D4.3.1 *Assessment of pilot activities through macroalgae deployments*

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1. Introduction

The aim of the BLUEISLANDS project is to address the seasonality of waste production generated by tourism on Mediterranean islands and propose solutions to overcome this problem. For this purpose, a study on the identification of the presence and the extent of anthropogenic nutrients into coastal waters of selected islands was carried out in 2017 in Cyprus and Sicily, and in 2018 in Rhodes. During the study phase, the presence of anthropogenic nutrients was detected by measuring the variation of nitrogen isotopic signature ($\Delta\delta^{15}N$) in macroalgal bioindicators exposed to the environmental conditions of highly tourist sites and control sites, before, during and after the tourist peak. What emerged from the study phase was that in all the islands investigated, the presence of anthropogenic nutrients, although not of great concern, increased during the summer in the tourist sites. Particularly, in Cyprus, ^{15}N-enriched nutrients were detected since the beginning of June, peaked in August and then decreased substantially in October. In Sicily the presence of anthropogenic nutrients was recorded mainly in August followed by a slight decrease in October, while in Rhodes, a negligible ^{15}N-enrichment was detected at the tourist beach compared to the other two islands investigated.

These results, which are detailed in the deliverable D3.5.1 ("Preliminary study for detection of anthropogenic nutrients in marine coastal areas"), were taken into account by local partners to set specific objectives and to draft a list of measures and action plans aiming at reducing and/or avoiding the increase in waste and nutrients of anthropic origin that reach the coastal areas. Hence, throughout summer 2019, a series of pilot activities were implemented in the same tourist beaches where the preliminary study was conducted. Pilot activities were calibrated on the specific needs of the islands and were focused mainly on raising public awareness and education through various media involving direct beach users, either tourists and locals, youths and adults, but also local authorities. Among the media used, informative campaigns were disseminated through posters, banners and leaflets, merchandise items, on-site dissemination events, online websites, social media and radio adverts. Main messages of the informative campaigns concerned i) the importance of efficient and adequate wastewater system to avoid organic contamination of seawater, ii) impacts and threats of litter to coastal marine ecosystems and marine life, iii) the importance of changing consumer habits and adopting sustainable behaviours to prevent litter entering the environment. Further details of the pilot actions implemented in the different islands are available in the deliverable D4.2.2 ("Pilot activities implementation").

To assess the effectiveness of the pilot activities aimed at reducing anthropogenic nutrients into coastal waters, the same macroalgae experiment conducted during the preliminary study phase was repeated in summer 2019, during the high tourist peak (i.e. August), in the same tourist sites, following the same methodology described in D3.5.1. Data collected were elaborated to calculate the $\Delta\delta^{15}N$ of the macroalgae deployed, and compared to the results previously acquired, in order to assess the effectiveness of the pilot actions implemented, in terms of achievement of the established targets. It was defined that the success of the pilot activities is achieved when the frequency of $\Delta\delta^{15}N$ values falling within the "Low" category ($\Delta\delta^{15}N$ < 0.5‰) of $\delta^{15}N$ enrichment (Tab. 1):

- remained stable, where it was already high (≥ 80%) in previous results (i.e. study phase);
- increased to ≥80% where it was lower (<80%) in previous results (i.e. study phase).

Table 1. Intervals of $\Delta\delta^{15}N$ values set to define the level of $\delta^{15}N$ enrichment of the macroalgae incubated during the short-term experiment.

$\Delta\delta^{15}N$	**Level of macroalgae $\delta^{15}N$ enrichment**
< 0.5 ‰	Low
0.5 – 1.0 ‰	Moderate
1.0 – 1.5 ‰	High

2. Results

2.1 Cyprus

Main physicochemical features of the study sites and experiment procedural control

Main physicochemical variables recorded during the experimental activities carried out in Cyprus in August 2019 were comparable across Impact and Control sites. Mean seawater temperature was 28.8 ± 0.2°C. Similarly, salinity was overall constant all over the sites, being on average 39.8 ± 0.2 psu. The mean $\delta^{15}N$ signature of the macroalga *Cystoseira humilis* collected at the Collection site varied slightly among days of collection, decreasing from 0.3 ± 0.2 ‰ at Day 0 to 0.1 ± 0.1 ‰ at Day 3 – Control *in situ* (procedural control). Permutational analysis of variance (PERMANOVA) did not reveal statistically significant differences in $\delta^{15}N$ values of the macroalgae among days of collection, confirming that the experimental procedure (e.g. cut, handling, deployment) did not affect the macroalgae performance (Tab. 2).

Table 2. Results of PERMANOVA (main test) testing for differences in $\delta^{15}N$ values of *Cystoseira humilis* collected at the Collection site of Cyprus, for the factor Collection day.

Source of variation	df	MS	Pseudo-F	P(perm)	Perms
Collection day	2	0.025	0.569	0.565	994
Residuals	12	0.045			

Macroalgae experiment

$\delta^{15}N$ values of the macroalgae deployed at increasing distance from the coastline at Impact and Control sites did not show overall significant differences compared to the baseline reference (i.e. the mean $\delta^{15}N$ value of the macroalgae from the Collection site at Day 0) (Fig. 1). The only exception is represented by the macroalgae deployed at 200 m at the Impact site, which showed a significant higher value than the baseline according to the t-test performed (mean values ± standard deviation respectively 0.7 ± 0.3‰ vs. 0.3 ± 0.2‰, *p*-value _= 0.045). $\delta^{15}N$ values recorded at the Impact site ranged between 0.1 and 1.3‰ and were overall higher than the baseline. On the contrary, $\delta^{15}N$ values of the macroalgae deployed at the Control sites were more distributed around the baseline value, and comprised between -0.8 and 0.9‰, indicating natural fluctuation in the uptake of nutrients present in the column water, especially at Control 1. Almost all the macroalgae deployed at 100 m distance from the coastline at Control 2 were lost, probably due to the passage of speedboats during the days of the experiment implementation, that might have had accidentally cut the structures ropes. Only one structure was found and macroalgae analysed.

