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Stable Isotope ($\delta^{13}C$, $\delta^{15}N$, $\delta^{18}O$, δD) Composition and Nutrient Concentration of Red Sea Primary Producers

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Measurements of isotopic composition of marine primary producers are a valuable tool to follow and trace the source and cycling of organic matter in the marine systems, as well to describe the physiological status of aquatic photosynthetic organisms. Although stable isotope data abounds in the literature, relatively limited information regarding the isotopic signatures of marine primary producers is available for the Red Sea. Here we present data on carbon concentration (and nitrogen when possible) of phytoplankton, macroalgae, seagrasses, mangroves and salt-marsh plants, and examine how their isotopic signatures differed among plant types across a north-south gradient in the Red Sea. We also tested the potential use of deuterium, δD, to distinguish among primary producers whose carbon isotopic values may overlap. Our findings showed a clear differentiation of carbon and nitrogen content between the different groups of primary producers, as well as between species. Seagrasses and mangroves had on average larger carbon (30 and 49% of C, respectively) and nitrogen content (1.8% N) than other groups. In terms of stable carbon isotopes, seagrasses, and macroalgae tended to be heavier (−7.3 and −13.3‰, respectively) than halophytes, mangroves, and phytoplankton, which showed statistically similar and lighter $\delta^{13}C$ values (between −24 and −26‰). There was a tendency for the nitrogen isotopic composition of seagrass and macroalgae to become lighter from the southern to the northern Red Sea, in parallel to a decline in nitrogen concentration in the tissues, indicative of a higher dependence of nitrogen fixation as a source of nitrogen toward the more oligotrophic northern Red Sea. Our results showed an overlap in the $\delta^{13}C$ and $\delta^{15}N$ values between macroalgae and seagrasses; however, their δD values were significantly different (seagrasses −56.6 ± 2.8‰ and macroalgae −95.7 ± 3.4‰). This remarkable difference offers a promising alternative for ecological studies where a similar range of isotopic values could mask different potential sources.

Keywords: stable isotopes, nitrogen, carbon, deuterium, oxygen

INTRODUCTION

The stable isotope composition of marine primary producers is a fundamental property that informs of their physiology and status (Farquhar et al., 1989; Descolas-Gros and Fontungne, 1990; Maberly et al., 1992; Hemminga and Mateo, 1996; Burkhardt et al., 1999), their sources of nitrogen (Dawson et al., 2002), and allows tracing their contribution in both supplying carbon to the marine food web (Peterson and Fry, 1987) and depositing carbon to the sediment (Kennedy et al., 2010). While in continental plants carbon isotope composition depends mainly of specific enzymatic mechanisms of primary production (e.g., C3, C4, or CAM) and WUE (Water Use Efficiency), in marine plants, all C3 (RuBisCO) depends on the supply and source of carbon (CO_2 or HCO_3-) and specific mechanisms of primary production (e.g., C3 or C4), light level, plant size and growth rate, among others (Farquhar et al., 1989; Descolas-Gros and Fontungne, 1990; Maberly et al., 1992; Hemminga and Mateo, 1996; Burkhardt et al., 1999; Raven et al., 2002). As such, the isotope composition differs significantly from phytoplankton, with $\delta^{13}C$ signatures of about −22‰, to seagrasses, often growing under CO_2-limitation leading to heavy isotopic signatures with values of around −8‰ (Hemminga and Mateo, 1996). Nitrogen isotopes give information of the source of nitrogen, with nitrogen fixation characterized by values similar to atmospheric N values (i.e., $\delta^{15}N$ around −2 to +1‰, Wada and Hattori, 1976), comparable to those derived from fertilizer applications (McClelland et al., 1997), and recycled nitrogen, including heavy (+5‰; Liu et al., 1996) nitrogen isotopes in nitrate in deep ocean waters, and even heavier isotopes associated with human and animal waste inputs (+10 to +20‰, McClelland et al., 1997). Recently, deuterium isotopes (δD) were identified as a way to improve the discrimination of sources of organic matter in food web studies, as the deuterium isotopic signature (δD) of marine plants varies greatly, although the reasons for this variability are not entirely understood (Wilkinson et al., 2015).

Whereas, stable isotope data abound for many ecosystems, these are relatively sparse for the Red Sea, including reports on the stable isotope composition of seston and coral reef biota (Kurten et al., 2014), and stable C and N isotope composition of primary producers in the central Red Sea (Almahasheer et al., 2017).

Here we report the stable isotope composition ($\delta^{13}C$, $\delta^{15}N$, $\delta^{18}O$, and δD) and carbon and nitrogen concentration of primary producers (phytoplankton, macroalgae, seagrass, mangroves, and salt-marsh plants) in the Red Sea. We do so by reporting the results of 597 samples collected along 93 sites distributed from 17.3° North to 28.1° South, and including 37 coastal plant species (8 seagrass species, 23 macroalgae species, 1 mangrove species, and 5 salt-marsh species) as well as mixed phytoplankton communities sampled as seston. We then examined whether their stable isotope signatures differ among plant types, species and across latitude in the Red Sea. Additionally, we assessed the potential of Deuterium isotopes, δD, to discriminate between macroalgae and seagrass, for which proximity in carbon isotope composition often makes their source discrimination difficult based on C isotopes (Kennedy et al., 2010). Specifically, we test the following hypothesis, (1) that the existence of a south to north gradient in nutrient supply and oligotrophy, with nutrient inputs occurring from the south and the waters becoming more oligotrophic toward the north (e.g., Ngugi et al., 2012), should be reflected in a gradient in nitrogen isotopic composition, from positive values in the south to values near 0, consistent with a prevalence of nitrogen fixation (e.g., Hemminga and Mateo, 1996; Kennedy et al., 2004; Papadimitriou et al., 2005) toward the north; (2) that mangroves, with a C3 photosynthetic pathway are likely to have the lightest carbon isotopic composition, whereas seagrasses, which are often carbon-limited, should show the heavier isotopic values (e.g., Hemminga and Mateo, 1996); and (3) that carbon and nitrogen isotopic values are likely to overlap between seagrass and macroalgae, in particular, so that additional isotopes, such as oxygen, $\delta^{18}O$, and deuterium isotopes, δD (e.g., Wilkinson et al., 2015), will be required to be able to discriminate organic matter derived from these primary producers in trophic studies.

METHODS

Study Site

We sampled 93 stations in five habitats along the Red Sea including 16 mangrove stations, 2 saltmarsh stations, 33 seagrass stations, 36 coral reef stations, and 6 pelagic open water stations (**Figure 1**).

