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Effects of Manure Enriched with Algae *Chlorella vulgaris* on Soil Chemical Properties

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Abstract

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The effect of the algal species *Chlorella vulgaris* at six different ratios (0, 1, 2, 3, 4, 5 g) of air dried algae biomass, mixed with 10.18 g of sheep manure, added to 50 g of soil, and incubated for a period of 15 weeks was studied in a laboratory in order to detect the role of adding small amounts of admixed algal biomass to soil in biodegradation of soil organic carbon. The obtained data showed that mineralization of soil organic carbon increased by 16.2–35.9% at all rates of algae addition compared to the control, while the highest increase was observed at the highest rates of algae addition. There was a 40–50% increase in the available form of potassium, while the highest increase was also observed at the highest rates of algae addition. The four times higher rates of added algae increased the corresponding content of nitrate nitrogen by 20–30%, while ammonium nitrogen contents decreased by 9.5–35.7% for all amounts of added algae in comparison with the control. The available forms of copper, manganese, and zinc were also increased for all amounts of added algae by 56.8–61.9%, 55.8–67.3%, and 34.1–40.6%, respectively. On the contrary, the addition of algae did not indicate significant differences among treatments as concerns organic or available phosphorus contents. The results proved the effect of the algae *Chlorella vulgaris* as an accelerator agent in biodegradation of soil organic matter, without any significant negative impact on soil chemical properties.

Keywords: algae; manure; soil chemical properties; soil organic carbon biodegradation

The addition of organic materials into soil affects largely the chemical properties of the fertilized soil surface, as well as the biological activity of microflora, which in turn determines the biochemical status of soil fertility. Particular attention is paid to the biochemical function of soil when organic fertilizers of different types and origins are applied (Gougoulias *et al.* 2013; Romero *et al.* 2013; Fernandez-Hernandez *et al.* 2014; Hernandez *et al.* 2014; Odlare *et al.* 2014), because organic materials dominate in microbial nutrition. As known, the addition of appropriate organic substances contributes to the maintenance of soil fertility.

In preliminary experiments, Gravanis *et al.* (2005) found that the presence of oregano (*Origanum vulgare* L.) dry matter decreased the soil organic carbon mineralization; on the contrary, Chouliaras *et al.* (2007) found that the presence of basil (*Ocimum basilicum* L.) dry matter increased the soil organic carbon mineralization.

Compared with conventional terrestrial crops (Chakradhar *et al.* 2008; Singh & Gu 2010), the cultivation of microalgae is characterized by a fast growth rate, with no need of insecticides and pesticides application; these products qualified with these referred advantages could be adopted also for organic

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farming applications. Some algae strains favour high biomass production and other high lipid concentration (Yoo *et al.* 2010). Biomass of microalgae in agriculture can be used also as bio fertilizer and as soil conditioner (Faheed & Fattah 2008; Mata *et al.* 2010; Coppens *et al.* 2015; Uysal *et al.* 2015; Abdel-Raouf *et al.* 2016; Renuka *et al.* 2016). Additionally, the role of blue-green algae in supplying N for rice growing and for improving physico-chemical properties of soil is well documented (Metting 1996; Mandal *et al.* 1999; Song *et al.* 2005).

The byproducts of wastewater treatment and particularly those of dairy products processing are suitable for the algae biomass production (Hoffmann 1998; Craggs *et al.* 2003); also, dried algal biomass resulting from animal manure treatment can substitute for the commercial fertilizers nitrogen and phosphorus used in potting (Mulbry *et al.* 2005).

The objective of this laboratory research was to find out if the application of small amounts of algae biomass into soil amended with sheep manure has any beneficial effect on soil chemical properties.