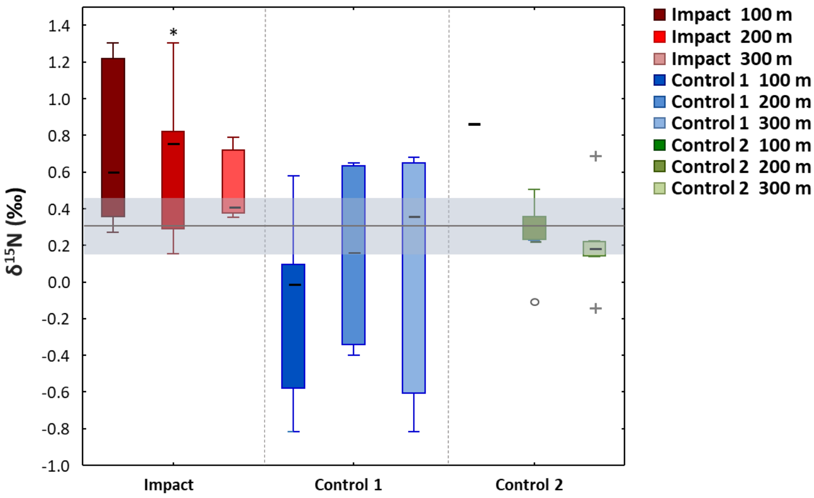

Figure 1. Boxplot of $\delta^{15}N$ values of the macroalgae deployed at different distance from the coastline (100, 200, 300 m) in the study sites of Cyprus: Impact (Sunrise beach), Control 1 and 2 (Cavo Greco) in August 2019 (i.e., tourist peak). Each box contains 50% of the data, the thick horizontal line indicates the median; lower and upper whiskers represent respectively the first and fourth quartiles of the total range and circles represent outliers of the distribution. Asterisks indicate the significance level of the differences between the $\delta^{15}N$ values of the deployed macroalgae and the baseline, according to t-test. *p*-values: * = p-value < 0.05. Horizontal line with shadow area overlaying the boxplots indicate the mean of the baseline and the related standard deviation.

Looking at the isotopic variation of the macroalgae during the deployment at the tourist site, $\Delta\delta^{15}N$ values in 2019 were overall lower than those in 2017, accounting for a range comprised respectively between -1.1 and 1.0‰ in 2019 and between -0.3 and +1.4‰ in 2017, with a reduction of the highest peak of 0.32‰ at the Impact site (Fig. 2). These results are clear also from the georeferenced maps (Fig. 3), where the moderate level of macroalgae $\delta^{15}N$ enrichment (0.5 - 1.0‰, i.e. mid-dark orange contours) reduced from the whole landward line (100 m from the coastline) in 2017, into two separated spots in 2019, surrounded by a majority of light orange contours indicating a low level of enrichment (<0.5‰).

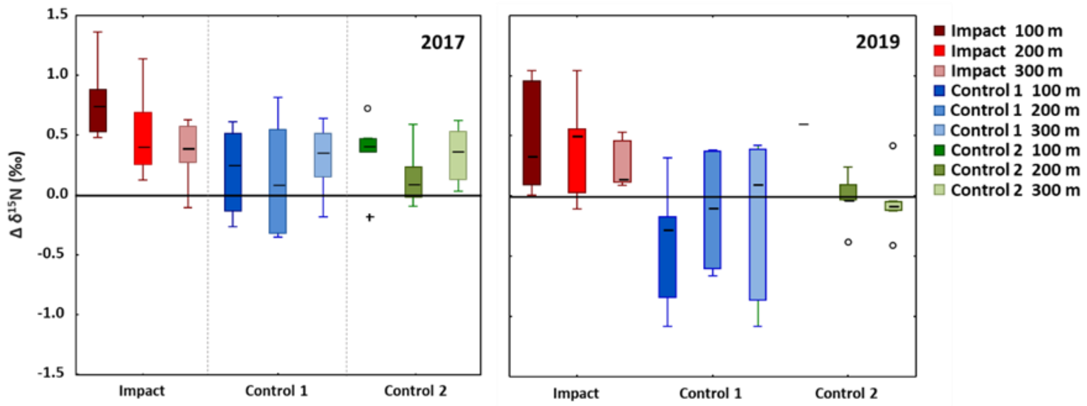

Fig. 2. Boxplot of $\Delta\delta^{15}N$ values (variation of $\delta^{15}N$ compared to the baseline) of the macroalga *Cystoseira humilis* deployed at different distance from the coastline (100, 200, 300 m) at the Impact site of Cyprus (Sunrise beach) in August 2017 and 2019. Each box contains 50% of the data, the thick horizontal line indicates the median; lower and upper whiskers represent respectively the first and fourth quartiles of the total range and circles represent outliers of the distribution. Black horizontal line overlaying the graph indicates the baseline; data above and below the baseline indicate ^{15}N-enrichment and ^{15}N-depletion of macroalgae respectively.