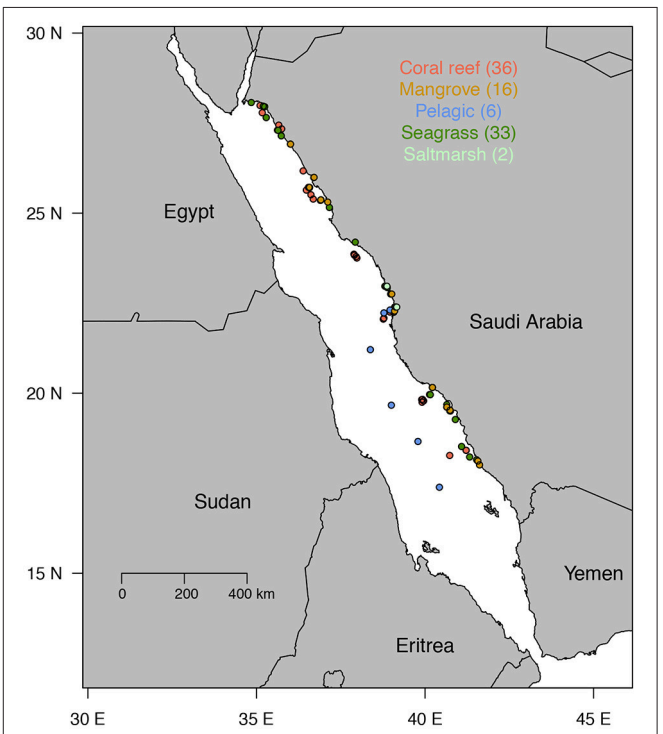

FIGURE 1 | Map indicating the study sites. Colors represent the different types of habitats with in parenthesis the number of sites surveyed for each habitat.

Coastal Habitats Sampling

Coastal habitats were sampled during four coastal cruises onboard the R/V Thuwal on February 2016, January 2017, March 2017, and July 2017 [for further details on cruises and survey methods see (Garcias-Bonet and Duarte, 2017; Anton et al., 2018a)]. Those stations located in the central Red Sea were sampled using small boats on November 2014, February 2016, January 2017, March 2017, and July 2017. We sampled one mangrove species (*Avicennia marina*), 5 halophyte species (*Anabasis setifera*, *Salicornia* sp., *Suaeda monoica*, *Zygophyllumm cocenium,* and one unknown species), 8 species of seagrasses (*Cymodocea nodosa*, *Enhalus acoroides*, *Halodule uninervis*, *Halophila decipiens*, *Halophila ovalis*, *Halophila stipulacea*, *Thalassia hemprichii,* and *Thalassodendron ciliatum*) and 23 species of macroalgae (*Amphiroa fragilissima*, *Caulerpa racemosa*, *Caulerpa serrulata*, *Caulerpa* sp., *Caulerpa taxifolia*, *Colpomenia sinuosa*, *Dictyosphaeria cavernosa*, *Dictyota* sp., *Galaxaura* sp., a green filamentous alga, *Halimeda tuna*, *Halymenia* sp., *Lobophora variegata*, *Mesophylium mesomorphum*, *Padina pavonica*, *Padina* sp., *Sargassum ilicifolium*, *Sargassum* sp., *Turbinaria ornata*, *Tydemania expeditionis*, *Udotea flabellum,* and 2 unknown macroalgal species) (**Table 1**). At each station, we collected macrophyte blades from the most common macrophyte species. We sampled 4 mangrove leaves, 4 halophyte leaves, 4 seagrass shoots for the small size seagrass species, 4 second-youngest leaves for the larger-sized seagrass species, and 4 blades for each macroalgal species. The samples were transported onboard or back to the laboratory and processed immediately.

Coastal Habitats Sample Processing

Seagrass and macroalgae blades were rinsed with seawater collected from the same location and epiphytes were carefully removed. Mangrove, halophyte, seagrass, and macroalgae blades were dried at 60°C until constant weight was reached. Dried macrophyte tissue samples were ground and acidified to remove inorganic carbon before the carbon (C) and nitrogen (N) content and stable isotopic composition ($\delta^{13}C$, $\delta^{15}N$, $\delta^{18}O$, and δD) analyses. Specifically, carbonates were removed from macrophyte tissues by exposing the samples to chloride baths and vapors (1M HCl) overnight after which samples were re-dried at 60°C.

Macrophyte Carbon and Nitrogen Content and Isotopic Composition ($\delta^{13}C$, $\delta^{15}N$, $\delta^{18}O$, and δD) Analyses

Elemental C and N content analysis of macrophyte blade samples were performed with an Organic Elemental Analyser Flash 200 (Thermo Fisher Scientific, Massachusetts, USA). The accuracy was <0.2, <0.1, and <0.08% for C, N, and H, respectively. The isotopic composition analyses of macrophyte blades were performed by elemental analysis isotope ratio mass spectrometry at the UH Hilo Analytical Laboratory (USA) and the Stable Isotope Laboratory of the Instituto Andaluz de Ciencias de la Tierra (CSIC-UGR, Spain) and by cavity ring-down spectroscopy at the Tarek Ahmed Juffali Research in Red Sea Ecology Laboratory (KAUST, Saudi Arabia). Stable isotope ($\delta^{13}C$ and $\delta^{15}N$) analysis of macrophyte samples collected on November 2014 were performed on Delta V mass spectrometer with Conflo III coupled to a Costech 4010 elemental analyzer (Thermo Scientific, Massachusetts, USA). Isotopic composition ($\delta^{13}C$, $\delta^{15}N$, $\delta^{18}O$, and δD) analyses of macrophyte samples collected from February 2016 to March 2017 were performed on a Carlo Elba NC1500 (Milan, Italy) elemental analyzer coupled on-line via a ConFlow III with a Delta Plus XP (Thermo-Finnigan, Bremen, Germany) mass spectrometer (EA-IRMS). Commercial CO_2 and N_2 were used as the internal standard for the C and N isotopic analyses.

For $\delta^{13}C$ analysis, we used internal standards of −30.63 and −11.65‰ (V-PDB), and for $\delta^{15}N$ analysis, we used internal standards of −1.02 and +16.01‰ (AIR). The analytical precision was better than ±0.1‰ for $\delta\ ^{15}N$ and $\delta\ ^{13}C$, calculated from standards systematically interspersed in sample batches and after the correction of the mass spectrometer daily drift. The standard for reporting C measurements is V-PDB (Vienna-PDB) and for N measurements atmospheric nitrogen (AIR).

Isotopic composition ($\delta^{13}C$) analysis of macrophyte samples collected on July 2017 was performed by cavity ring-down spectroscopy (CM-CRDS G2201-I, Picarro, California, USA) attached to a combustion module (Costech Analytical Technologies Inc., California, USA). Dried and acidified macrophyte tissue samples were encapsulated in tin capsules. We used internal standards and three certified solid C standards from the Reston Stable Isotope Laboratory (United States Geological Survey, USGS, Virginia, USA): USG62 (Caffeine, $\delta\ ^{13}C = -14.79$ ‰), USG40 (glutamic acid, $\delta\ ^{13}C = -26.39$ ‰), and USG41a (L-glutamic acid enriched in ^{13}C, $\delta\ ^{13}C = +36.55$‰). The analytical precision and accuracy of $\delta^{13}C$ measurements were ±0.13 and ±0.69‰, respectively.

