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Article *in* Journal of Plant Nutrition and Soil Science · August 2006

DOI: 10.1002/jpln.200521842

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Marine benthic macrophytes as possible nitrogen source in agriculture

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Accepted January 15, 2006 PNSS P184/2B

Summary
Marine benthic macrophytes were examined as possible nitrogen (N) sources for agriculture in Greece in terms of N mineralization and available standing stocks. Net N mineralization patterns were determined over a 30-week incubation period under aerobic conditions. *Ulva* sp., *Cystoseira barbata C.* Agardh, and *Posidonia oceanica* (L.) Delile storm-cast material (SC) and harvested leaves (L) were incorporated into acidic, slightly acidic after liming, and alkaline soils and incubated at 35 °C. From the initial stage until the 12th week of incubation, ammonification was higher than nitrification in most cases. The decreasing order of mineralized N originating from macrophytes was: *Ulva* sp. > *P. oceanica* (SC) > *C. barbata* > *P. oceanica* (L). Rates of net N mineralization of the macrophytes were the highest in the alkaline soil, whereas the lowest values were recorded in the strongly acidic soil. Application of Ca into the acidic soil revealed a considerable increase of N mineralization. Immobilization and ammonification were observed after incorporation of *Ulva* sp. into the acidic soil. As *Ulva* sp. proved to be the only potential alternative N source for arable crops, its standing stocks in two geographically different Greek lagoons, the Vassova Lagoon (N Greece) and Papas Lagoon (S Greece) were estimated. It was estimated that 2 ha of *Ulva* could supply adequate N to cultivate approximately 1 ha cotton. *Posidonia* and *Cystoseira* did not show potential as an alternative N source for agriculture and especially in plant nutrition.

Key words: mineralization / immobilization / nitrification / ammonification / macrophytes

1 Introduction

Marine macrophytes, especially seaweeds, are widely used in agriculture (*Guiry* and *Blunden*, 1991). Dried seaweeds are used as animal food, fertilizers, and soil conditioners. Liquid seaweed extracts are used as growth promoters and crop protectants against pests and diseases (*Guiry* and *Blunden*, 1991; *Verkleij*, 1992). In the Mediterranean Sea, seaweed stocks are generally limited. However, recent proliferation of fast growing green algae, usually *Ulva* sp., is becoming an increasingly common phenomenon in many shallow marine ecosystems undergoing eutrophication (*Sfriso* and *Pavoni*, 1994; *Schramm* and *Nienhuis*, 1996). Fast growing macroalgae such as *Ulva* assimilate and store N in excess of their growth requirements (*Herbert*, 1999). A consequence of the elevated nutrient content is that their decomposition is more rapid due to their narrow C : N ratios (*Enriquez* et al., 1993). These stands affect biodiversity and ecosystem function and require expensive management practices such as biomass harvesting that provides nutrient-bioremediations capabilities (*Schramm*, 1991). This harvested material can be utilized in many different ways such as for fertilizers and bioenergy production (*Guiry* and *Blunden*, 1991). The increasing trends of eutrophication (*De Jonge* et al., 2002) as well as the usage of *Ulva* sp. as a biofilter of fish-culture effluents (*Vandermeulen* and *Gordin*, 1990; *Neori* et al., 1991) could in future increase the available biomass of this species. Unlike seaweeds, the seagrass *P. oceanica* forms extensive meadows along the Mediterranean coastline. These meadows are highly productive systems, and when leaves senesce, large amounts of storm-cast organic material (OM) are displaced by the wave action and deposited close to the beach (Pergent et al., 1994). *Posidonia oceanica* influences the geomorphology of sandy shores (*Jeudy de Grissac* and *Boudouresque*, 1985), and dead leaves are also frequently thrown up on beaches forming “banquettes” which protect them from erosion (*Jeudy de Grissac*, 1984).