MATERIAL AND METHODS

Algae cultivation. The algal species *Chlorella vulgaris* was obtained from the Experimental Phycology and Culture Collection of Algae at the University of Göttingen, Germany (EPSAG). It was kept at 4°C and each vial was used within 3 months. The composition of the used growth medium was as suggested by Andersen *et al.* (1991). For *Chlorella vulgaris* each 50 l of growth medium contained: 0.2 g KNO_3/l, 0.02 g K_2HPO_4/l, 0.02 g $MgSO_4{\cdot}7H_2O$/l, 30 ml of soil extract/l, and 5 ml/l of solution containing the following micronutrients per litre: 1 mg $ZnSO_4{\cdot}7H_2O$, 2 mg $MnSO_4{\cdot}4H_2O$, 10 mg H_3BO_3, 1 mg $Co(NO_3)_2{\cdot}6H_2O$, 1 mg $MoO_4{\cdot}2H_2O$, 0.005 mg $CuSO_4{\cdot}5H_2O$, 700 mg $FeSO_4{\cdot}7H_2O$ and 800 mg EDTA.

The bioreactors were rectangular Teflon vessels sizing 30 × 35 × 60 cm. The experiment was carried out in a greenhouse of the Technological Educational Institute (TEI) of Thessaly for a period of 82 days. Two bioreactors were exposed to exactly the same conditions such as light, orientation, stirring, and temperature, then natural fluctuation of pH and electrical conductivity (EC) in the growth media was monitored. Throughout the cultivation process, the EC ranged from 0.517 to 0.730 dS/m and pH from 9.00 to 10.69 (Spiliotis *et al.* 2014). Mass concentrations on a dry basis (mg dry algal mass/l of growth medium) were determined from the volume of medium and the weight of algal mass after water evaporation and drying. Figure 1 shows the increase of algae mass concentration during the crop season. In days 16–32 of the crop season, the concentration of algae mass stabilized at 200 mg /l of growth medium, in days 37–65 it stabilized at 400 mg/l of growth medium, and from day 68 to the final crop it increased to 600 mg/l of growth medium. At the end of the growing season, air-dried algae was collected in order to be used.

Incubation experiment. In this study, 10.18 g of sheep manure containing 2.90 g of organic carbon (Table 1), obtained from the farming establishments of TEI of Thessaly, were added to 50 g of air-dried, sandy loam textured soil with 0.28% of organic carbon, derived from the same region. Into 50 g of this soil plus 10.18 g of the above mentioned sheep manure, 0, 1, 2, 3 ,4, and 5 g of air dried and well milled algae *Chlorella vulgaris* were incorporated. Thus an experimental unit constituted of 50 g soil, 10.18 g sheep manure, and a variable amount of algae. The control (C) was formed only by 50 g soil enriched with 10.18 g sheep manure; treatments A1, A2, A3, A4 or A5, control and algae 1, 2, 3, 4, or 5 g, respectively. These treatments were maintained under the same conditions in an incubator. The treatments were prepared in four replicates and kept at 28°C for a period of 15 weeks. In the beginning of the incubation period, all treatments were supplemented with distilled water at two-thirds of the field capacity. 22.9, 23.6, 24.3, 25.0, 25.6, and 26.2 g distilled water was added for each treatment, respectively. During the first three weeks of the incubation period, loss of moisture was supplemented every Monday and Thursday, but for the next three weeks the soils were left to dry. This process was repeated until the

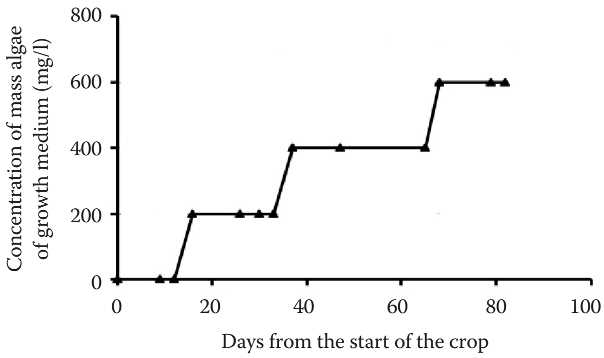

Figure 1. Variation of dry algal mass content in the growth medium during culture (mg dry weight/l)

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end of the incubation period according to Wu and Brookes (2005), reporting that the alternation of drying and rewetting soil samples enhances mineralization of both soil biomass organic matter and non-biomass organic matter. Drying and rewetting of soil is an important process in soil aggregation, metabolic activity of the soil microbial biomass, and soil organic matter (SOM) decomposition (Mikha *et al.* 2005; Gordon *et al.* 2008; Xiang *et al.* 2008). Figure 2 shows the periodic fluctuation of moisture during of the incubation period for all treatments; while, at the beginning (Table 2) and at the end of the incubation period, the soil samples and soil mixtures were analyzed.

