3 \pm 22.6 (14)	29.4 \pm 9.42 (6)	20.1 \pm 17.5 (6)	0.963 \pm 0.610 (3)
Co	0.423 \pm 0.195 (14)	3.89 \pm 8.15 (6)	0.640 \pm 0.164 (6)	1.32 \pm 0.358 (3)
Fe	524 \pm 193 (14)	1972 \pm 4035 (6)	302 \pm 59.4 (6)	609 \pm 32.5 (3)
Mn	5.41 \pm 3.06 (14)	31.7 \pm 51.9 (6)	8.24 \pm 2.12 (6)	59.2 \pm 41.2 (3)
Zn	12.5 \pm 23.2 (14)	46.4 \pm 35.5 (6)	165.8 \pm 108.2 (6)	13.9 \pm 4.71 (3)

Table 3: Proposed set of threshold values when using $\delta^{15}\text{N}$ in *Ulva* species. as an indicator of nitrogen source type for decision support in managing green tides in coral reef environments, based on coral reef literature and *Ulva* blooms that affected New Caledonia's reefs in 2018-2019.

Value	Conclusion	Reference
< 2	<i>Ulva</i> Bloom amplified by inorganic fertilizers	This study
[2; 5]	<i>Ulva</i> Bloom triggered by natural factors or a diversity of sources	This study
[5; 20]	<i>Ulva</i> Bloom amplified by sewage water	Mwaura <i>et al.</i> (2017); Abaya <i>et al.</i> (2018)
> 20	<i>Ulva</i> Bloom exclusively triggered by sewage water	This study; Dailer <i>et al.</i> (2012)

Figures legends:

Figure 1: Photographs (© Ifremer) of the sampled algae used for elemental analyses. A) *Ulva batuffolosa*; B) *Halimeda cylindracea*.

Figure 2: Location of the stations in Noumea (A), PGD and Moindou (B), and Boulouparis (C), where the macroalgae *Ulva ohnoi*, *U. batuffolosa*, *U. siganiphyllia* and *Halimeda cylindracea* were sampled for elemental analyses. The details of the species sampled at each station are provided in Table 1.

Figure 3: Cell quotas of nitrogen (A), phosphorus (D), carbon (B) and their ratios (C, E, F), in *Ulva* spp. collected at different stations. PGD: Poe-Gouaro-Deva; Moin: Moindou; Boul: Boulouparis; Nou: Noumea.

Figure 4: Mean (\pm standard deviation) of nitrogen (A) and carbon (B) isotope ratios for *Ulva* spp. samples collected at different stations.

Figure 5: Mean (\pm standard deviation) nitrogen isotope ratio for *Ulva* spp. and *Halimeda cylindracea* samples collected at common stations. The numbers of samples used for computing means at station scale are provided in Table 1.

Figure 6: Mean (\pm standard deviation) nitrogen isotope ratio for *Halimeda cylindracea* samples collected at different stations. Nou: Noumea.

Figures :

Figure 1 (should be printed in colours)



Figure 2 (should be printed in colours)