The stable isotopic composition was calculated as:

$$\delta\ =\ (R_{sample}/R_{standard} - 1)^{*}1000$$

where:

$$R\ =\ {}^{13}C/{}^{12}C \text{ for } \delta^{13}C \text{ values}$$
$$R\ =\ {}^{15}N/{}^{14}N \text{ for } \delta^{15}N \text{ values}$$
$$R\ =\ {}^{18}O/{}^{16}O \text{ for } \delta^{18}O \text{ values}$$
$$R\ =\ D/H \text{ for } \delta D \text{ values}$$

For the $\delta^{18}O$ and δD analysis of macrophyte tissues, samples were loaded in silver capsules, subjected to vacuum (10^{-3} mbar) and heat (70°C) for 48 h in a metallic carousel. Then, argon was introduced to reach atmospheric pressure, avoiding any contamination with atmospheric water vapor. Samples were passed through a ceramic column containing a glassy carbon tube at 1450°C to produce H_2 and CO gases (Sharp et al., 2001) in a high-temperature reactor (TC/EA) coupled on-line via a ConFlow III interface to a Delta XP isotope ratio mass spectrometer (Thermo-Finnigan, Bremen). These gases were separated by chromatography using a helium carrier gas stream. To avoid memory effects, each sample was analyzed 6 times, discarding the first 3 analyses and averaging the

TABLE 1 | Summary with information of the 93 surveyed study sites in the Arabian Red Sea.

Lat	Long	Habitat	Date	Depth (m)	T (°C)	S (PSU)	Species collected
18.117	41.560	Coral reef	10/3/17	2	29.20	37.94	*Dictyota* sp., *Sargassum ilicifolium*
18.413	41.222	Coral reef	12/3/17	3	28.86	38.38	*Caulerpa serrulata, Halimeda tuna, Turbinaria ornata,*
18.416	41.216	Coral reef	27/02/16	3	29.10	38.82	*Caulerpa racemosa, Halimeda tuna, Halymenia* sp., *Sargassum ilicifolium*
19.505	40.753	Coral reef	28/02/16	3	28.79	38.91	*Sargassum ilicifolium, Turbinaria ornata*
19.611	40.644	Coral reef	29/02/16	5	28.79	38.91	*Halimeda tuna, Udotea flabellum*
19.750	39.911	Coral reef	20/7/17	10	30.00	38.02	*Turbinaria ornata*
19.788	39.956	Coral reef	20/7/17	10	31.20	37.94	*Tydemania expeditionis*
19.788	39.957	Coral reef	17/03/17	10	28.18	38.91	*Dictyosphaeria cavernosa, Lobophora variegata, Lobophora variegata, Mesophylium mesomorphum*
19.821	39.901	Coral reef	17/03/17	10	27.18	38.82	*Turbinaria ornata, Tydemania expeditionis,Udotea flabellum*
19.828	39.926	Coral reef	17/03/17	10	27.18	38.85	*Amphiroa fragilissima, Galaxaura* sp.
22.057	38.762	Coral reef	21/03/17	10	24.77	39.49	*Amphiroa fragilissima, Galaxaura* sp., *Halimeda tuna, Turbinaria ornata, Tydemania expeditionis*
22.085	38.781	Coral reef	16/8/17	10	31.48	39.44	*Turbinaria ornata, Tydemania expeditionis*
22.253	38.961	Coral reef	4/4/16	2	27.77	38.73	*Halimeda tuna, Turbinaria ornata*
22.253	38.961	Coral reef	17/4/17	3	29.95	38.48	*Turbinaria ornata*
23.754	37.977	Coral reef	15/7/17	10	31.67	38.35	*Turbinaria ornata*
23.793	37.956	Coral reef	15/7/17	10	31.67	38.29	*Tydemania expeditionis*
23.832	37.904	Coral reef	27/03/17	10	25.76	39.58	*Turbinaria ornata*
23.860	37.890	Coral reef	27/03/17	10	24.71	39.54	*Amphiroa fragilissima, Tydemania expeditionis*
25.355	36.895	Coral reef	22/01/17	10	24.58	40.12	*Tydemania expeditionis*
25.363	36.912	Coral reef	22/02/16	6	23.35	40.21	*Caulerpa serrulata, Tydemania expeditionis*
25.391	36.683	Coral reef	21/01/17	10	24.51	40.12	*Turbinaria ornata, Tydemania expeditionis*
25.510	36.616	Coral reef	13/7/17	10	30.90	38.84	*Tydemania expeditionis*
25.640	36.480	Coral reef	13/7/17	10	30.30	39.00	*Turbinaria ornata*
25.704	36.568	Coral reef	21/02/16	4	23.87	40.53	*Dictyosphaeria cavernosa*
25.717	36.542	Coral reef	21/02/16	6	24.13	40.12	*Turbinaria ornata*
25.990	36.699	Coral reef	6/3/17	2	25.21	40.45	*Halimeda tuna*
26.173	36.385	Coral reef	6/3/17	1	24.15	40.39	*Galaxaura* sp., *Turbinaria ornata*
26.918	36.006	Coral reef	5/3/17	3	25.17	40.48	*Galaxaura* sp.
27.300	35.640	Coral reef	11/7/17	10	31.27	38.76	*Tydemania expeditionis*
27.304	35.623	Coral reef	11/7/17	10	31.51	39.08	*Turbinaria ornata*
27.339	35.747	Coral reef	19/01/17	10	23.61	40.23	*Tydemania expeditionis*
27.444	35.661	Coral reef	19/01/17	10	23.46	40.24	*Turbinaria ornata, Tydemania expeditionis*
27.654	35.289	Coral reef	3/3/17	1	22.77	40.28	*Amphiroa fragilissima*
27.789	35.171	Coral reef	3/3/17	1	24.02	40.32	*Sargassum ilicifolium, Turbinaria ornata*
27.989	35.106	Coral reef	19/02/16	3	23.22	40.53	*Turbinaria ornata*
18.009	41.617	Mangrove	11/3/17	1	27.78	44.42	*Avicennia marina*
18.118	41.567	Mangrove	10/3/17	1	30.77	38.65	*Avicennia marina*
19.528	40.741	Mangrove	28/02/16	0	–	–	*Avicennia marina*
19.616	40.646	Mangrove	29/02/16	0	30.46	39.36	*Avicennia marina*
20.160	40.220	Mangrove	26/02/16	0	26.61	40.16	*Avicennia marina*
20.160	40.220	Mangrove	28/02/16	0	30.04	39.40	*Avicennia marina*
22.282	39.097	Mangrove	10/11/14	0.5	–	–	*Avicennia marina, Padina pavonica, Sargassum* sp., *Turbinaria ornata,* Unknown spp 2, *Unknown* spp *3*
22.392	39.130	Mangrove	12/11/14	0.5	–	–	*Avicennia marina, Padina pavonica*
22.753	39.013	Mangrove	16/11/14	0.5	–	–	*Avicennia marina, Suaeda monoica, Unknown* spp *1*
22.971	38.846	Mangrove	17/11/14	0.5	–	–	*Anabasis setifera, Avicennia marina, Colpomenia sinousa, Padina pavonica, Sargassum* sp., *Turbinaria ornata, Unknown* spp *3*
25.307	37.114	Mangrove	7/3/17	0	19.48	41.98	*Avicennia marina*
25.368	36.909	Mangrove	23/02/16	0	22.28	40.70	*Avicennia marina*

(Continued)