Although seaweeds applied as organic fertilizers enrich the soil with nutrients, little attention has been paid to this process. Field and laboratory studies have been conducted to explain the complexity of these processes (*Parnas*, 1975; *Smith*, 1979; *Cabrera* and *Kissel*, 1988; *Manfongoya* et al., 1998; *Honeycutt*, 1999). The mineralization process depends mainly on temperature, moisture, aeration, acidity, form, and content of the soil organic matter (SOM) (*Stanford* et al., 1973; *Nyborg* and *Hoyt*, 1978). Soil acidity is often regarded as the limiting factor restricting the conversion of organic N to its mineral form. When acidic soils are limed, a portion of the SOM becomes more susceptible to mineralization (*Black*, 1968).

The aim of this study was to test *Ulva* sp. and storm-cast material of *P. oceanica* (SC) as an alternative N source for arable crops in terms of N mineralization. Healthy leaves of *P. oceanica* (L) and thalli of the brown seaweed *Cystoseira barbata* were also analyzed for comparative purposes rather than as an alternative N source. Another aim was to investigate the dynamics of net N mineralization and immobilization from macrophytes in alkaline, strongly acidic, and acidic soil after liming.

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2 Materials and methods

2.1 Soils

Acidic and alkaline soils were collected in the spring of 2000 from 0–50 cm, where the majority of roots of annual crops is situated. According to Soil Taxonomy (1999), the acidic soil was classified as *Alfisol* and the alkaline belongs to the order of *Vertisols.* The samples were air-dried and sieved to pass through a 2 mm mesh. A soil-distilled water suspension (1:1 w:v) was prepared and the soil pH determined with a glass electrode (*McLean*, 1982). Particle-size distribution was determined by the Bouyoucos hydrometer method (*Gee* and *Bauder*, 1986). The effect of liming on N mineralization was investigated by mixing 4.78 g $CaCO_3$ kg^{-1} to acidic soil. Two days after the liming, the value of pH was increased to 6.7 (*Shoemaker* et al., 1961). Organic matter in the soils was determined by a modified method of Walkley-Black (*Nelson* and *Sommers*, 1996) in which the soil-particle sample was grinded <0.15 mm. Total N in the soils was determined by the salicylic-sulfuric acid digestion (*Page* et al., 1982).

Net N mineralization was measured under laboratory conditions at 35 °C. Nitrate-N and NH_4^+-N were determined by a FIAstar 9000 Analyzer (FOSS TECATOR, Sweden). All results reported were averages of triplicate measurements and are expressed on a dry-weight basis.

2.2 Marine benthic macrophytes

Three marine benthic macrophytes (*Ulva* sp.*, C. barbata,* and *P. oceanica*) were used. *Ulva* sp. and *C. barbata* were collected from the shallow lagoon Vassova, Nestos Delta (N Greece) in spring 1998. *Posidonia oceanica* leaves (L) that originated from a 14–16 m deep meadow and storm-cast material (SC) were collected from the coast of Strymonikos Gulf, (N Greece) in winter 1997. The plant material was washed with distilled water, dried at 60° for 48 h and then pulverized. The OM and total N in the plant material was determined as described for the soil samples and results reported were averages of duplicate determinations.

2.3 Incubation procedure and kinetic parameters

Homogenous subsamples were obtained from each air-dried soil sample and net N mineralization was determined in incubation experiments by using the method of *Stanford* and *Smith* (1972). For each of the three soil treatments, triplicate 15 g of air-dried soil were mixed with equal quantity of quartz sand (20 mesh) and 0.3 g of dried plant material. In the control treatment, no plant material was added to the soil, and the initial inorganic N was removed by leaching with 100 mL $CaCl_2$. The soil, sand, and plant mixtures were moistened with a fine spray of distilled water. Soil moisture was adjusted by applying a suction of 80 kPa. The soil columns were placed vertically, in the dark at 35 °C. Soil water content was monitored by weighing the samples three times a week and replacing the water up to field water capacity. Nitrogen mineralization was determined after 2, 4, 8, 12, 16, 22, and 30 weeks. Leaching was performed by adding 100 mL of 0.01 M $CaCl_2$ followed by 25 ml of N-free nutrient solution (0.002 M $CaSO_4 . 2H_2O$; 0.002 M $MgSO_4$; 0.005 M $Ca(H_2PO_4)_2 . H_2O$, and 0.0025 M K_2SO_4). The leachates were collected, and the total volume was measured.