Methods of analyses. Samples were analyzed using the following methods.

Organic carbon was determined with wet oxidation by 1 mol/l $K_2Cr_2O_7$ and titration of the remaining reagent with 0.5 mol/l $Fe(NH_4)_2(SO_4)_2$ $6H_20$, according to the method described by Walkley and Black (1934).

Soil pH was determined in (1 : 5) soil/water extract, being measured by glass electrode pH meter (Vogel 1994). The EC was measured in (1 : 5) soil/water extract using conductivity meter (Rhoades *et al.* 1999). Exchangeable forms of potassium and sodium were extracted with 1 mol/l CH_3COONH_4 and measured by flame photometer (Corning 400, Sherwood Scientifics, Halstead, UK), using the ammonium acetate method (Page 1982). Both ammonium and nitrate nitrogen were extracted with 0.5 mol/l $CaCl_2$ and estimated by distillation in the presence of MgO and Devarda's alloy, respectively (Bremner & Keeney 1966).

Available P forms (Olsen P) were extracted with 0.5 mol/l $NaHCO_3$ and measured colorimetrically according to the method described by Watanabe and Olsen (1965).

Organic phosphorus was determined by the difference in phosphorus content of the 1 mol/l H_2SO_4 extract measured before and after ignition of the samples at 550°C, according to the method described by Anderson (1980). Available forms of Mn, Zn, and Cu were extracted with DTPA (diethylenetriaminepentaacetic acid 0.005 mol/l + $CaCl_2$ 0.01 mol/l + triethanolamine 0.1 mol/l) and measured by atomic absorption. For the determination of total metals Mn, Cu, and Zn, 1 g of material was digested at 350°C + 10 ml HNO_3 + 5 ml $HClO_4$. According to the method described by Allen *et al.* (1974) and Varian (1989), the samples were analyzed by a SpectrAA 10Plus Varian Atomic Absorption Spectrometer (Melbourne, Australia), with the use of flame and air-acetylene mixture.

The C-organic mineralization was determined as the difference between the soil organic carbon content of mixtures at the start and at the end of the incubation period. Results were expressed as C-organic mineralization/kg initial soil organic carbon of mixtures, at the start of incubation period.

Table 1. Chemical properties of soil, sheep manure, and algae species *Chlorella vulgaris* air-dried samples

Property	Soil	Sheep manure*	*Chlorella vulgaris*
Texture	Sandy loam		
Organic carbon (%)	0.28 ± 0.01	28.46 ± 1.24	
$CaCO_3$ (%)	7.4 ± 0.36		
SP	51.0		
N-total (g/kg)	1.56 ± 0.07	24.36 ± 1.21	22.7 ± 1.17
$N\text{-}NH_4^+$ (mg/kg)	34.7 ± 5	133 ± 14	
$N\text{-}NO_3^-$ (mg/kg)	60.7 ± 9	487.2 ± 33	
K-exchangeable (mg/kg)	284.9 ± 16		
P-Olsen (mg/kg)	9.72 ± 3.2		
P-organic (mg/kg)	76.4 ± 4.5		
P-total (mg/kg)	291.9 ± 9.5	8490 ± 353	6840 ± 304
Na-exchangeable (mg/kg)	219.7 ± 13		
CEC (mmol/kg)	20.95 ± 1.12		
pH	7.85 ± 0.05	7.04 ± 0.04	
EC (dS/m)	0.42 ± 0.03	0.74 ± 0.06	
Cu-DTPA (mg/kg)	0.94 ± 0.04		
Zn-DTPA (mg/kg)	0.23 ± 0.01		
Mn-DTPA (mg/kg)	1.08 ± 0.05		
Na-total (mg/kg)	557.0 ± 23	11350 ± 414	2180 ± 114
K-total (g/kg)	5.1 ± 0.25	22.43 ± 0.61	6.9 ± 0.31
Cu-total (mg/kg)	46.05 ± 2.4	74.3 ± 3.4	52.3 ± 2.6
Zn-total (mg/kg)	43.99 ± 2.4	267.0 ± 14	203.4 ± 9.2
Mn-total (mg/kg)	558.2 ± 28	328.7 ± 19	6.14 ± 0.7
Mg-total (mg/kg)	7137 ± 342	8400 ± 364	92.3 ± 4.4
Fe-total (g/kg)	18.3 ± 0.9	2.25 ± 0.1	4.78 ± 0.3
Ca-total (g/kg)	31.5 ± 1.2	13.0 ± 0.7	3.8 ± 0.1