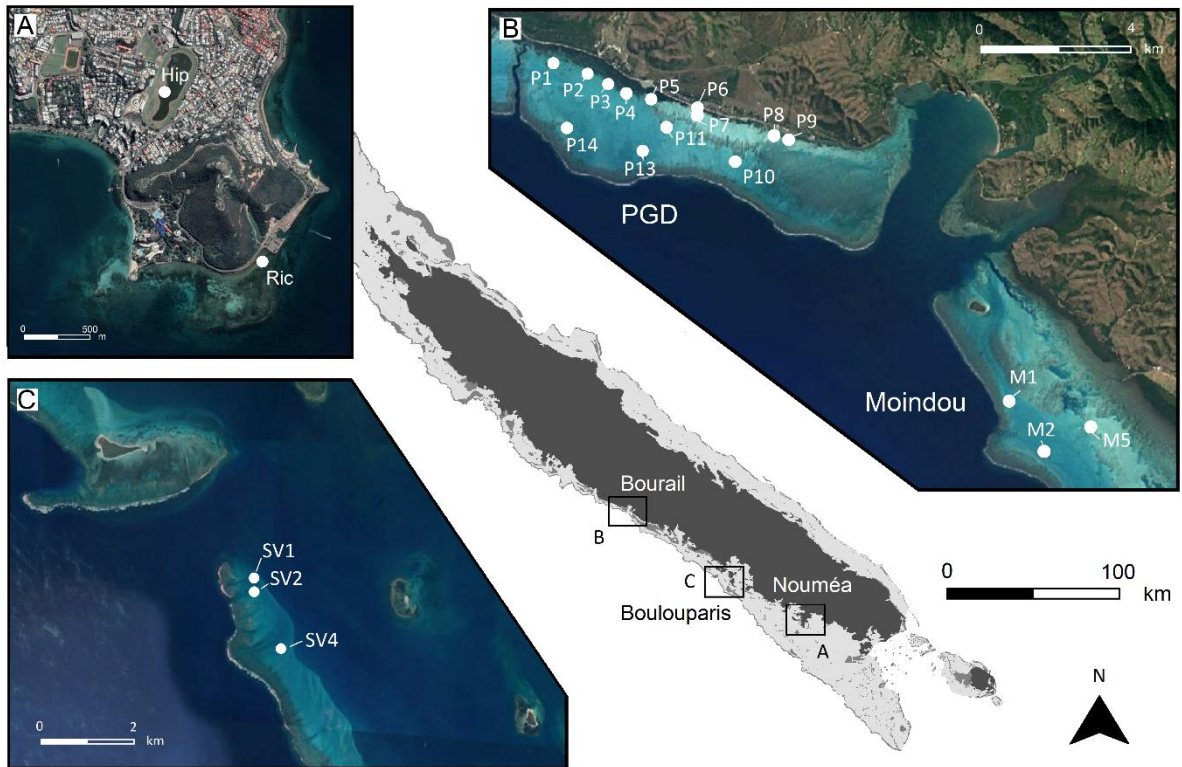


Figure 3