The N-mineralization potential (N_0) was estimated by the following nonlinear equation of *Stanford* and *Smith* (1972): $N_t = N_0 (1 - e^{-kt})$, where N_t is the cumulative amount of N mineralized at a specific time interval (t, weeks), N_0 is the potentially mineralized N, and k is the first-order rate constant.

The results were analyzed using the statistical package Statistica version 7.1 (edition 1999). Cluster analysis was performed by the Euclidean distance algorithm.

2.4 Standing stocks of *Ulva* sp.

The standing stocks of *Ulva* sp. were determined in two different lagoons, the Lagoon Vassova (N Greece) and the Lagoon Papas (S Greece), between spring 1998 and summer 1999.

The Lagoon Vassova is a highly compartmentalized lagoon comprising of three basins: a shallow main basin not deeper than 0.5 m, a basin of 13 channels artificially constructed up to 2 m deep, and the over-wintering and stocking basins up to 4 m deep (Fig. 1). It is connected with the sea through a narrow channel (30 m wide, 1.5 m deep) and covers an area of ha. Nutrients enter the lagoon with freshwater from a neighboring drainage channel and by precipitation. The main commercial use of the lagoon is traditional fish aquaculture. Fish immigrants are prevented from returning to the sea by a system of mesh frames (metal grids) and a stationary entrapment system (fish barrier). Macroalgae are the dominant components of the benthic vegetation, and *Ulva* sp. often forms dense populations in a relatively isolated area of ha. *Ulva* sp. population was sampled destructively from this area using a 30 cm × 50 cm × 100 cm (width × length × height) metallic stainless frame and a net between March 1998 and December 1999 (3 random samples per month, 30 samples in total) (Figure 1).

Lagoon Papas consists of a relatively uniform shallow area (0.2–1.5 m depth) of ha (Fig. 1). Its connection with the sea is limited to three narrow channels that isolate the lagoon, especially its southernmost parts that ends as a swamp. The lagoon is enriched with nutrients from agricultural run-off from the neighboring cultivated areas. Semi-intensive fish aquaculture with food additions of *Mugil cephalus* and *Sparus aurata* is the main commercial use of the lagoon. *Ulva* sp. is the main component of benthic vegetation that covers of the lagoon area during spring and summer. *Ulva* sp. populations were sampled by using a Ponnar–0.05 m Grab in three sites between summer 1998 and summer 1999 (3 random samples per season, 45 samples in total; Fig. 1).

The biomass of *Ulva* sp. from each sampling site was estimated by drying the sorted and cleaned fresh material at 60 °C until constant weight was obtained. All mean standing stocks and total biomass values (area × mean standing stock) were estimated in tonnes of dry biomass per hectare and in tonnes of dry biomass, respectively.