SP – saturation percentage moisture; CEC – cation-exchange capacity; EC – electrical conductivity; DTPA – diethylenetriaminepentaacetic acid 0.005 mol/l + $CaCl_2$ 0.01 mol/l + triethanolamine 0.1 mol/l; *digested sheep manure three months; data represent average means and standard deviation (n = 4)

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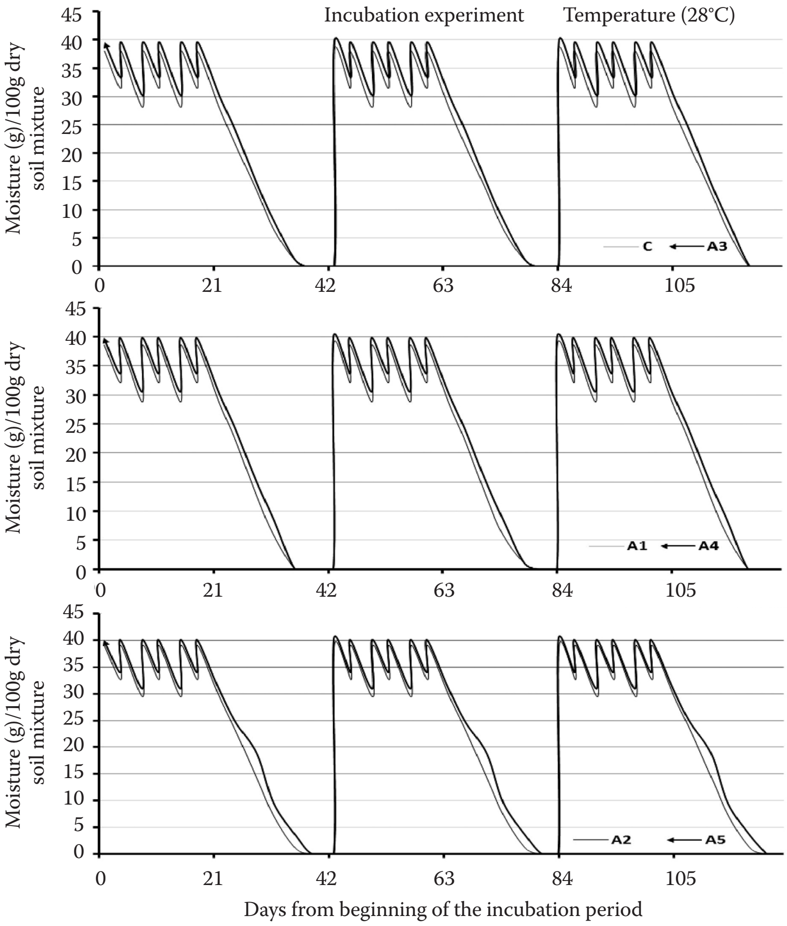

Figure 2. Periodic moisture fluctuations of each treatment during the incubation period
C – control (50 g soil and 10.18 g sheep manure); A1, A2, A3, A4 or A5 – control and algae 1, 2, 3, 4, or 5 g, respectively; data for each treatment represent average means for four replicates

Statistical analysis. The incubation experiment was realized in four repetitions for each treatment. Tukey's procedure was used to detect and separate the mean values (significant differences at $P = 0.05$), according to Minitab statistical software (5^{th} Edition, 2005) (Ryan *et al.* 2005), in order to conduct the analysis of variance. Linear correlation analysis between the decomposition of soil organic carbon and the differences estimated in the treatments with algae, concerning the concentrations of N-NH_4^+, N-NO_3^-, K-exchangeable, P-Olsen and P-organic, was also performed using the Minitab software (significance level $P = 0.05$).