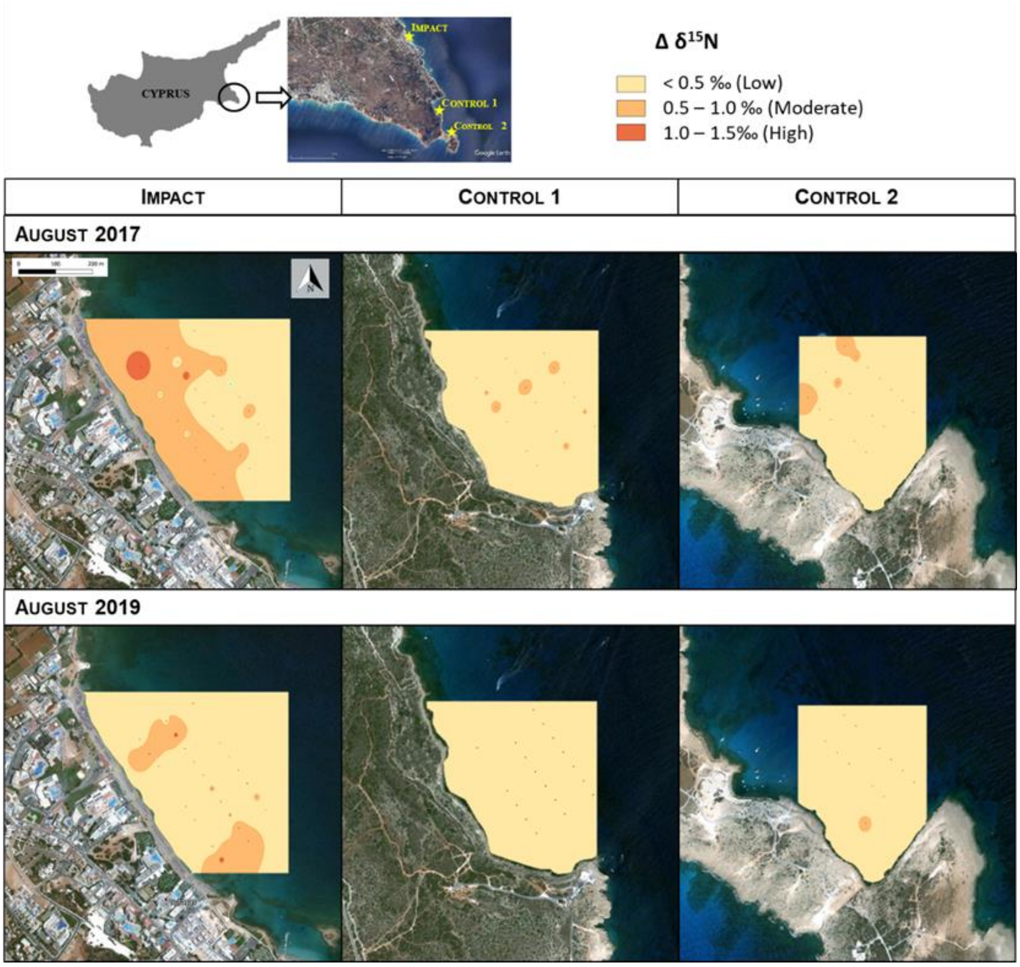

Figure 3. Georeferenced maps of $\Delta\delta^{15}N$ values in August 2017 and 2019 at the Impact site (Sunrise beach, Protaras) and Control sites (Cavo Greco) of Cyprus.

Overall, the frequency of $\Delta\delta^{15}N$ data of the deployed macroalgae included in the "Low" category of $\delta^{15}N$ enrichment increased from 56% in 2017 to 75% in 2019 after pilot activities implementation in the Impact site (Fig. 4a). Therefore, the target defined to assess the effectiveness of the pilot actions (80% of $\Delta\delta^{15}N$ data in "Low" category) was not reached in Cyprus. However, the increase by 19% of $\Delta\delta^{15}N$ data in the "Low" category is noteworthy. Looking at the results of the different distances from the coastline, the target was only reached at 300 m distance (Fig. 4d), but the highest improvement was recorded landward (i.e. at 100 m distance, Fig. 4b), with a increase by 46% of frequency of $\Delta\delta^{15}N$ data included in the "Low" category, indicating a consistent reduction of the anthropogenic nutrients sources deriving from the coastline.

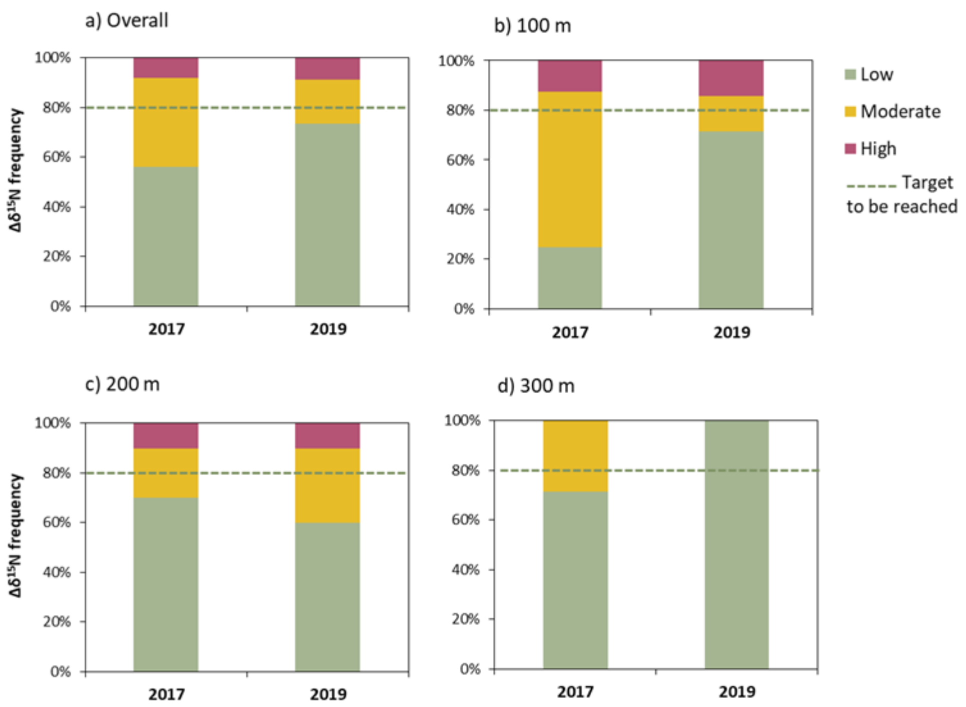

Fig. 4. Comparison of the frequency of $\Delta\delta^{15}N$ data of *Cystoseira humilis* included in the enrichment categories "Low", "Moderate" and "High", resulted from deployment experiment at the Impact site in 2017 and 2019. Comparisons are presented in separate panels, if considering data of the macroalgae deployed in the whole site (overall, a), and at different distance from the coastline: 100 m (b), 200 m (c) and 300 m (d).

2.2 Sicily

Main physicochemical features of the study sites and experiment procedural control

Temperature and salinity of surface seawater recorded in August 2019 in the experimental sites of Sicily were constant across Impact and Control sites and were on average 23.1 ± 1.9°C and 38.8 ± 0.1 psu respectively. $\delta^{15}N$ signatures of the thalli of *Cystoseira amentacea* collected at the Collection site were comparable among the different days of collection (Day 0, Day 3 and Day 3 – Control *in situ*). Overall mean was 6.8 ± 0.3‰ and no significant differences were recorded by statistical test (PERMANOVA p-value > 0.05, Tab. 3), suggesting that the experimental procedures did not influence the $\delta^{15}N$ signature of the macroalgae.

Table 3. Results of PERMANOVA (main test) testing for differences in $\delta^{15}N$ values of *Cystoseira amentacea* collected at the Collection site of Sicily, for the factor Collection day.