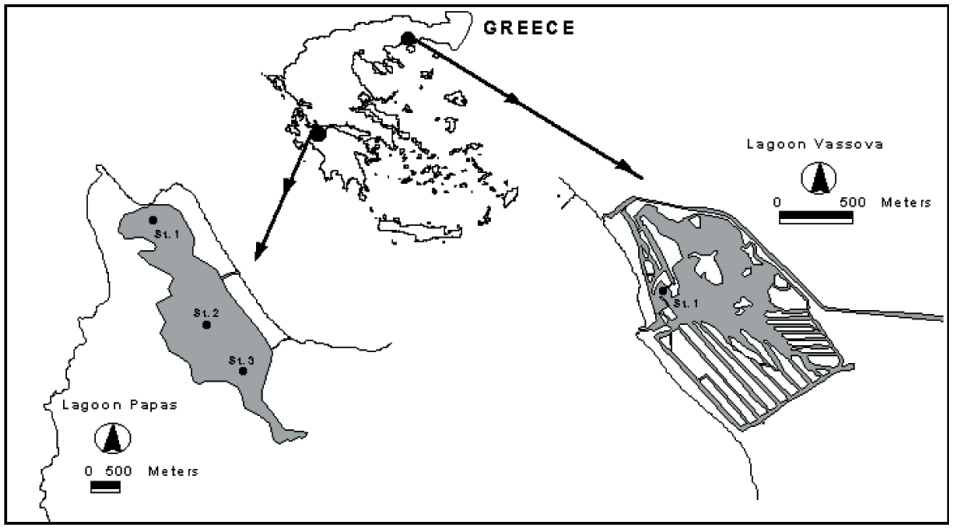

Figure 1: Map of the studied lagoons showing the sampling sites (st).

3 Results

Acidic and alkaline soils varied substantially in their chemical properties. Soil texture in the examined *Alfisol* is loamy, whilst in the *Vertisol*, it is clayey (Tab. 1).The pH in the acidic soil was very low, and the OM and N content was higher than in the alkaline soil (Tab. 1). Marine macrophytes also varied in their chemical nature. *Posoidonia oceanica* (SC) had the highest OM content, *C. barbata* the lowest content (Tab. 2), whilst total N was highest in *Ulva* sp. and lowest in *P. oceanica* (SC). Accordingly, the soil/plant mixtures were different in their OM and N content (Tab. 2).

The cumulative amount of mineralized N from the soil/plant mixtures as a function of incubation period is shown in Fig. 2, and the kinetic parameters for the various treatments are given in Tab. 3.

Cumulative net N mineralization and immobilization were also affected by soil acidity. Net N mineralization in the acidic soil was 96.3 mg N (kg soil)$^{-1}$ during the incubation period and was increased to 171.1 mg N (kg soil)$^{-1}$ in the acidic limed soil (Tab. 3). Highest mineralization values were observed in the alkaline-soil treatments for all plant materials and lowest values in the acidic-soil treatments. Liming of the acidic soil did improve mineralization. Mineralization from *Ulva* sp. was also affected after liming and increased from 27.0 to 33.8 mg N (kg plant material)$^{-1}$. Net N mineralization was recorded in all the alkaline-soil treatments except *P. oceanica* (L). In the acidic soil, only *Ulva* sp. showed net N mineralization while N immobilization occurred in all the other acidic-soil treatments with the other macrophytes. Lime application in the acidic soil enhanced the cumulative net N mineralizion of *Ulva* sp. and *P. oceanica* (SC) treatments and decreased N immobilization of in the *C. barbata* and *P. oceanica* (L) treatments (Fig. 2).

Table 1: Physico-chemical properties of soils used in this study.

Soils	**Clay** (%)	**S.E.**	**Sand** (%)	**S.E.**	**Silt** (%)	**S.E.**	**Organic matter** (g kg^{-1})	**S.E.**	**Total N** (g kg^{-1})	**S.E.**	**pH**	**S.E.**
Acidic (*Alfisol*)	25.3	0.12	38.3	0.15	37.4	0.09	58.4	0.3	4.04	0.05	4.5	0.0
Alkaline (*Vertisol*)	45.3	0.09	32.3	0.12	23.4	0.14	9.4	0.2	0.67	0.02	8.0	0.1
Acidic after liming											6.7	0.1

Table 2: Organic matter and total N of the dried marine plants and soil/plant mixtures.