TABLE 1 | Continued

Lat	Long	Habitat	Date	Depth (m)	T (°C)	S (PSU)	Species collected
25.715	36.577	Mangrove	22/02/16	0	21.75	40.49	*Avicennia marina*
25.717	36.560	Mangrove	21/02/16	0	24.45	40.44	*Avicennia marina*
25.993	36.708	Mangrove	6/3/17	0	21.50	41.58	*Avicennia marina*
26.915	36.011	Mangrove	5/3/17	0	24.67	41.06	*Avicennia marina*
17.388	40.426	Pelagic	20/3/18	2	–	–	Seston
18.660	39.790	Pelagic	19/3/18	2	–	–	Seston
19.667	39.000	Pelagic	18/3/18	2	–	–	Seston
21.209	38.378	Pelagic	17/3/18	2	–	–	Seston
22.230	38.780	Pelagic	22/3/18	2	–	–	Seston
22.309	38.961	Pelagic	17/3/18	2	–	–	Seston
22.392	39.160	Saltmarsh	12/11/14	0.5	–	–	*Salicornia* sp., *Zygophyllumm cocenium*
22.971	38.870	Saltmarsh	17/11/14	0.5	–	–	*Anabasis setifera, Salicornia* sp., Unknown spp 1
18.119	41.565	Seagrass	10/3/17	0.5	32.16	38.24	*Caulerpa* sp., *Halodule uninervis, Halophila ovalis*
18.150	41.531	Seagrass	11/3/17	0.7	29.90	38.47	*Caulerpa* sp., *Padina pavonica, Thalassia hemprichii,*
18.229	41.323	Seagrass	11/3/17	6.2	29.63	38.27	*Halodule uninervis, Thalassia hemprichii*
18.520	41.084	Seagrass	12/3/17	1.5	29.73	38.53	*Caulerpa taxifolia, Padina pavonica, Thalassia hemprichii*
19.270	40.899	Seagrass	28/02/16	0.5	29.01	38.89	*Caulerpa racemosa, Halophila decipiens, Thalassia hemprichii*
19.509	40.735	Seagrass	28/02/16	1	29.06	37.45	*Thalassia hemprichii*
19.682	40.645	Seagrass	29/02/16	0.5	29.47	38.86	*Halodule uninervis, Halophila ovalis, Halophila stipulacea, Thalassia hemprichii, Udotea flabellum*
19.959	40.155	Seagrass	26/02/16	0.5	29.53	38.68	*Halodule uninervis, Halophila stipulacea, Thalassia hemprichii*
20.159	40.215	Seagrass	18/03/17	1	28.52	39.58	*Halimeda tuna, Halodule uninervis, Padina pavonica, Thalassia hemprichii*
20.159	40.217	Seagrass	26/02/16	0.5	26.23	40.58	*Halimeda tuna, Halodule uninervis, Halophila decipiens, Padina pavonica, Thalassia hemprichii*
22.249	39.072	Seagrass	21/03/17	2.5	24.83	39.94	*Halophila ovalis, Halophila stipulacea*
22.249	39.072	Seagrass	16/8/17	2.5	33.63	40.00	*Halophila ovalis, Halophila stipulacea*
22.282	39.060	Seagrass	10/11/14	0.5	–	–	*Halophila stipulacea*
22.392	39.100	Seagrass	12/11/14	0.5	–	–	*Enhalus acoroides, Halodule uninervis, Thalassia hemprichii, Thalassodendron ciliatum*
22.393	39.131	Seagrass	10/4/16	1.5	29.44	41.43	*Enhalus acoroides*
22.393	39.131	Seagrass	17/4/17	1.5	–	39.55	*Cymodocea nodosa, Enhalus acoroides, Halodule uninervis*
22.753	38.980	Seagrass	16/11/14	0.5	–	–	*Halodule uninervis, Thalassia hemprichii, Thalassodendron ciliatum*
22.934	38.880	Seagrass	5/4/16	1.5	27.90	41.30	*Halophila stipulacea*
22.971	38.810	Seagrass	17/11/14	0.5	–	–	*Halodule uninervis, Halophila stipulacea, Thalassia hemprichii, Thalassodendron ciliatum*
24.194	37.930	Seagrass	28/03/17	2	24.26	39.96	*Halodule uninervis, Halophila ovalis, Thalassia hemprichii*
24.194	37.930	Seagrass	13/8/17	2	31.16	39.11	*Halodule uninervis, Halophila ovalis, Halophila stipulacea, Thalassia hemprichii*
25.158	37.163	Seagrass	7/3/17	3.5	22.68	40.45	*Dictyosphaeria cavernosa, Dyctiota* sp., *Halophila stipulacea, Padina* sp., *Thalassodendron ciliatum,*
25.362	36.911	Seagrass	22/02/16	1	22.85	40.36	*Halodule uninervis, Halophila decipiens, Halophila ovalis*
25.371	36.914	Seagrass	23/02/16	1	22.85	40.36	*Halodule uninervis, Halophila decipiens, Padina pavonica*
25.702	36.569	Seagrass	21/02/16	4	23.87	40.53	*Halophila decipiens, Halophila ovalis, Halophila stipulacea*
25.989	36.699	Seagrass	6/3/17	2	22.92	40.48	*Halodule uninervis, Halophila decipiens*
26.917	36.008	Seagrass	5/3/17	1	25.21	40.45	*Halodule uninervis, Halophila ovalis, Halophila stipulacea, Thalassia hemprichii*
27.146	35.736	Seagrass	20/01/17	3.5	23.06	40.32	*Halophila stipulacea,*
27.300	35.640	Seagrass	11/7/17	7	30.27	39.40	*Thalassodendron ciliatum,*
27.654	35.289	Seagrass	3/3/17	6	22.77	40.28	*Padina pavonica, Thalassodendron ciliatum*
27.955	35.245	Seagrass	20/02/16	2	23.32	40.59	*Thalassia hemprichii, Thalassodendron ciliatum*
27.969	35.201	Seagrass	20/02/16	8	22.55	40.65	*Halophila stipulacea*
28.072	34.846	Seagrass	4/3/17	1	17.41	41.12	*Green filamentous, Halodule uninervis, Halophila ovalis, Halophila stipulacea, Thalassia hemprichii*

The full data set can be found in Anton et al. (2018b).

last 3 measurements. Commercial CO and H_2 bottles and different standards, previously calibrated vs. V-SMOW (IAEA international standards), were used as internal standards for the hydrogen isotopic analysis. The analytical precision and accuracy of $\delta^{18}O$ and δD were ± 0.3 and $\pm 1‰$, respectively, calculated from standards systematically interspersed in sample batches and after the correction of the mass spectrometer daily drift. The standard for reporting oxygen and hydrogen is V-SMOW (Vienna Standard Mean Ocean Water).

Pelagic Open Water Sampling

Red Sea pelagic open water samples were collected during an offshore scientific cruise on board the R/V Al Azizi along a latitudinal transect (17–21° N) on March 2018 and from a station located off-shore the central Red Sea, between May to December 2017. During the transect, water samples were taken at five different depths (100, 60, 22, 8, and 1% of the photosynthetic active radiation, PAR) using a multi-sample/carousel rosette system equipped with 12 Niskin bottles. The samples from the central Red Sea were collected from surface waters (~2 m) with a manually deployed Niskin bottle.