Source of variation	df	MS	Pseudo-F	P(perm)	Perms
Collection day	2	0.065	0.739	0.486	851
Residuals	12	0.088			

Macroalgae experiment

The comparison of the $\delta^{15}N$ values of *Cystoseira amentacea* deployed in the study sites with that one of the baseline revealed a certain degree of overlapping between the macroalgae deployed in the three sites and the baseline range, which was comprised between 6.4 and 7.1‰ (Fig. 5). However, the macroalgae deployed at 200 m distance from the coastline at the Impact site and those at 250 m at the Control 2 site showed significantly higher $\delta^{15}N$ values than the baseline (t-test *p*-value respectively <0.01 and <0.05). Overall, $\delta^{15}N$ values across sites and distances ranged between 6.4 and 7.7‰, with no remarkable differences between sites, except for the macroalgae deployed at 200 m distance at the Impact site. It should be noted that all the macroalgae deployed at 250 m and 300 m distances at Control 1 were lost, hence the analyses were carried out only on the macroalgae deployed at 200 m distance from the coastline.

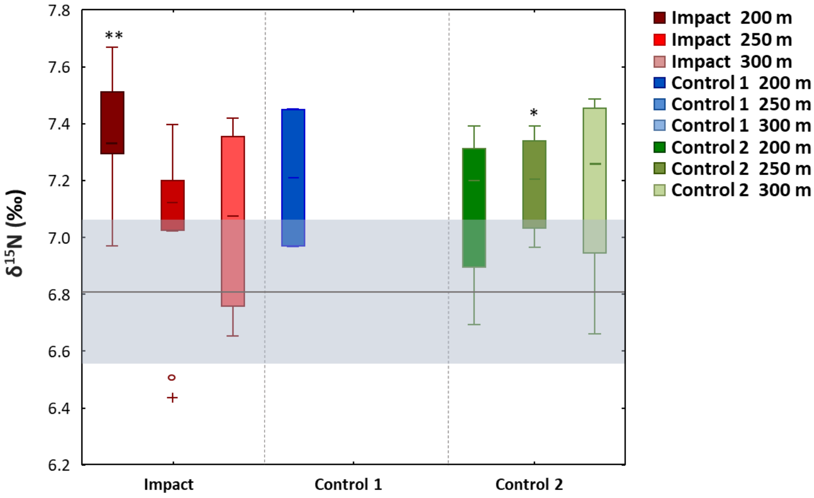

Figure 5. Boxplot of $\delta^{15}N$ values of the macroalga *Cystoseira amentacea* deployed at different distance from the coastline (200, 250, 300 m) in the study sites of Sicily: Impact (Giardini Naxos Bay), Control 1 and 2 (Fondaco Parrino beach) in August 2019 (i.e., tourist peak). Each box contains 50% of the data, the thick horizontal line indicates the median; lower and upper whiskers represent respectively the first and fourth quartiles of the total range and circles represent outliers of the distribution. Asterisks indicate the significance level of the differences between the $\delta^{15}N$ values of the deployed macroalgae and the baseline, according to t-test. *p*-values: * = *p*-value< 0.05, ** = *p*-value< 0.01. Horizontal line with shadow area overlaying the boxplots indicate the mean of the baseline and the related standard deviation.

Overall, $\Delta\delta^{15}N$ values of the macroalgae deployed in August 2019, after pilot activities implementation, were restricted to a much narrower range compared to those of macroalgae deployed in 2017 (Fig. 6). In fact, while in 2017 macroalgae $\Delta\delta^{15}N$ values spanned from -1.2 to 1.2‰, in 2019 $\Delta\delta^{15}N$ ranged between -0.4 and 0.80. Such trend is reflected in the georeferenced maps (Fig. 7), where the light orange contour (low level of $\delta^{15}N$ enrichment) was homogeneously distributed in the whole Impact site in 2019, clearly indicating a reduced influence of nutrients of anthropic origin compared to 2017, when, in contrast, the southern part of the bay showed a moderate level of $\delta^{15}N$ enrichment. A comparable situation between the two years, was evident in Control 2, while it was not possible to interpolate $\Delta\delta^{15}N$ data in Control 1 in 2019, due to the loss of macroalgae structures.

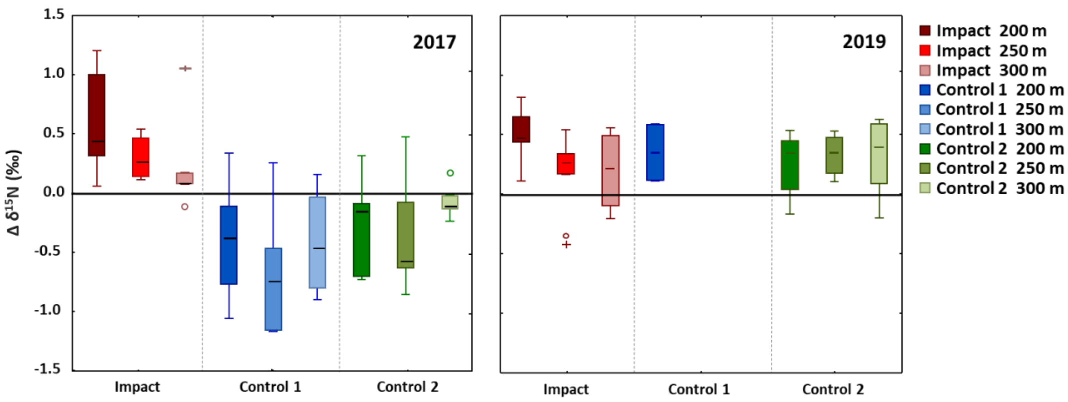

Figure 6. Boxplot of $\Delta\delta^{15}N$ values (variation of $\delta^{15}N$ compared to the baseline) of the macroalga *Cystoseira amentacea* deployed at different distance from the coastline (200, 250, 300 m) in the study sites of Sicily: Impact (Giardini Naxos Bay), Control 1 and 2 (Fondaco Parrino beach) in August 2017 and 2019. Each box contains 50% of the data, the thick horizontal line indicates the median; lower and upper whiskers represent respectively the first and fourth quartiles of the total range and circles represent outliers of the distribution. Black horizontal line overlaying the graph indicates the baseline; data above and below the baseline indicate ^{15}N-enrichment and ^{15}N-depletion of macroalgae respectively.