Materials	**Sampling area**	**Organic matter** (g kg^{-1})	**S.E.**	**Total N** (g kg^{-1})	**S.E.**	**Total N** acid soil/ plant mixture (g kg^{-1})	**S.E.**	Total N alkaline soil/ plant mixtures (g kg^{-1})	**S.E.**
Ulva sp.	Vassova Lagoon	569.5	0.5	29.0	0.2	4.53	0.2	1.22	0.02
C. barbata	Vassova Lagoon	519.2	0.4	20.7	0.1	4.37	0.2	1.06	0.03
P. oceanica (SC)	Strymonikos Gulf	639.8	0.5	3.4	0.1	4.03	0.04	0.72	0.03
P. oceanica (L)	Strymonikos Gulf	569.0	0.4	14.0	0.3	4.23	0.03	0.93	0.02

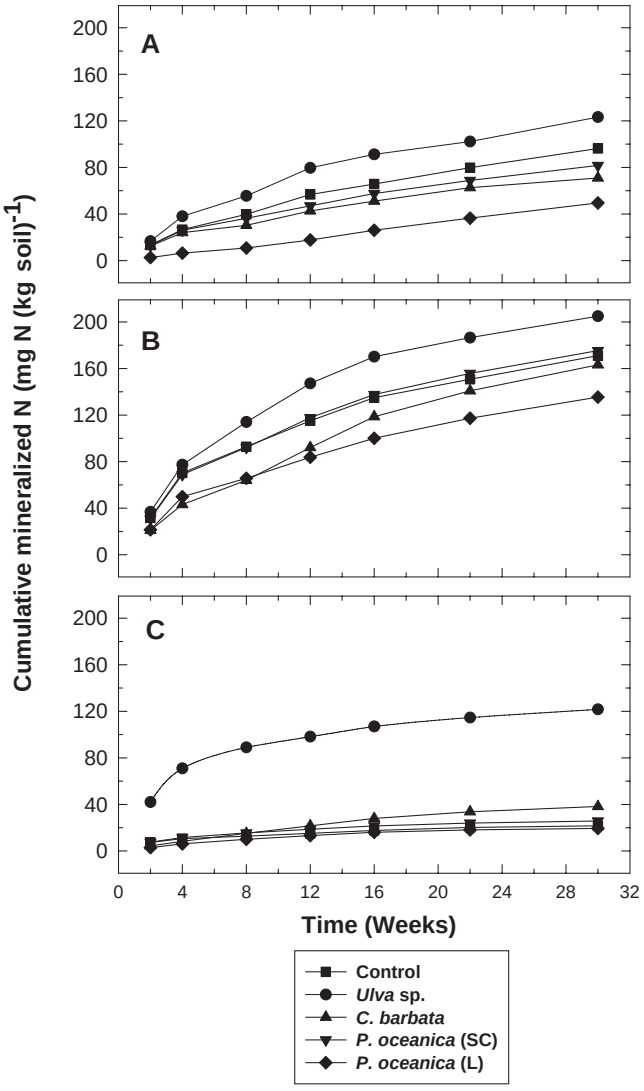

Figure 2: Cumulative nitrogen mineralization of marine macrophytes incorporated in acidic (A), limed acidic (B), and alkaline (C) soils. (SC) = storm-cast and (L) = leaves.

The potentially mineralizable N in the soil columns (no plant material was added) was a small percentage of the total N in the acidic soil, whilst in limed acidic soil, it was higher (Tab. 3). The N_0 in the acidic soil was 168.3 mg N kg^{-1} and after liming, it increased to 324.1 mg N kg^{-1}. N_0 in the alkaline soil was the lowest at 47.2 mg N kg^{-1} (Tab. 3). Figure 4 shows the values of immobilization, nitrification, and ammonification during the incubation period in the various *Ulva* sp./soil mixtures. *Ulva* sp. was strongly influenced by soil acidity. In the acidic soil, both immobilization and ammonification were recorded. After liming, ammonification was much higher than nitrification. In the alkaline soil, mineralization (nitrification + ammonification) was much higher in comparison to the acidic soil treated with lime, and nitrification was the dominant inorganic form.