Pelagic Open Water Sample Processing and Carbon Isotope ($\delta^{13}C$) Analysis

After water collection, a 1-L sample from each depth was filtered onto a pre-combusted 15 mm Whatman GF/F filter and then placed in a small Petri dishes that remained overnight with 100–150 μL of 50% HCl to remove inorganic carbonates. Analyses were performed on desiccated filters, for 24 h, in a desiccator. Filters were encapsulated in tin capsules, and carbon isotope ($\delta^{13}C$) composition was analyzed by cavity ring-down spectroscopy (CM-CRDS G2201-I, Picarro, California, USA) attached to a combustion module (Costech Analytical Technologies Inc., California, USA). Cavity ring-down spectrometer calibration was performed as described in the macrophyte analysis section. The stable isotopic composition was calculated as described in the macrophyte analysis section.

Statistical Analyses

Differences among types of primary producers were tested using ANOVA, with significant differences between specific groups assessed using Tukey *post-hoc* HSD test, and the relationships between pairs of variables were assessed using linear regression analysis, after checking the compliance of the data with the assumptions of the analysis. Discriminant analysis was used to test the power of combinations of stable isotopes to resolve differences between primary producer types, specifically seagrass and macroalgae. Principal component analysis was used to explore the contribution of the different tissue properties measured (concentration and isotopic composition of elements) to variability within and among primary producer groups. All analyses were conducted in the JMP vs. 13 statistical analysis software.

RESULTS

The nitrogen concentration in the plant tissues increased with increasing carbon concentration (**Figure 2**, $R^2 = 0.42$, $P < 0.0001$), and the different primary producer types differed significantly both in carbon and nitrogen concentration (**Table 2**, ANOVA, $P < 0.0001$). The carbon and nitrogen concentrations increased from macroalgae, which had the lowest C and N concentration, to seagrass and mangroves, which had the highest C and N concentrations (**Table 2**). Seagrass had the lowest C/N ratio, followed by macroalgae, while halophytes and mangroves had similar high C/N ratios (**Table 2**, ANOVA and Tukey *post-hoc* HSD test, $P < 0.0001$).

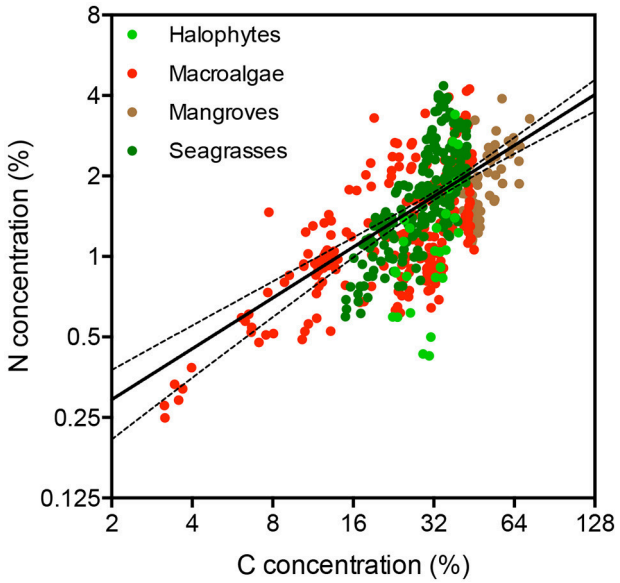

FIGURE 2 | Relationship between nitrogen and carbon concentration in the tissues of Red Sea mangroves (brown dots), halophytes (light green dots), seagrasses (dark green dots), and macroalgae (red dots). The solid line shows the fitted regression line across all plant types ±95% CI of the slope.

Macroalgae and, particularly, seagrasses presented heavy carbon isotopic composition, while seston, halophytes, and mangroves had similar light values (**Table 2**, ANOVA, $P < 0.0001$, and Tukey *post-hoc* HSD test, $P < 0.01$). Seagrasses had the lightest nitrogen isotopic composition, whereas macroalgae and mangroves had similar, intermediate nitrogen isotopic composition and halophytes had the heaviest composition (**Table 2**, ANOVA, $P < 0.0001$, and Tukey *post-hoc* HSD test, $P < 0.0001$). Oxygen isotopic composition, $\delta^{18}O$, ranged broadly among seagrass and macroalgae, but did not differ significantly among these two groups of primary producers (**Table 2**). Carbon and nitrogen isotopes were analyzed using two different instruments each. Whereas standards were used to ensure the accuracy of results, the differences in instrumentation may have introduced some bias in the comparison of isotopic values among primary producers (Mill et al., 2008). However, provided the broad ranges of isotopic values covered (**Tables 1**, **2**), these possible biases are unlikely to affect the comparisons drawn here.

Deuterium isotopes clearly discriminated seagrasses (−56.6 ± 2.8‰) from the lighter δD values of macroalgae (−95.7 ± 3.4‰), with a mean difference in excess of 39‰ units (**Table 2**,

TABLE 2 | Mean ± SE of the concentration of carbon (C) and nitrogen (N) and the C/N and the stable isotopes of carbon ($\delta^{13}C$), nitrogen ($\delta^{15}N$), oxygen ($\delta^{18}O$), and hydrogen (δD) measured in the blades of the different taxa of primary producers.

Taxa	C (%)	N (%)	C/N	$\delta^{13}C$	$\delta^{15}N$	$\delta^{18}O$	δD
Halophyte	31.48 ± 1.12	1.22 ± 0.14	37.63 ± 3.45	−24.21 ± 1.1	4.19 ± 0.56	–	–
	(27) A	(27) A	(27)	(27) A	(27) A		
	22.46–39.62	0.42–3.4	13.18–84.44	−29.36 to −11.44	−1.89–7.93	–	–
Macroalgae	26.28 ± 0.79	1.37 ± 0.05	24.55 ± 0.76	−13.38 ± 0.3	1.74 ± 0.12	23.39 ± 0.51	−95.7 ± 3.6
	(220) B	(220) A	(220)	(185) B	(165) B	(33) A	(33) A
	3.15–45.27	0.25–4.23	6.17–56.76	−25.6 to −2.09	−2.3–8.98	12.95–27.11	−136 to −57
Mangrove	49.39 ± 0.94	1.89 ± 0.07	32.42 ± 0.9	−26.58 ± 0.13	1.72 ± 0.29	–	–
	(64) C	(64) B	(64)	(64) C	(64) B		
	40.89–73	1.12–3.89	16.02–46.0	−28.91 to −24.24	−1.36–9.44	–	–
Seagrass	30.89 ± 0.52	1.84 ± 0.80	21.47 ± 0.41	−7.73 ± 0.11	0.19 ± 0.24	22.35 ± 0.37	−56.6 ± 2.7
	(213) A	(213) B	(222)	(188) C, D	(155) C	(44) A	(50) B
	14.98–42.58	0.6–4.35	9.33–40.83	−12.41 to −4.1	−14.75–4.44	17.2–26.8	−93 to −18
Seston	–	–	–	−25.43 ± 0.42	–	–	–
				(38) D			
	–	–	–	−29.54 to −19.49	–	–	–

Sample size is given in parenthesis with the range of values indicated below. Primary producers with different letters (A, B, C, D) display statistically significant differences in the properties examined (Tukey post-hoc HSD test).