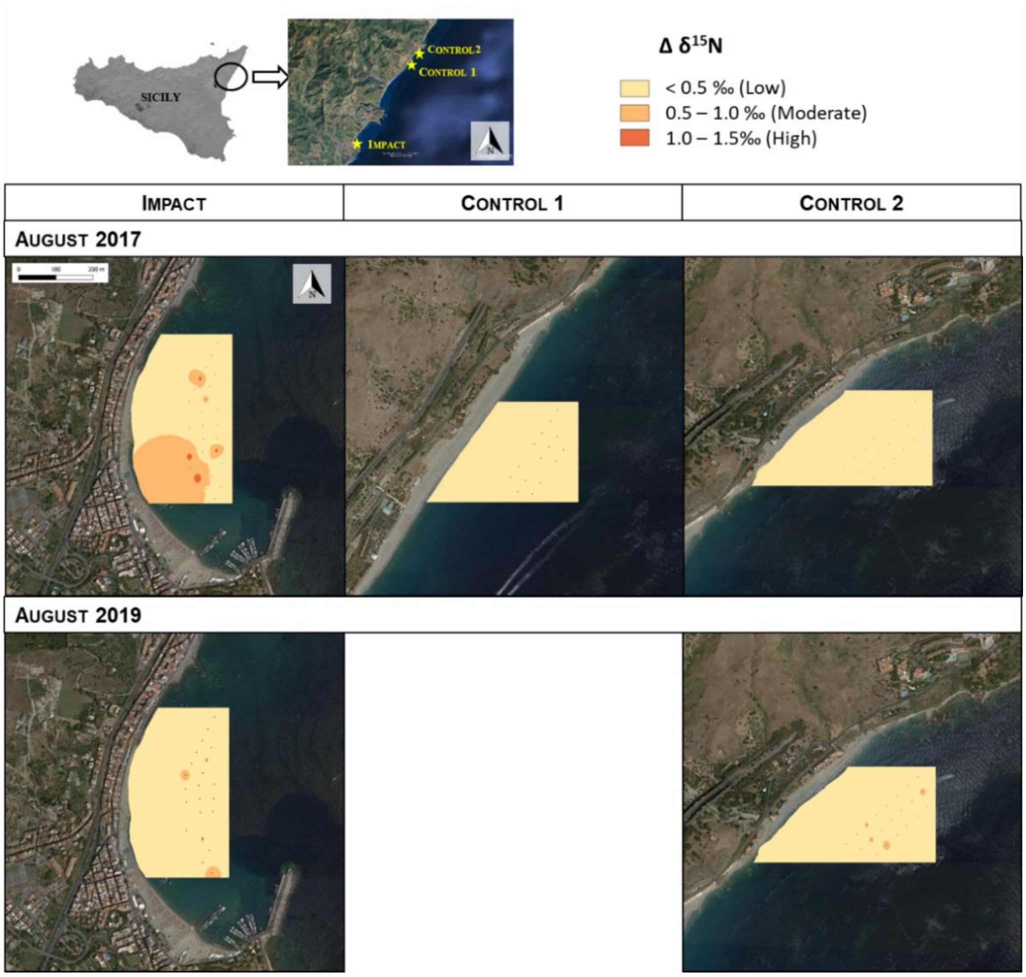

Figure 7. Georeferenced maps of $\Delta\delta^{15}N$ values in August 2017 and 2019 at the Impact site (Giardini Naxos Bay) and Control sites (Fondaco Parrino beach) of Sicily.

The frequency of $\Delta\delta^{15}N$ data of *Cystoseira amentacea* included in the “Low” category of $\delta^{15}N$ enrichment at the Impact site increased from 73% in 2017 to 82% in 2019, following the pilot activities implementation (Fig. 8a), indicating a full achievement of the target (i.e. 80%). In addition, no data in the enrichment category “High” were recorded in 2019, compared to the 15% found in 2017. Looking at the results at the different distances from the coastline, the target was actually reached both at 250 and 300 m (Fig. 8c and d), but not at 200 m distance from the coastline (Fig. 8b), suggesting still a slight influence of anthropogenic nutrients deriving from the coastline.

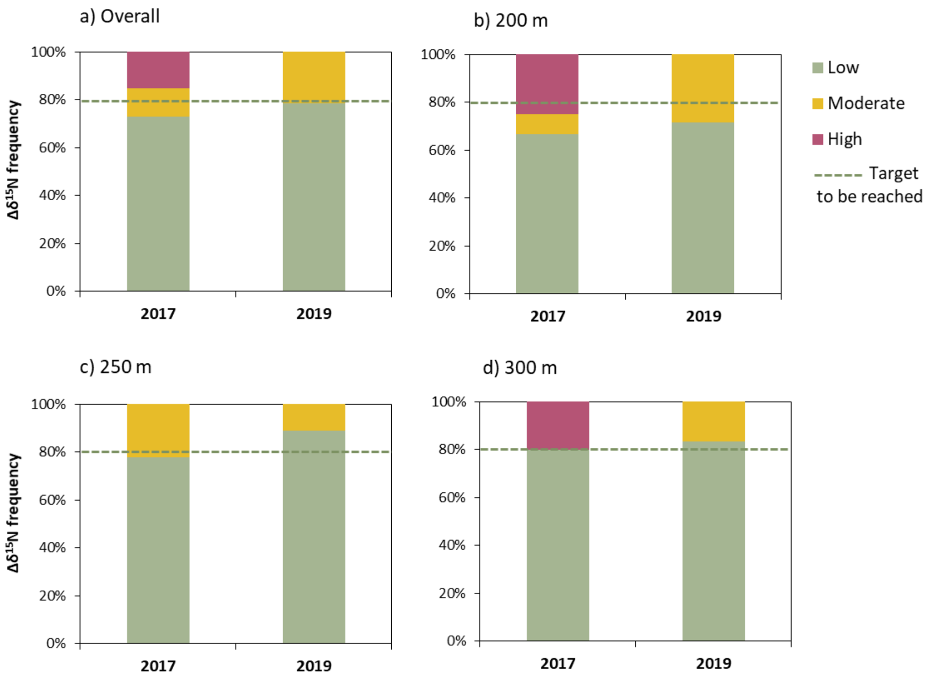

Fig. 8. Comparison of the frequency of $\Delta\delta^{15}N$ data of *Cystoseira amentacea* included in the enrichment categories “Low”, “Moderate” and “High”, resulted from deployment experiment at the Impact site in 2017 and 2019. Comparisons are presented in separate panels if considering data of the macroalgae deployed in the whole site (overall,, and at different distance from the coastline: 100 m (b), 200 m (c) and 300 m (d).