RESULTS AND DISCUSSION

The results of the laboratory experiment showed that the decomposition of soil organic carbon, added as sheep manure or as pre-existed organic carbon in soil, was significantly affected by algal air dried biomass addition, and also an increase in mineralization of soil organic carbon by 16.2–35.9% at all algae addition rates was observed (Figure 3). The increased soil organic carbon biodegradation was probably due to the increased microbial activity. The organic carbon decomposition in the soil, where algae was incorporated at 5 g per 50 g soil, was about 1.6 times higher than that obtained in the algae-free soil (control), after a long incubation period. Similar studies showed that the presence of foliar tissues could either decelerate the degradation of organic fertilizers added to soil (oregano or neem) (Gravanis *et al.* 2005; Gougoulias *et al.* 2010), or accelerate it (presence of basil) (Chouliaras *et al.* 2007).

The ammonium forms of nitrogen decreased by the addition of algae in all treatments in comparison with the control, at the end of the incubation period. These values are lower in comparison to

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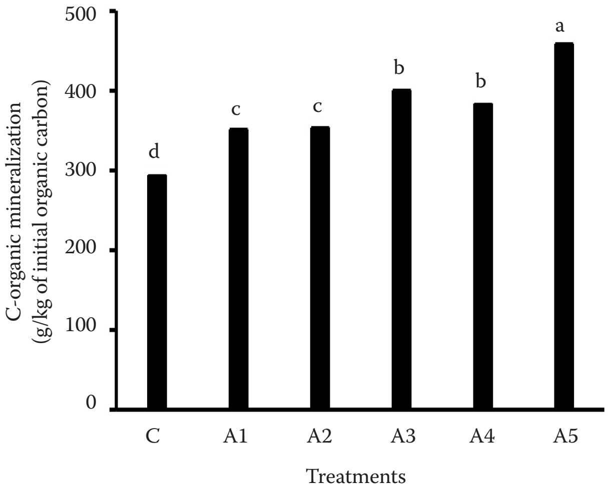

Figure 3. Effect of algae dry matter concentration on organic carbon mineralization
C – control (50 g soil and 10.18 g sheep manure); A1, A2, A3, A4 or A5 – control and algae 1, 2, 3, 4, or 5 g, respectively; values in columns with the same letter do not differ significantly according to Tukey's test (P = 0.05)

corresponding values found at the start of the incubation period (Table 2). Contrarily, the nitrate nitrogen content increased at the end in comparison to the beginning of the incubation period. The four times higher rates of added algae increased the corresponding content of nitrate-nitrogen by 20–30% in comparison with the control at the end of the incubation period (Table 3), then the nitrification effect on soil proceeded normally after the algae addition. The decrease of soil organic carbon and of ammonium forms with a simultaneous increase of nitrate forms at the end of the incubation period indicates that the mineralization process proceeded properly. Nevertheless, the final balance of N-content at the end of the incubation period also depends on nitrogen gasification (denitrification), taking place even under partially reduction conditions into soil microstructure (Chouliaras 1994).

The addition of algae at all rates increased available K by 40–50% at the end of the incubation period, while the highest increase was observed with the high-

Table 2. Chemical properties of soil mixtures at the beginning of the incubation period