ANOVA, $P < 0.0001$). The combined use of δD and $\delta^{13}C$ isotopes improved the capacity to discriminate between seagrass and macroalgae (Discriminant analysis, $R^2 = 0.72$, misclassification error 9.6%, **Figure 3**). Indeed, δD and $\delta^{13}C$ isotopes thresholds corresponding to the lower values found here for seagrass leaves (δD >−92.89‰ and $\delta^{13}C$ >−9.93‰) largely discriminates between seagrass (heavier isotopic composition) and macroalgae tissues (lighter isotopic composition), including only 4 (i.e., 12%) seaweed samples in isotopic ranges characterized by isotopic signatures (δD >−92.89‰ and $\delta^{13}C$ >−9.93‰) above those thresholds (**Figure 3**).

Macroalgal N was significantly lower for macroalgae growing in coral reefs than for those growing in seagrass meadows or mangrove forests (ANOVA and Tukey HSD test, $P < 0.001$), whereas their carbon concentration was the highest when growing in mangrove forests (ANOVA and Tukey HSD test, $P < 0.001$). There was no difference in the C, N, and D isotopic composition of macroalgae with habitat ($P > 0.05$). There was a tendency for the nitrogen isotopic composition of seagrass and macroalgae, but not that of mangroves, to become lighter from the southern to the northern Red Sea, in parallel to a decline in nitrogen concentration of the tissues, indicative of a higher dependence of nitrogen fixation as a source of nitrogen toward the more oligotrophic northern Red Sea (**Figure 4**).

Principal component analysis showed that the combination of carbon and nutrient concentration and stable isotope composition separated the different primary producers (**Figure 5**). The first principal component had the highest positive loadings for %C and %N and the most negative loadings for $\delta^{18}O$ and $\delta^{13}C$, accounting for 36.1% of the variance in the data. The second principal component had the highest positive loadings for δD and %N and the most negative loadings for C/N and $\delta^{15}N$, accounting for 25.8% of the variance in the elemental composition of Red Sea primary producers (**Figure 5**).

Much of the variability within plant types was attributable to differences among species. Nitrogen and carbon isotopes differed significantly among halophyte species (**Figure 6**). Carbon isotopes differed greatly between macroalgal species, which had similar nitrogen isotopic composition, except for *Dystiosphaeria cavernosa*, which had significantly lighter N isotopic composition than most other macroalgal species (**Figure 6**). Deuterium isotopes also differed significantly ($P < 0.05$) between macroalgal species. Seagrass species also differed significantly in C, N, and Deuterium isotopes, with *Enhalus acoroides* characterized by remarkably heavy carbon isotopic composition and *H. uninervis* having the heaviest deuterium isotope composition (**Figure 6**).

DISCUSSION

The results provide a comprehensive resource on carbon and nitrogen of primary producers for isotopic studies in the Red Sea, and characterize the variability in C and N concentration, which was coupled across primary producers, and isotopic composition among plant types as well as within species among the same group, except mangroves, for which *A. marina* comprises over 99% of the Red Sea cover, and seston, for which species-specific analyses were not possible. Much of the variability within the different primary producers reported here was attributable to differences among species, but differences among types of primary producers were also important. However, seagrass and macroalgal nitrogen concentration tended to decline with increasing latitude, reflecting the south to north oligotrophication gradient derived from the role of the inflow of Indian Ocean waters through the Bab al Mandel straight as the main nutrient supply to the Red Sea (e.g., Ngugi et al.,

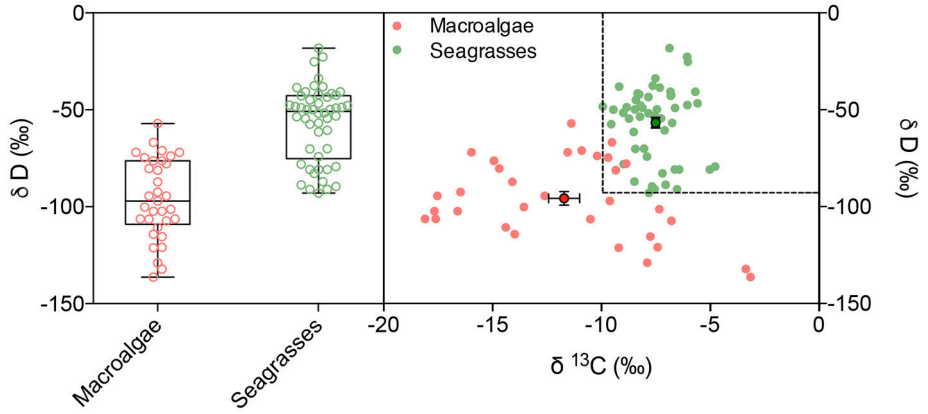

FIGURE 3 | Deuterium stable isotope concentration **(left)** and the relationship between $\delta^{13}C$ and δD **(right)** on the blades of macroalgae and seagrasses, indicated in red and green, respectively. Dashed lines indicate the minimum values of $\delta^{13}C$ and δD for seagrass tissues. Mean ± SE values of $\delta^{13}C$ and δD values are shown with dots and error bars.

2012). The variability in isotopic composition of Red Sea primary producers may, however, be larger than represented here, as variability may occur with depth and season (Fourqurean et al., 2007; Viana and Bode, 2015), which were not comprehensively sampled here.

Seagrasses tended to be isotopically heavy in terms of carbon, consistent with previous results (Hemminga and Mateo, 1996; Kennedy et al., 2004; Papadimitriou et al., 2005). This reflects the fact that their photosynthesis tends to be carbon-limited, and consequently, have less isotopic discrimination of ^{13}C. In addition, almost all samples were collected in shallow waters, where most seagrass habitats are found in the Red Sea, and where seagrass carbon isotopic composition is expected to be heavier (e.g., Fourqurean et al., 2007). Their nitrogen isotopic composition suggests nitrogen fixation as the main source of nitrogen for these plants, in agreement with previous reports on seagrass stable N isotopic composition (Hemminga and Mateo, 1996; Kennedy et al., 2004; Papadimitriou et al., 2005; Garcias-Bonet et al., 2016). Nitrogen fixation rates are typically high in seagrass sediments, due to mutualistic or symbiotic association exists between the seagrasses and heterotrophic nitrogen fixers in the rhizosphere (Welsh, 2000), with additional, but much smaller, contributions of epiphytic diazotrophs (e.g., Moriarty and O'Donohue, 1993). The trend toward a lighter, closer to atmospheric signal, of the nitrogen isotopic composition of macroalgae and seagrass from south to north, in parallel with a decline in nitrogen concentration in the tissues, indicates that the importance of nitrogen fixation as a source of nitrogen increases toward the more oligotrophic waters of the northern Red Sea. A recent study from the UK also shows variation in the $\delta^{15}N$ signal of seagrass tissue in response to exposure to eutrophication (Jones et al., 2018). The consistent latitudinal pattern of decline in $\delta^{15}N$ signal of Red Sea primary producers, together with the parallel decline in nitrogen concentration on plant tissues, represents a isoscape (*sensu* Bowen, 2010) that can be used to predict or interpolate expected isotopic values at Red Sea locations not sampled here (Bowen, 2010).