2.3 Rhodes

Main physicochemical features of the study sites and experiment procedural control

Main physicochemical variables recorded during the experimental activities were comparable across Impact and Control sites in Rhodes in August 2019. Seawater temperature was on average 28.2 ± 0.6 °C across sites. Similarly, salinity was similar in the three sites, being on average 39.4 ± 0.3 psu.

Mean $\delta^{15}N$ of the macroalga *Cystoseira compressa* collected at the Collection site varied slightly among days of collection, ranging from 2.1 ± 0.3 ‰ at Day 3 to 2.5 ±0.2 ‰ at Day 0. Permutational analysis of variance (PERMANOVA) did not reveal significant differences among days of collection, confirming that the experimental procedure (e.g. cut, handling, deployment) did not affect the macroalgae performance (Tab. 4).

Table 4. Results of PERMANOVA (main test) testing for differences in $\delta^{15}N$ values of *Cystoseira compressa* from the Collection site of Rhodes, for the factor Collection day.

Source of variation	df	MS	Pseudo-F	P(perm)	Perms
Collection day	2	0.336	3.326	0.068	996
Residuals	12	0.101			

Macroalgae experiment

Most of $\delta^{15}N$ values of the macroalgae deployed at increasing distance from the coastline at Impact and Control sites showed lower values than the baseline (Fig. 9). According to the t-test performed, almost all groups of macroalgae had significantly lower $\delta^{15}N$ (p-value < 0.05) than the baseline (2.5 ± 0.2 ‰), with the exception of those deployed at 200 m at the Impact site and at 300 m at the Control 2 site.

Overall, $\delta^{15}N$ values ranged between 0.6 and 3.0‰, with no substantial differences among sites.

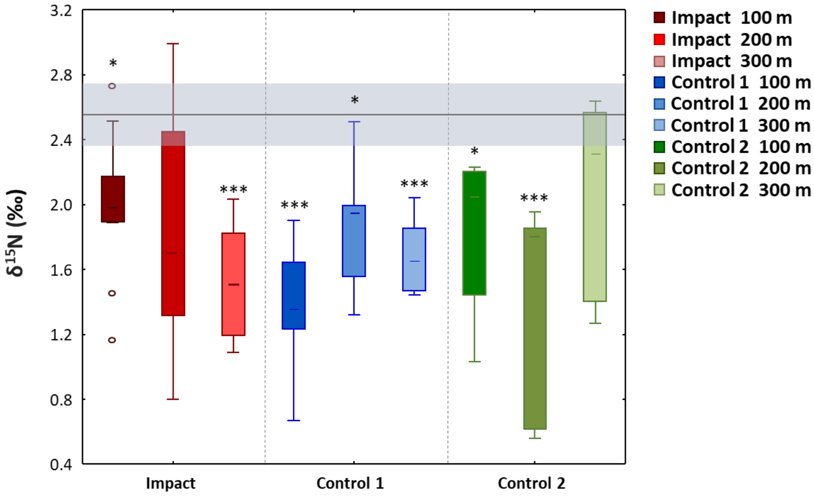

Figure 9. Boxplot of $\delta^{15}N$ values of the macroalgae *Cystoseira compressa* deployed at different distance from the coastline (100, 200, 300 m) in the study sites of Rhodes: Impact (Faliraki beach), Control 1 and 2 (Afandou beach) in August 2019 (i.e., tourist peak). Each box contains 50% of the data, the thick horizontal line indicates the median; lower and upper whiskers represent respectively the first and fourth quartiles of the total range and circles represent outliers of the distribution. Asterisks indicate the significance level of the differences between the $\delta^{15}N$ values of the deployed macroalgae and the baseline, according to t-test. *p*-values: * = *p*-value< 0.05, ** = *p*-value< 0.01, *** = *p*-value< 0.001. Horizontal line with shadow area overlaying the boxplots indicate the mean of the baseline and the related standard deviation.

Looking at the isotopic variation of the macroalgae deployed in the tourist site, there was an evident decrease of $\Delta\delta^{15}N$ values in the 2019 experiment compared to 2018 (Fig. 10). Moreover, most $\Delta\delta^{15}N$ values were negative, ranging from -2.0 to 0.4‰, while in 2018 they ranged from -0.9 to 0.7‰. The results of the experiment carried out in 2019 showed also a higher data variability, suggesting greater fluctuations in the uptake of nutrients present in the water column at all sites.

Only slight differences emerged comparing the georeferenced maps of 2018 and 2019, since in both years the vast majority of data of all sites was included in the "Low" category of $\delta^{15}N$ enrichment (< 0.5 ‰) (Fig. 11).

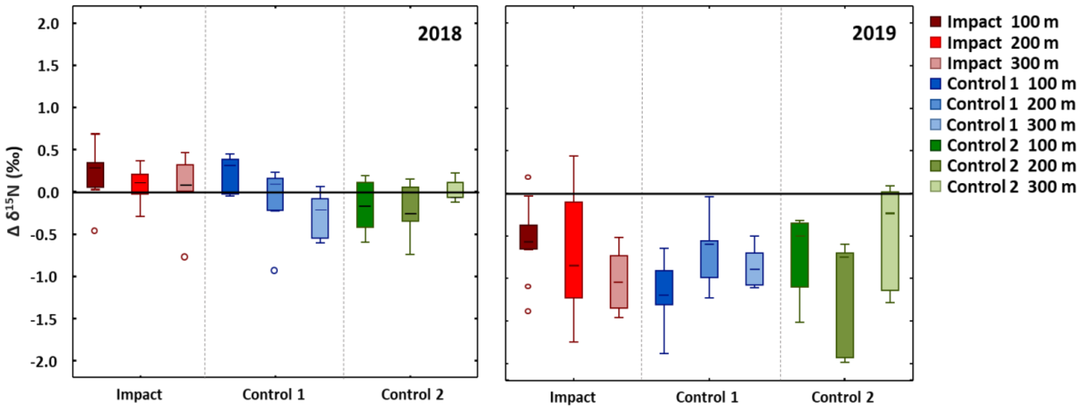

Fig. 10. Boxplot of $\Delta\delta^{15}N$ values (variation of $\delta^{15}N$ compared to the baseline) of the macroalga *Cystoseira compressa* at different distance from the coastline (100, 200, 300 m) in the study sites of Rhodes: Impact (Faliraki beach), Control 1 and 2 (Afandou beach) in August 2018 and 2019. Each box contains 50% of the data, the thick horizontal line indicates the median; lower and upper whiskers represent respectively the first and fourth quartiles of the total range and circles represent outliers of the distribution. Black horizontal line overlaying the graph indicates the baseline; data above and below the baseline indicate ^{15}N-enrichment and ^{15}N-depletion of macroalgae respectively.