Statistical analysis showed significant correlations between cumulative mineralized N and nitrification, ammonification, and N_0 after 30 weeks of incubation. These relationships are described by the following equations:

$$N_{mineralization} = 2.13 + 0.51\ N_0\ (n = 15, R^2 = 0.976^{***}) \quad (1)$$

$$N_{nitrification} = 18.8567 + 0.2414\ N_{mineralization}\ (n = 15, R^2 = 0.592^{***}) \quad (2)$$

$$N_{mineralization} = 29.7587 + 1.2321\ N_{ammonification}\ (n = 15, R^2 = 0.935^{***}) \quad (3)$$

$$N_0 = -12.47 + 1.73\ N_{ammonification} + 2.42\ N_{nitrification}\ (n = 15, R^2 = 0.982^{***}) \quad (4)$$

The population of *Ulva* sp. in the studied areas varied seasonally. Highest biomass production was observed during spring in Vassova (1.67 Mg ha^{-1}, 1.64 Mg in total) and summer in Papas (3.22 Mg ha^{-1}, 951 Mg in total) lagoons (Fig. 4). Taking into account that 1 kg of air-dried *Ulva* sp. contains 29 g N (Tab. 2), the estimated total N available from this source is 48 kg N ha^{-1} and 93.38 N ha^{-1}, respectively. According to Greek practice, the N dose recommended for cotton varies between 110 and 130 kg N ha^{-1} (*Karyotis* et al., 2002). This

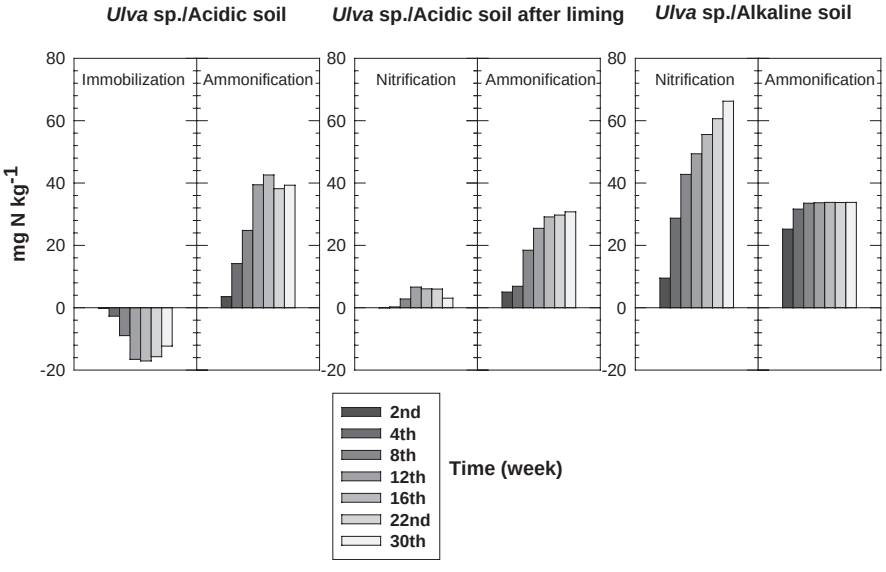

Figure 3: Cumulative net-nitrification and ammonification of *Ulva* sp.

Table 3: Kinetic parameters, cumulative N mineralization, and potentially mineralized N of the soil/plant mixtures (t = 30 w).

Soil/plant mixtures	Constant k (weeks^{-1})	N_0 (mg N kg^{-1})	$N_{cum.}$ (mg N kg^{-1})	N_{min} : tot. soil N(%)	N_0 : tot. soil N (%)
Acidic soil (control)	0.039	168.3	96.25	2.38	4.16
Acidic Soil/*Ulva sp.*	0.040	223.1	123.21	2.72	4.92
Acidic Soil/C. barbata	0.047	125.1	70.97	1.62	2.86
Acidic Soil/*P. oceanica* (SC)	0.045	141.6	81.72	2.03	3.51
Acid Soil/*P. oceanica* (L)	0.015	90.6	49.50	1.17	2.14
Limed Acidic Soil (control)	0.050	324.1	171.06	4.23	8.02
Limed Acidic Soil/*Ulva sp.*	0.048	397.1	204.88	4.52	8.77
Limed Acid Soil/C. barbata	0.035	299.1	163.13	3.73	6.84
Limed Acidic Soil/*P. oceanica* (SC)	0.049	329.5	175.32	4.35	8.18
Limed Acidic Soil/*P. oceanica* (L)	0.046	244.1	135.40	3.20	5.77
Alkaline Soil (control)	0.062	47.2	21.67	3.23	7.04
Alkaline Soil/*Ulva sp.*	0.063	290.9	121.65	9.97	23.84
Alkaline Soil/*C.* barbata	0.028	78.0	38.24	3.61	7.36
Alkaline Soil/*P. oceanica* (SC)	0.059	54.4	25.76	3.58	7.56
Alkaline Soil/*P. oceanica* (L)	0.041	37.2	19.46	2.09	4.00