The nitrogen isotopic composition of Red Sea seagrass ranged widely ($-14.75 > \delta^{15}N < +4.44$‰ vs. AIR) including some extremely negative values. High variability in the $\delta^{15}N$ signal of seagrass tissue has been shown in the past, in particular in relation to eutrophication (Jones et al., 2018) and sewage inputs ($\delta^{15}N > 6$‰, Costanzo et al., 2001). None of the seagrass sampled here were collected near human populations, which are limited along the Saudi coast of the Red Sea, so sewage inputs are unlikely to affect seagrass $\delta^{15}N$ values. Values approaching +5‰ (AIR) are characteristic of nitrate from deep ocean waters (Sigman et al., 1999, 2009), where NO_x may exceed 20 μmol L^{-1} (Churchill et al., 2014; Zarokanellos et al., 2017), as well as waters dominated by recycled processes where an enrichment in ^{15}N in the dissolved nitrate pool occurs due to Rayleigh fractionation by preferential uptake of ^{14}N (Churchill et al., 2014). These positive values may also result from denitrification in oxygen-depleted waters (Somes et al., 2010) as a source of reduced nitrogen, which typically dominate in the seagrass rhizosphere, for seagrass (NH_4^+, NO_2^-) since this process involves an important isotopic fractionation between 15 and 35‰ (Robinson, 2001). Likewise, nitrogen inputs with atmospheric dust deposition may also support negative isotopic values, as nitrogen isotopic values in adjacent desert areas are characterized by values ranging from −6.9 to +1.9‰ (Wankel et al., 2010). $\delta^{15}N$ values next to zero or slightly positive are consistent with nitrogen fixation as the main nitrogen source for these plants, as often reported for seagrass tissues (Hemminga and Mateo, 1996; Kennedy et al., 2004; Papadimitriou et al., 2005; Garcias-Bonet et al., 2016). There was no relationship between the $\delta^{15}N$ composition of the seagrass tissues and their C/N ratio, which were expected from relationships between nitrogen fixation rates, which affect the δ15N composition of the plants, and their C/N ratio reported in the past (Cook et al., 2015; Russell et al., 2016).

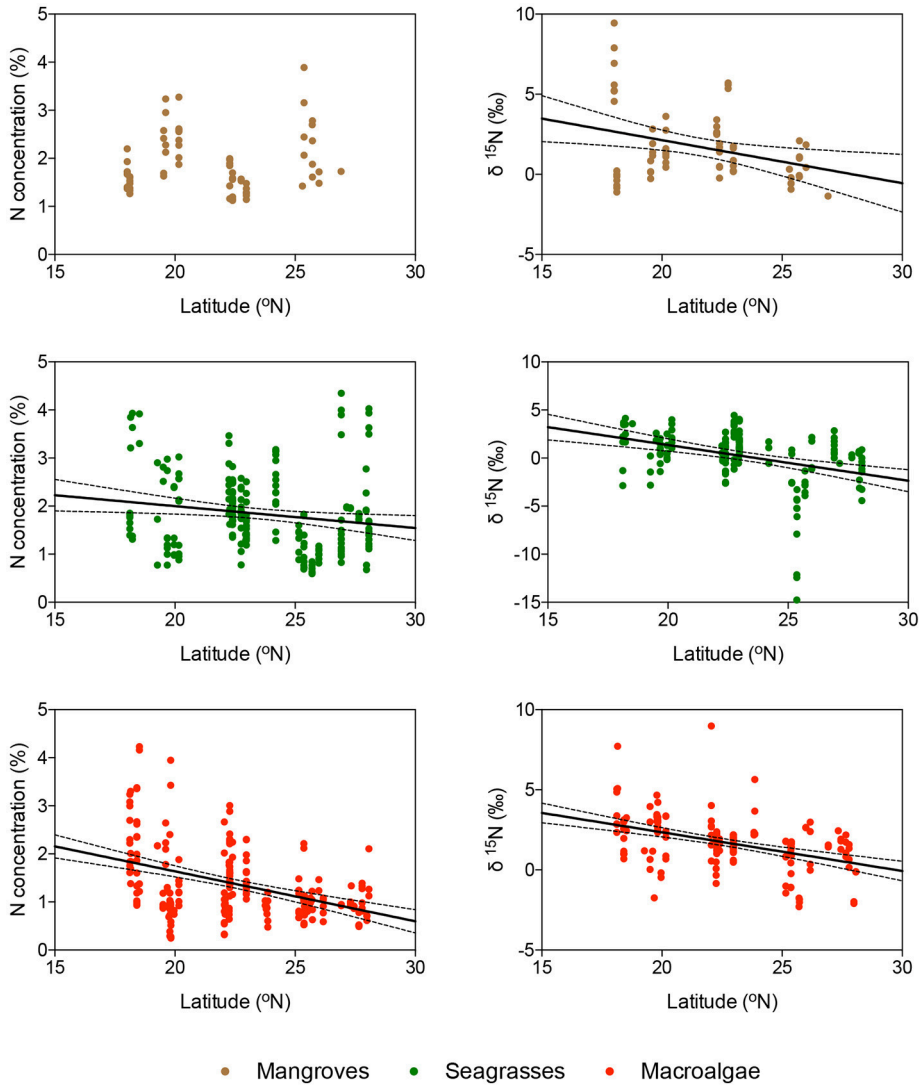

FIGURE 4 | Relationship between latitude and the concentration of nitrogen (panels on the left) and the δ^{15}N composition (panels on the right) of the blades of (from top to bottom) mangroves, seagrasses, and macroalgae, indicated in brown, green, and red, respectively. Solid lines show the fitted linear regression equations and dotted lines show the 95% c.l. of the slope. Only significant regressions are shown.

Seagrass, macroalgae, and mangroves showed a trend toward declining nitrogen isotopic values from south to north, spanning from positive (+3 to +15‰) values in the south to values around 0 to +1‰ in the Northern Red Sea (**Figure 4**). The positive values in the south correspond to nitrate inputs from the Indian Ocean (Brandes et al., 1998) through the Strait of Bab-EI-Mandab, specifically Gulf of Aden Intermediate Water, which inflow is higher during summer/autumn (Wafar et al., 2016). In contrast, the values near 0 in the Northern Red Sea signal at a prevalence of nitrogen fixation as a source of nitrogen.

Macroalgae overlapped with seagrass in both carbon and nitrogen isotopic composition but showed a broader range consistent with the greater diversity of phylogenetic form and physiology of this polyphyletic group. Hence, stable isotopes of C and N used elsewhere to apportion the sources of carbon in food webs (e.g., Peterson and Fry, 1987) and sediment carbon stocks (e.g., Kennedy et al., 2004; Papadimitriou et al., 2005), cannot discriminate seagrass from macroalgae-derived carbon in the Red Sea (e.g., Almahasheer et al., 2017). However, the exploratory analyses of deuterium isotopes conducted here confirmed earlier reports from lakes (Cole et al., 2011) and coastal studies (Wilkinson et al., 2015), in that deuterium isotopes can discriminate among primary producers that overlap in carbon isotope values. Indeed, deuterium isotopes differed significantly between Red Sea seagrass and macroalgae and, together with carbon isotopes, helped discriminate seagrass from macroalgae. Carbon isotopes combined with deuterium