Properties	C	A1	A2	A3	A4	A5
N-NH_4^+ (mg/kg)	49.1[a]	45.2[a]	54.3[a]	41.9[a]	47.2[a]	43.4[a]
N-NO_3^- (mg/kg)	99.1[d]	119.1[cd]	140.1[bc]	175.9[b]	230.9[a]	244.8[a]
K-exchangeable (mg/kg)	3677.7[b]	3683.4[b]	3763.1[b]	4253.7[a]	4398.3[a]	4465.2[a]
P-Olsen (mg/kg)	357.5[a]	311.6[a]	354.2[a]	333.3[a]	337.3[a]	314.9[a]
P-organic (mg/kg)	353.0[c]	331.7[c]	393.6[b]	400.9[ab]	415.0[ab]	433.7[a]
Na-exchangeable (mg/kg)	676.7[a]	684.3[a]	689.5[a]	694.3[a]	696.3[a]	702.4[a]
CEC (mmol/kg)	67.6[b]	67.9[b]	68.3[b]	73.1[ab]	76.9[a]	77.2[a]
pH	7.84[a]	7.82[a]	7.73[ab]	7.52[ab]	7.45[b]	7.48[b]
EC (dS/m)	0.69[b]	0.71[b]	0.75[b]	0.82[ab]	0.86[a]	0.91[a]
Cu-DTPA (mg/kg)	2.92[a]	2.12[b]	2.98[a]	2.03[a]	2.48[ab]	2.07[b]
Zn-DTPA (mg/kg)	7.33[b]	7.68[a]	7.42[ab]	7.53[ab]	7.76[a]	7.89[a]
Mn-DTPA (mg/kg)	8.23[a]	8.42[a]	8.03[a]	8.13[a]	7.95[a]	7.42[b]
Na-total (mg/kg)	762.0[e]	785.2[de]	807.6[cd]	829.3[bc]	850.4[ab]	870.9[a]
K-total (g/kg)	8.03[a]	8.01[a]	8.00[a]	7.98[a]	7.96[a]	7.94[a]
Cu -total (mg/kg)	50.83[a]	50.85[a]	50.88[a]	50.90[a]	50.92[a]	50.94[a]
Zn-total (mg/kg)	80.53[f]	82.54[e]	84.48[d]	86.36[c]	88.19[b]	89.96[a]
Mn-total (mg/kg)	479.4[a]	471.7[a]	464.1[a]	456.9[a]	449.9[a]	443.1[a]
Mg-total (g/kg)	7.35[a]	7.23[a]	7.12[a]	7.06[a]	6.90[a]	6.79[a]
Fe-total (g/kg)	15.59[a]	15.41[a]	15.24[a]	15.07[ab]	14.91[b]	14.76[b]
Ca-total (g/kg)	25.9[c]	27.97[a]	27.58[a]	27.20[a]	26.84[b]	26.49[b]

CEC – cation-exchange capacity; EC – electrical conductivity; DTPA – diethylenetriaminepentaacetic acid 0.005 mol/l + $CaCl_2$ 0.01 mol/l + triethanolamine 0.1 mol/l; for each chemical property of soil mixtures, lines of table with the same letter do not differ significantly according to Tukey's test (P = 0.05); C – control (50 g soil and 10.18 g sheep manure); A1, A2, A3, A4 or A5 – control and algae 1, 2, 3, 4, or 5 g, respectively

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est rates of algae additions. Similarly, Chouliaras *et al.* (2007) reported an increase of available K in the case of basil dry matter addition. Additionally, Renuka *et al.* (2016) reported that dried microalgal biomass used as a biofertilizer in wheat crop (*Triticum aestivum* L.) increased the availability of nitrogen, phosphorus, potassium, and improved yields of wheat crop. Nevertheless, concerning the phosphorus forms (organic or bio available), the results indicated that there was no significant difference between treatments by the added algae at the end of the incubation period in comparison with the control (Table 3); then phosphorus biosynthesis compared to organic phosphorus biodegradation was balanced after algal addition to manure during the incubation period; as far as it concerns biodegradation P-availability, it is also affected by the soil P-fixation capacity. In a previous research of similar soils (slightly alkaline, calcareous, and loamy soils) in the given area, the P-fixation effects were always verified, while the bridging role of Ca^{2+} cations between P-anions and soil colloidal forms was adopted as predominating (Chouliaras *et al.* 1990). In similar studies, Gougoulias *et al.* (2010) reported an increase of available phosphorus forms in the case of oregano dry matter addition. Additionally, the use of algal biomass as a slow release fertilizer has been reported (Mulbry *et al.* 2005).

The comparison of differences estimated between the control values and the treatments with algae, concerning the concentrations of N-NH_4^+, N-NO_3^-, K-exchangeable, P-Olsen and P-organic, showed statistically significant linear correlation with the decomposition of soil organic carbon only in the case of available potassium, for the lower dose additions of algae (Table 4). In a similar comparison between the differences of the concentrations and the algae dosages, any significant correlation ($P > 0.05$) was not found.