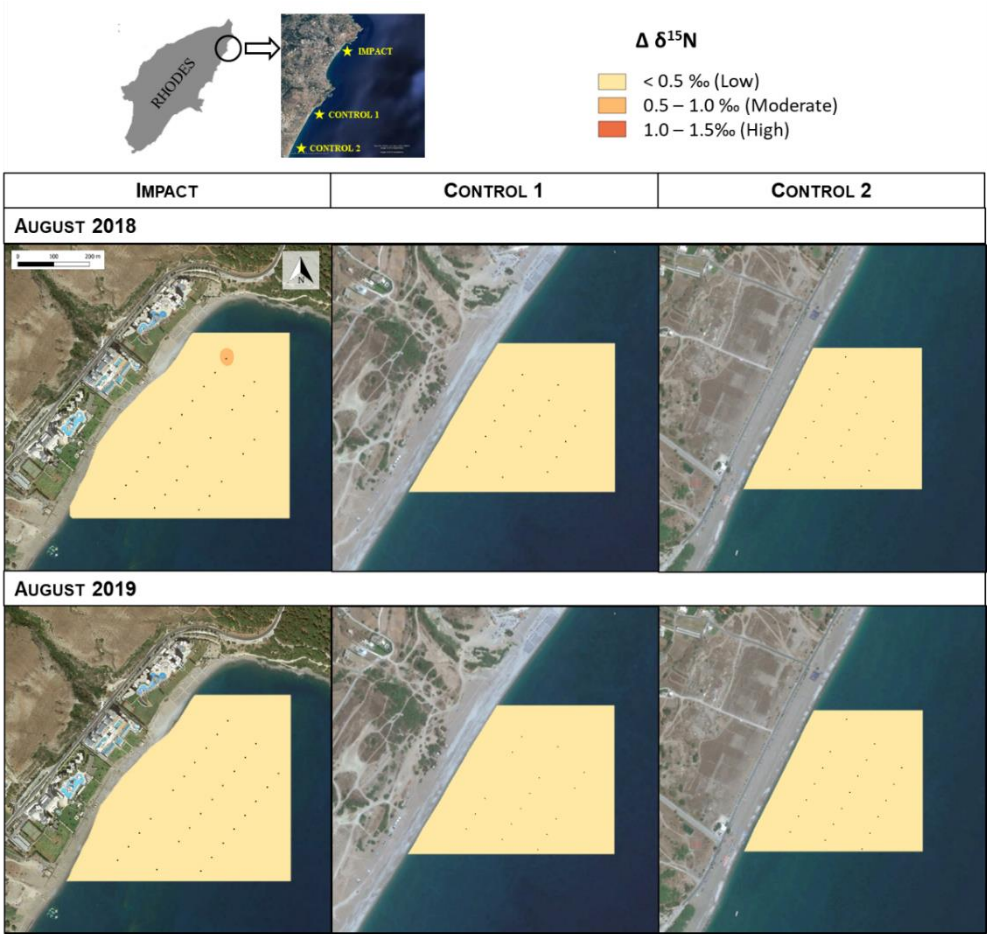

Figure 11. Georeferenced maps of $\Delta\delta^{15}N$ values in August 2018 and 2019 at the Impact site (Faliraki beach) and Control sites (Afandou beach) of Rhodes.

Comparing the frequency of $\Delta\delta^{15}N$ data included in the "Low" category of $\delta^{15}N$ enrichment before and after pilot activities implementation in Rhodes, the target set to consider successful the pilot activities (80% of data) was already detected in 2018, and was maintained also in 2019. In fact, the percentage of data falling in the "Low" category increased from 96 to 100% considering the Impact site as a whole. This was actually due to the reduction of anthropogenic nutrients detected at 100 m distance from the coastline, where the frequency of $\Delta\delta^{15}N$ data included in the "Low" category of $\delta^{15}N$ enrichment increased from 91 to 100%, indicating a lower input from the coastline. In contrast, more seaward, at both 200 m and 300 m distance from the coastline, the totality of data was already scarcely ^{15}N-enriched in the 2018, and in 2019 the situation remained unchanged (Fig. 12).