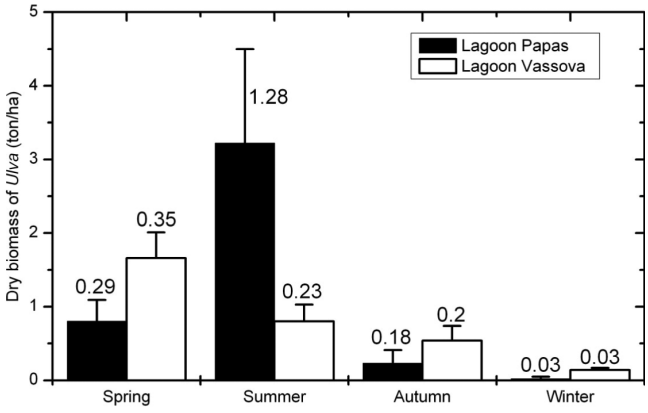

Figure 4: Seasonal variation of *Ulva*'s sp. biomass production in the Vassova and Papas Lagoons (vertical bars indicate confidence interval at 95%).

means that 1 ha of cotton would require N from 2 ha of *Ulva* sp. Therefore, *Ulva* sp. from the Lagoons Vassova and Papas can fertilize 0.5 and 122 ha of cotton plantations, respectively.

4 Discussion

The results of this study indicated that N mineralization was influenced by the chemical composition of the marine macrophytes as well as by soil properties. It should be emphasized that this study was carried out under controlled laboratory conditions (*i.e.*, constant temperature and moisture) and plant materials were ground and mixed thoroughly with soil. In the field, conditions are more variable and activity of soil microorganisms can considerably be inhibited by temperature and moisture fluctuation (*Smith* et al., 1976; *Purnomo* et al., 2000).

In general, plant material with N concentration >17.0 g kg^{-1} is considered to be desirable for organic fertilizers (*Frankenberger* and *Abdelmagid*, 1985). Of the tested macrophytes, the green alga *Ulva* sp. had the highest N content (29 g kg^{-1}), which was liberated by means of mineralization. Although tissue levels of nutrients in macroalgae are influenced by seasonal changes related to local water-nutrient regimes, the estimated N content of *Ulva* sp. is in agreement and close to the average of other published data (*Villares* and *Carballeira*, 2003). In the Greek lagoons Vassova and Papas, *Ulva* sp. reached biomass standing stocks (Fig. 4) similar in magnitude to most other European transitional waters (*Schramm* and *Nienhuis*, 1996; *Malta* et al., 2002). The exception is the Lagoon Venice, where the standing stocks of *Ulva* sp. surpassed the 2 kg dry wt m^{-2} (*Sfriso* and *Pavoni*, 1994). Standing-stock differences between the Lagoons Vassova (1.67 t ha^{-1}) and Papas (3.22 t ha^{-1}) could be attributed to wind- and wave-induced transport and accumulation of floating macroalgae in specific sites (*Malta* et al., 2002). Regarding *Ulva* sp. standing stocks and tissue N content, it was estimated that 2 ha of *Ulva* sp. could supply adequate N to cultivate 1 ha of cotton. Thus adequate biomass quantities of *Ulva* sp. are available only in the Papas Lagoon.