Non-significant changes of cation-exchange capacity (CEC) values were found at the end of the incubation period; the digestion of manure before

Table 3. Chemical properties of soil mixtures at the end of the incubation period

Properties	C	A1	A2	A3	A4	A5
N-NH_4^+ (mg/kg)	29.4^a	18.9^c	26.5^b	26.9^b	20.3^c	22.4^c
N-NO_3^- (mg/kg)	421.6^d	401.7^e	539.0^a	536.2^{ab}	519.4^b	497.0^c
K-exchangeable (mg/kg)	3276.0^d	4590.3^c	4609.8^c	4772.3^b	4953.0^a	4792.3^{ab}
P-Olsen (mg/kg)	332.4^{ab}	339.0^a	333.0^{ab}	326.6^{ab}	324.9^{ab}	321.1^a
P-organic (mg/kg)	515.0^{ab}	530.7^{ab}	501.6^{ab}	530.6^{ab}	577.3^a	429.7^b
Na-exchangeable (mg/kg)	1028.0^a	940.7^b	991.3^b	968.3^b	945.3^b	991.3^b
CEC (mmol/kg)	40.6^a	38.3^a	40.7^a	39.9^a	37.9^a	33.4^b
pH	7.41^a	7.13^b	6.88^d	7.05^c	7.01^c	7.00^c
EC (dS/m)	0.90^c	1.01^{cb}	1.05^{ab}	1.12^a	1.08^{ab}	1.05^{ab}
Cu-DTPA (mg/kg)	1.44^c	3.78^a	3.70^a	3.70^a	3.33^b	3.70^a
Zn-DTPA (mg/kg)	6.89^c	10.45^b	10.57^b	10.62^b	11.12^{ba}	11.59^a
Mn-DTPA (mg/kg)	4.52^d	12.07^b	10.23^c	13.10^a	10.74^c	13.81^a
Na-total (mg/kg)	1054^a	1026^a	1005^a	1037^a	1058^a	1058^a
K-total (mg/kg)	8207^c	9083^b	9909^a	9496^{ab}	9483^{ab}	9258^{ab}
Cu-total (mg/kg)	57.44^b	57.41^b	59.82^{ab}	59.26^{ab}	59.26^{ab}	65.56^a
Zn-total (mg/kg)	85.49^b	88.00^b	88.20^b	86.00^b	92.20^a	93.30^a
Mn-total (mg/kg)	491.2^c	818.4^b	1125.3^a	1133.7^a	1108.3^a	1014.3^{ab}
Mg-total (g/kg)	8.10^d	21.73^{bc}	21.59^{bc}	23.64^a	23.10^{ab}	23.10^{ab}
Fe-total (g/kg)	18.8^a	19.3^a	20.9^a	19.8^a	20.3^a	19.4^a
Ca-total (g/kg)	26.6^b	29.2^b	29.4^b	34.0^a	31.0^a	32.4^a

CEC – cation-exchange capacity; EC – electrical conductivity; DTPA – diethylenetriaminepentaacetic acid 0.005 mol/l + $CaCl_2$ 0.01 mol/l + triethanolamine 0.1 mol/l; for each chemical property of soil mixtures, lines of table with the same letter do not differ significantly according to Tukey's test ($P = 0.05$); C – control (50 g soil and 10.18 g sheep manure); A1, A2, A3, A4 or A5 – control and algae 1, 2, 3, 4, or 5 g, respectively

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its use had already a stabilization effect on the CEC property. By the addition of algae the exchangeable Na content decreased in all treatments at the end of the incubation period compared to the control. Contrarily, compared to the control, the exchangeable K content increased in all treatments. This was probably due to the stronger adsorbing intensity of exchangeable K compared to exchangeable Na in the soil colloids.

The soil pH decreased by algae dry matter addition, while the highest decrease was observed at the highest rates of algae additions, although in the case of basil dry matter addition the pH was not significantly affected (Chouliaras *et al.* 2007). The decrease of soil pH at the end of the incubation period was probably due to the stronger decomposition of soil organic matter and the NH_4^+-N oxidation to NO_3^--N.

The EC, with the addition of algae at four higher rates compared to the control, increased by 14.3 to 19.6% at the end of the incubation period. The EC for the control was 0.90 ms/cm, while for the remaining treatments it ranged from 1.05 to 1.12 ms/cm (Table 3). These values are higher than the corresponding values at the start of the incubation period (Table 2). This increase is due to the biodegradation of soil organic matter and the addition of algae, without creating serious risk of soil salinity.