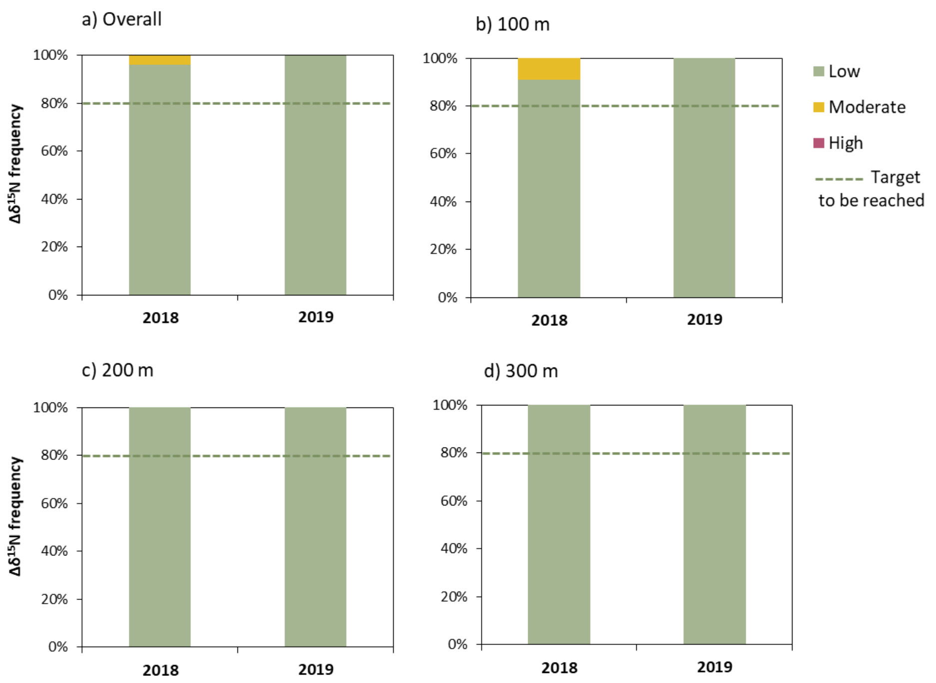

Fig. 12. Comparison of the frequency of $\Delta\delta^{15}N$ data of *Cystoseira compressa* included in the enrichment categories "Low", "Moderate" and "High", resulted from deployment experiment at the Impact site in 2017 and 2019. Comparisons are presented in separate panels, if considering data of the macroalgae deployed in the whole site (overall, a), and at different distance from the coastline: 100 m (b), 200 m (c) and 300 m (d).

3. Conclusion

Coastal tourism is one of the most fast growing sectors of the global economy, bringing important benefits to local economies and moving millions of people worldwide. However, together with tourism growth, increasing environmental impact affects coastal waters every year during summertime due to high input of anthropogenic nutrients and organic matter. In summer 2019, the local project partners of Cyprus, Sicily and Rhodes, namely the Department of Environment of the Ministry of Agriculture, Rural Development and Environment of Cyprus, the Taormina Etna Consortium and the Municipality of Rhodes, implemented a series of pilot actions aimed at reducing both marine litter and anthropogenic nutrients in coastal sites mostly frequented by tourists. Extensive awareness campaigns were conducted involving direct beach users and all levels of stakeholders of the horeca sector. Among the activities carried out, informative posters and leaflets were distributed at the entrance of the beaches, in bars, restaurants and cafes, inviting to conduct a proper behaviour to guarantee a good status of the environmental conditions.

Macroalgae experiments were carried out after several weeks of pilot activities implemented in the three islands, allowing to test their effectiveness in limiting the presence of anthropogenic nutrient in the water column. Results obtained showed that two out of three islands reached the target fixed for the achievement of successful pilot actions. While in Sicily and Rhodes, respectively 82 and 100% of $\Delta\delta^{15}N$ values fell within the “Low” category of $\delta^{15}N$ enrichment level of macroalgae, Cyprus was close (75%), but did not reach the threshold of 80% (summary in Fig. 13). Although the result obtained was not completely successful, it is worth to note that the improvement reached was remarkable also in Cyprus. The highest improvement (46% increase of data falling in the category "Low" of $\delta^{15}N$ macroalgae enrichment) was recorded, indeed, at 100 m distance from the coastline, indicating a consistent reduction of the anthropogenic nutrients deriving from the coastline, which was the ultimate aim of the of the activities carried out.

Concluding, these results show how important and encouraging is the implementation of concrete actions on-site to improve the environmental conditions of tourist sites, and how the involvement of direct users is fundamental to spread necessary awareness on environmental dynamics.

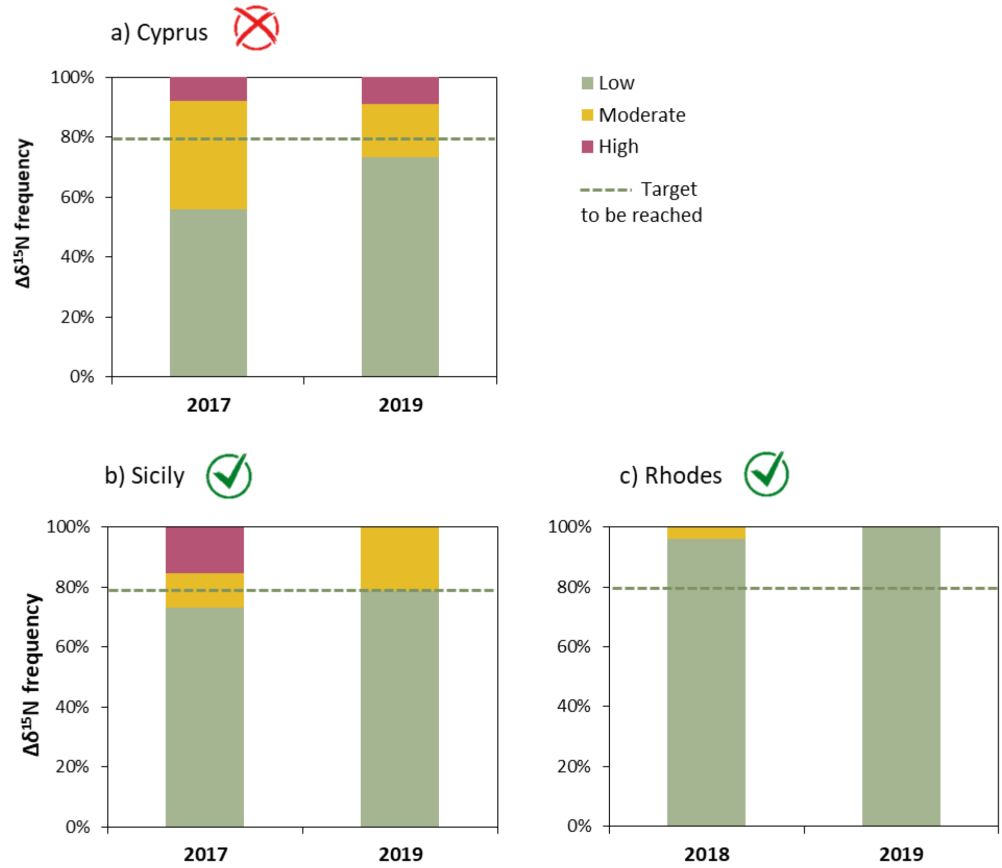

Fig. 13. Comparison of the frequency of $\Delta\delta^{15}N$ data of *Cystoseira* spp. falling in the "Low", "Moderate" and "High" categories of $\delta^{15}N$ enrichment, resulted from deployment experiment at the Impact sites of all islands before (2017 in Cyprus and Sicily, 2018 in Rhodes) and after pilot activities implementation (2019).