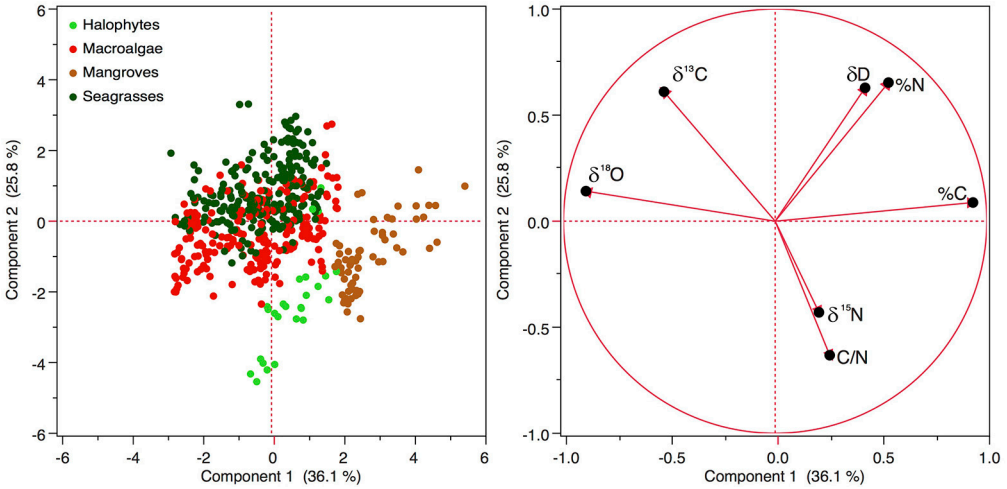

FIGURE 5 | Principal component analysis of the 5 types of primary producers according to the concentration of the stable isotopes ($\delta^{13}C$, $\delta^{15}N$, $\delta^{18}O$, and δD) and carbon and nitrogen and the ratio C/N in the plant tissues. Seston samples were excluded from the analysis as it was missing $\delta^{18}O$ and δD, so the loadings of seston on the axis are biased.

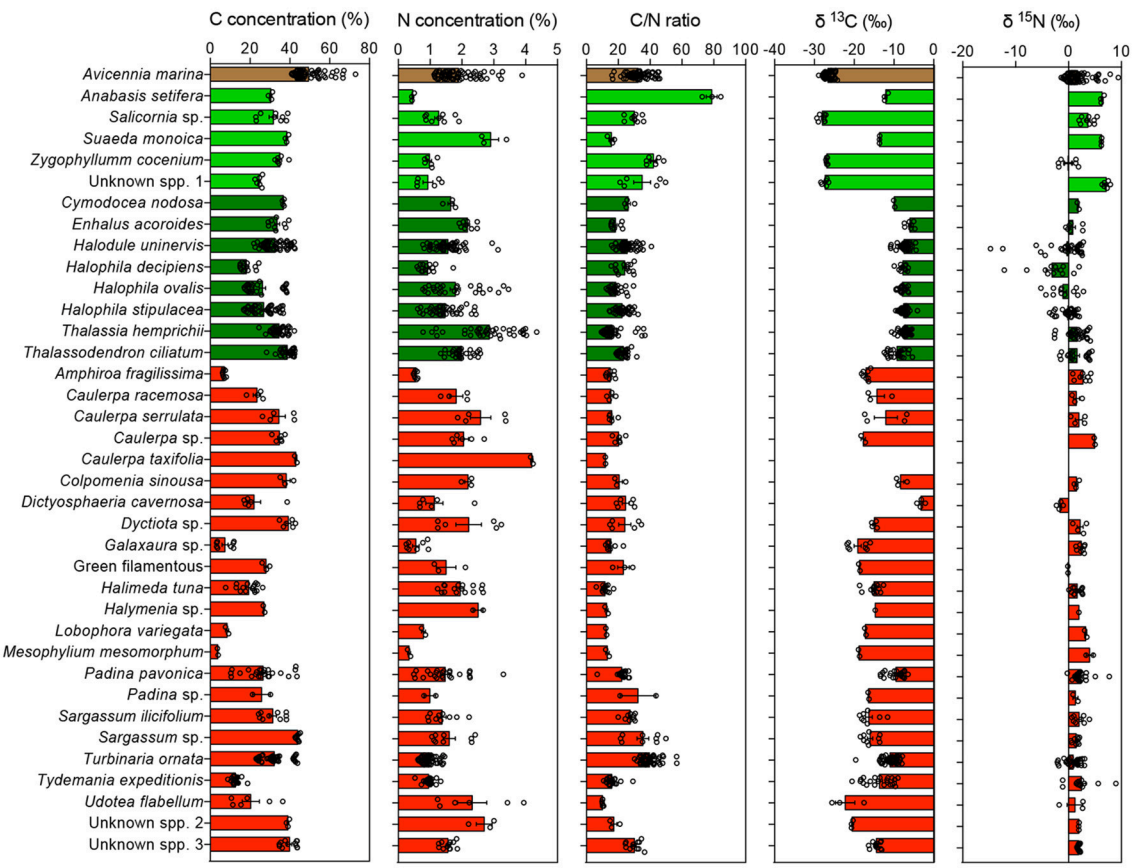

FIGURE 6 | Mean ± SE of the concentration of carbon (C), the concentration of nitrogen (N), the C/N ratio, the carbon ($\delta^{13}C$), and nitrogen ($\delta^{15}N$) isotopic composition measured in the blades of the different species of primary producers, color-coded by taxa. Mangroves, halophytes, seagrasses, and macroalgae are indicated in brown, light green, dark green, and red, respectively. Empty dots indicate individual measurements for each species and location.

isotopes could, therefore, be useful in discriminating the sources of organic matter in food webs or sediment deposits. This requires extreme care for sediments, as any traces of water, which contains large amounts of deuterium, may bias the values. Hence, relatively long desiccation times are required for studies involving plant stem and sediment samples (West et al., 2006).

Mangroves, salt-marshes and seston had similar ranges of $\delta^{15}N$ values but differed in $\delta^{13}C$ values, with halophytes having in general heavier values (−29.36 to −11.44‰ vs. V-PDB) than mangroves (−28.91 to −24.24‰ vs. V-PDB) and seston (−29.54 to −19.49‰ vs. V-PDB). Whereas the nitrogen isotopic composition of seston was not resolved here, earlier analyses of Red Sea seston point at nitrogen isotopic values of, on average, +2 to + 4.8‰ (Kurten et al., 2014; Almahasheer et al., 2017), within the range found for halophytes and mangroves in this study (**Table 2**).

Overall, isotopic composition values of Red Sea primary producers were consistent with values reported elsewhere (Hemminga and Mateo, 1996; Kennedy et al., 2004; Papadimitriou et al., 2005; Fourqurean et al., 2007). However, seston values tended to show isotopically-lighter carbon values compared to previous reports while those of seagrass tended toward the isotopically-heavier range of values reported for seagrasses in the past. The exploratory evaluation of deuterium isotopes confirms that its use holds promise to overcome the limitations of carbon isotopes (Wilkinson et al., 2015) in source apportioning using mixing models to discriminate between isotopically-overlapping plant types (e.g., macroalgae and seagrass in the Red Sea), supporting a range of applications including food web studies (e.g., Layman et al., 2012) and studies of the contributions of different primary producers to sediment organic carbon pools.

AUTHOR CONTRIBUTIONS

All authors designed the research, conducted sampling and analyses, contributed to writing the manuscript and approved the submission. CD, AA, and NG-B analyzed the data.

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