The levels of available forms of copper, zinc, and manganese were significantly increased by algae addition; all five algae rates increased available copper by 130–160%, available zinc by 50–70%, and available manganese by 120–200%; in particular, the higher rate of algae resulted in a higher increase of copper, zinc or manganese available forms in soil (Table 3). All these increasing values of available metal forms are related to more intensively mineralized soil organic matter. Moreover, in all treatments, the levels of available forms of copper, zinc, and manganese were significantly increased at the end of the incubation period compared with its onset (Table 2). In contrast, Gougoulias *et al.* (2010) found that dry plant matter amendment, in that case oregano (*Origanum vulgare* L.), did not affect the available Zn.

The levels of total forms of copper, zinc, and calcium at the end of the incubation period were increased by higher rates of algae addition compared with the control and with the lowest rates of algae addition. Total iron content by algae addition did not show

Table 4. Linear correlation between the concentrations of chemical properties of soil mixtures, that are determined as the difference between the contents at the end and at the start of the incubation period, and of the decomposition of organic carbon

Properties	C	A1	A2	A3	A4	A5
C-organic mineralization (g/kg initial organic carbon)	294.4^{d}	351.1^{c}	354.2^{c}	401.3^{b}	384.0^{b}	458.9^{a}
N-NH_4^+ (mg/kg)	–19.7^{ab}	–26.3^{b}	–27.8^{b}	–15.0^{a}	–26.9^{b}	–21.0^{ab}
r	0.927	0.932	0.630	0.875	0.968	0.736
p	0.245	0.237	0.566	0.321	0.160	0.473
N-NO_3^- (mg/kg)	+322.5^{bc}	+282.6^{cd}	+398.9^{a}	+360.3^{ab}	+288.5^{cd}	+252.2^{d}
r	0.770	0.998	0.902	0.972	0.935	0.995
p	0.440	0.039	0.284	0.150	0.231	0.065
K-exchangeable (mg/kg)	–401^{e}	+906.9^{a}	+846.7^{b}	+518.6^{c}	+554.7^{c}	+327.1^{d}
r	–0.998	0.998	1.00	0.987	0.770	0.980
p	0.035	0.042	0.013	0.105	0.440	0.127
P-Olsen (mg/kg)	–25.1^{e}	+27.4^{a}	–21.2^{d}	–6.7^{c}	–12.4^{cd}	+6.2^{b}
r	1.00	0.982	0.933	0.722	0.999	0.929
p	0.009	0.121	0.235	0.486	0.023	0.242
P-organic (mg/kg)	+162^{b}	+199^{a}	+108^{c}	+129.7^{c}	+162.3^{b}	–4.0^{d}
r	0.889	0.999	0.879	0.998	0.985	0.917
p	0.303	0.028	0.316	0.039	0.109	0.261

For each chemical property of soil mixtures, lines of table with the same letter do not differ significantly according to Tukey's test (P = 0.05); C – control (50 g soil and 10.18 g sheep manure); A1, A2, A3, A4 or A5, control and algae 1, 2, 3, 4, or 5 g, respectively; r – coefficients of linear correlation; p – level of significance (P = 0.05).

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statistically significant differences in all treatments at the end of the incubation period. All the algae amounts added increased the total magnesium and manganese contents at the end of the incubation period (Table 3). Moreover, the levels of total forms of copper, zinc, calcium, iron, magnesium, and manganese were significantly increased at the end of the incubation period compared with its onset for all treatments (Table 2).

CONCLUSION

Five different rates of algae biomass species *Chlorella vulgaris* were applied into a soil amended with sheep manure, and after a 15-week incubation period the corresponding effects on the soil chemical properties were attested.

The obtained data indicated that algae *Chlorella vulgaris* could be applied to the soil as a stimulating agent for soil organic carbon biodegradation. Moreover, a dried algae biomass applied in small amounts into a sandy loam soil amended with sheep manure ensured nitrification process and had positive effects on the availability of nutritional elements K, Cu, Zn, and Mn without any significant negative impact on soil chemical properties. These results could contribute to the replacement of usual chemical fertilizers in future alternative farming.

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