Posidonia oceanica (SC) had a low N content (3.4 g kg^{-1}). Nitrogen was immobilized in the acidic soil, and low N mineralization was observed in the limed acidic and alkaline soils (Fig. 2). *Posidonia oceanica* (L) had a higher N content (14.0 g kg^{-1}) than the *P.* oceanica (L) material but N immobilization occurred in all treatments. Nitrogen content, k values, and net mineralization values gave the decreasing order of macrophytes' N mineralization, as an average of all treat-

ments as *Ulva* sp. > *P. oceanica* (SC) > *C. barbata* > *P. oceanica* (L).

Soil acidity has a large effect on N mineralization of the macrophytes. When the acidic soil was limed and pH increased from 4.5 to the cumulative mineralized N was increased (Fig. 2). This was most pronounced for the *P. oceanica* (SC) material, where the net cumulative mineralized N after liming changed from –14.53 mg kg^{-1} (immobilization) to 4.26 mg (kg soil)$^{-1}$. The impact of liming in N mineralization has also been described by several authors (*Black*, 1965; *Nyborg* and *Hoyt*, 1978; *Raath* and *Saayman*, 1996; *Karyotis* et al., 2002).

Although liming increased N mineralization, higher amounts were determined when macrophytes are incorporated into alkaline soil. Net cumulative mineralized N originated by *Ulva* sp. was approximately 3 times higher in alkaline soil than in acidic and limed acidic soils (Fig. 2). Higher mineralization values reported by other authors (*Deng* and *Tabatabai*, 2000) may have been an effect of the cropping systems and origin of SOM. In this study, liming affected the mineralization constant k that increased from 0.039 to 0.050 $week^{-1}$ after liming. Our findings are difficult to be interpreted based on soil acidity only, because N content of acidic and alkaline soils varied greatly (Tab. 1). The decreasing order of soil importance to net mineralization of macrophytes was alkaline > limed acidic > very acidic soils.

Soil acidity is known to restrict nitrification in soil (*Raath* and *Saayman*, 1996). Net nitrification of *Ulva* sp. was ca. 6 times higher in alkaline soil compared to acidic soil. In contrast to net nitrification, net ammonification of *Ulva* sp. during the same incubation period was not considerably affected by soil pH suggesting that ammonification is a less pH-sensitive process. Although the nitrifying bacteria are pH sensitive (*Alexander*, 1977), the amount of nitrification also depends on the ammonification rate.

The cumulative amounts of mineralized N from macrophytes as a function of incubation time are illustrated in Fig. 2. A nonlinear relationship is obvious for all treatments, and our results are similar to that of *Stanford* and *Smith* (1972) as well as to *Frankenberger* and *Abdelmagid* (1985). After 12 weeks of incubation, of N content of macrophytes was mineralized. Equation 1 describes the relationship between mineralized N over 30 weeks of incubation and N_0. Potentially mineralized nitrogen (N_0) is of great importance indicating the N-supplying capacities of incorporated plant residues and is extremely useful especially for arid and semiarid rural areas where soils are poor in N and SOM. The practical utility in estimating N-fertilizer demands based on the calculated N_0 values requires further research because soil characteristics, climatic conditions, and composition of residues are involved in the release of mineral N (*Frankenberger* and *Abdelmagid*, 1985).

5 Conclusions

Ulva sp. can easily be decomposed even though immobilization occurred in the strongly acidic soil. Ammonification was the main process in the acidic and slightly acidic soils to which *Ulva* sp. material was added, whilst higher nitrification was recorded in alkaline soils. The use of *Ulva* sp., an abundant nuisance species in the Pappas Lagoon, as an alternative N source for arable crops would offer economical and ecological benefits for farmers, especially in soils with a low OM content. *Posidonia oceanica* (SC) is not a promising material to enrich soil with N, but due to its large available biomass, it could potentially be used to increase SOM.

Acknowledgments

We would like to thank the *Greek Ministry of Agriculture* for the financial support. The authors are also very grateful to Prof. *F. Taube* (University of Kiel) for his assistance and especially for the translation of English summary in German language.

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