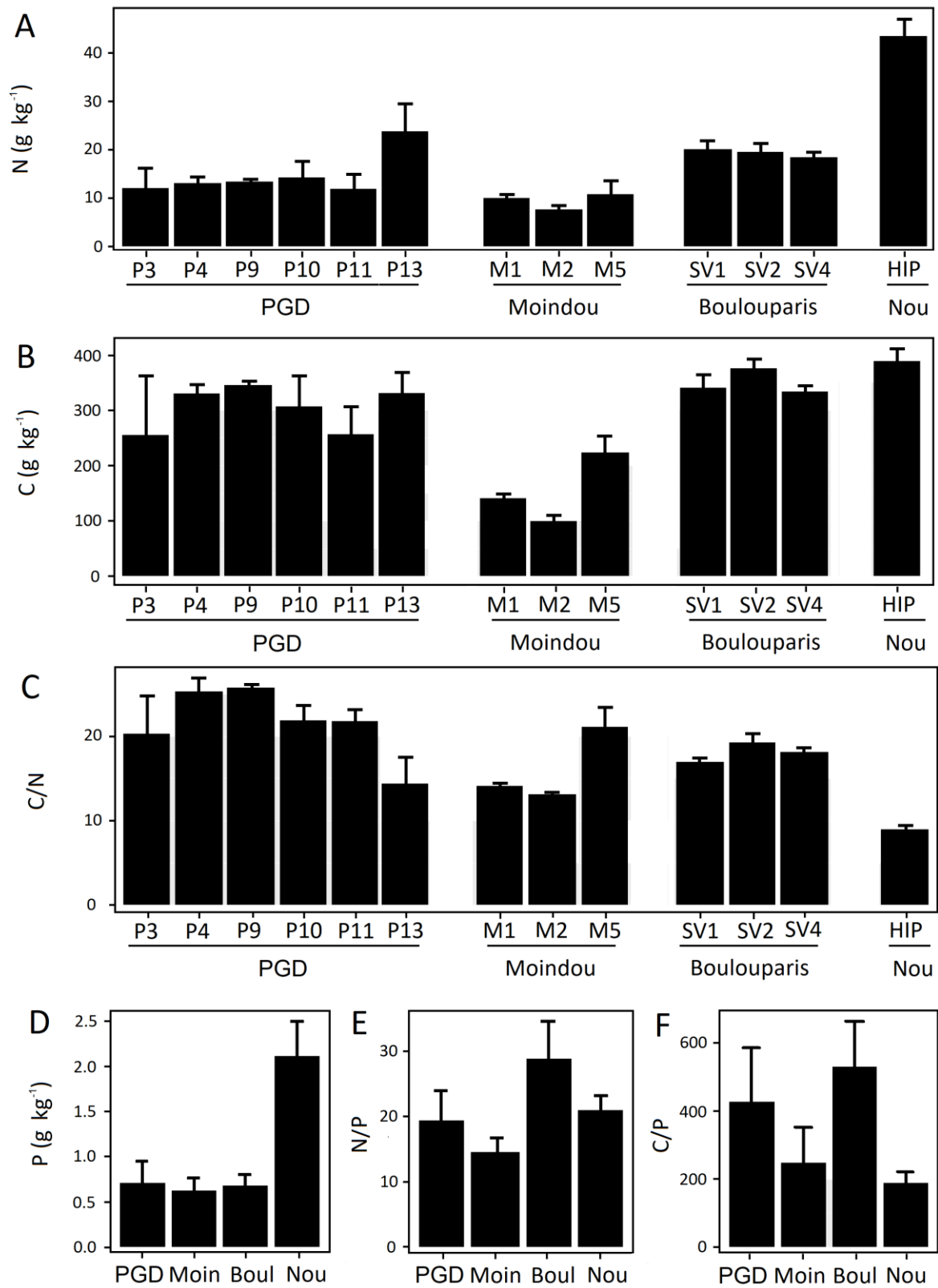


Figure 4

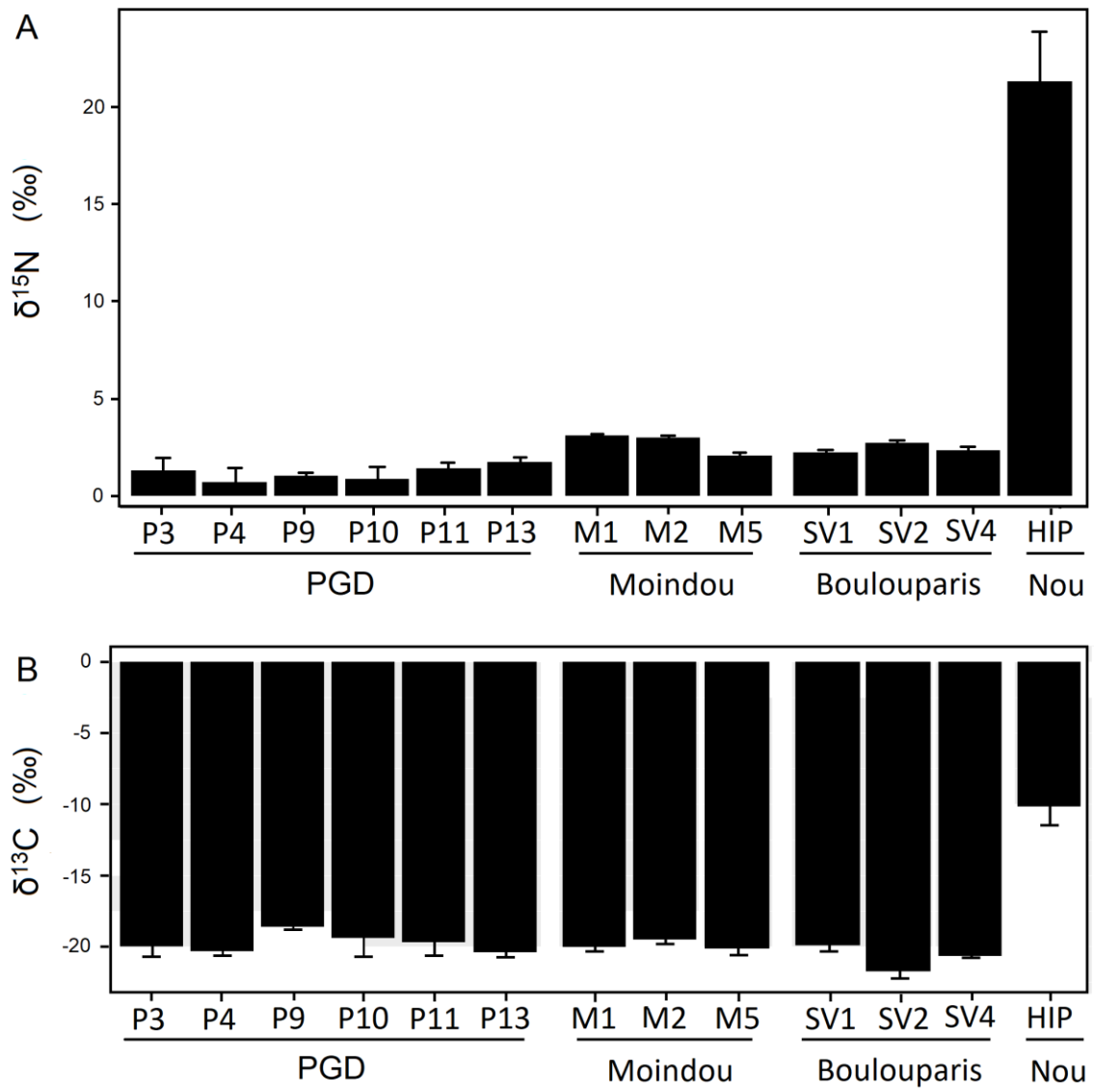


Figure 5

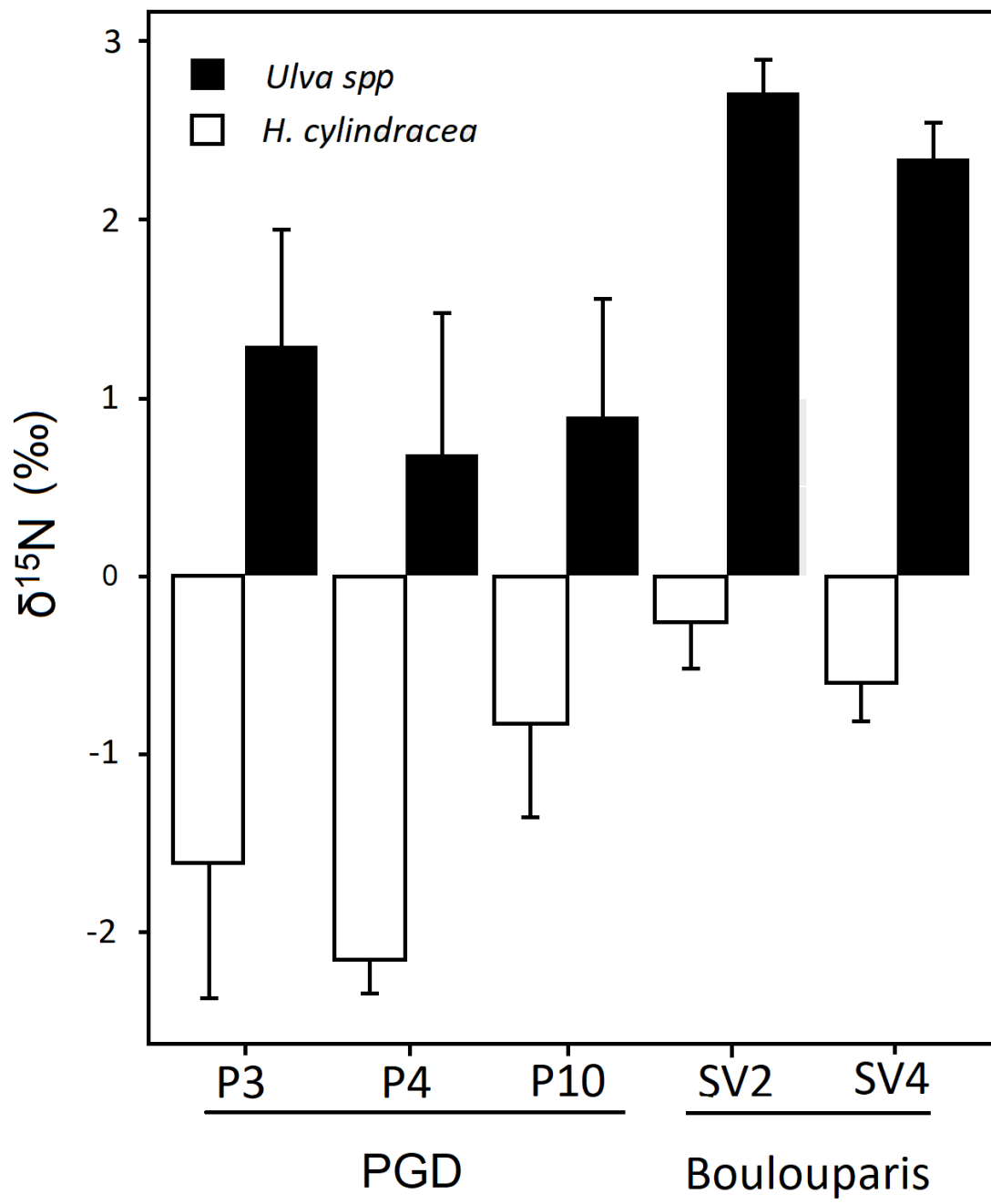


Figure 6

