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Development of soil chemical and biological properties in the initial stages of post-mining deposition sites

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Abstract The aim of this study was to assess the seasonal development of the physicochemical (pH, organic C, organic N, extractable P, Ca^{2+}, Mg^{2+}) and biological soil properties (microbial biomass, activities of urease, dehydrogenase and alkaline phosphatase) of the topsoil of mine deposition sites that differed based on the material used exclusively for their creation: (a) marlstones, (b) red-grey formations (RGF), and (c) fly ash (FA), during the first year after their creation. Our hypothesis was that all deposition sites, regardless the material they consist of, present equal opportunities for the establishment of spontaneous vegetation. All macronutrients concentrations (P, Ca^{2+}, and Mg^{2+}) remained constant with time and were found to be higher in the FA sites. Organic C, organic N, all enzyme activities, and microbial biomass were higher in the RGF and marl depositions, with marl sites presenting the highest values. All values of biological variables, with the exception of alkaline phosphatase, increased with time. The alkaline environment along with the slow improvement in soil biological properties of the FA sites seemed to present the most unfavorable conditions for spontaneous vegetation growth. On the contrary, the other two spoil materials presented significant improvement in the initial stages of soil formation in terms of soil functionality.

Keywords Enzyme activities · Microbial biomass · Secondary succession · Soil development

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Introduction

Greece is the fifth largest world producer of lignite and the second largest within the European community. This intensive exploitation of lignite requires the excavation of about 250 million m^3 of overlying coal layers which is deposited on spoil heaps creating a mosaic of soils structurally different due to the excavation depth layer they come from. Moreover, the total annual fly ash production, the principal by-product of the burnt lignite, is about 7.6×10^6 tones most of which (85–90 %) is being dumped to nearby areas as fly ash deposition heaps (Skodras et al. 2007).

An increase in the concerns for environment has made concurrent post-mining reclamation of the degraded land an integral feature of the whole mining spectrum (Ghose 1989). Conservation and reclamation efforts to ensure continued beneficial use of land resources are essential. There are two approaches for the reclamation or the restoration of a disturbed site: (1) allowing the establishment of spontaneous vegetation or (2) using technical reclamation with sowing or planting target species, accompanied by restoration or improvement of site conditions. Studies have shown that spoil heaps which have developed naturally (i.e., without reclamation actions) generally achieved richer plant communities than the reclaimed stands (Prach and Pysek 2001; Hodacova and Prach 2003). Therefore, natural plant succession in post-mining areas is promoted in modern restoration practice as a proper alternative to conventional reclamation, and it has been implemented in restoration projects in many European countries (Schulz and Wiegleb 2000).

The initial stages of soil development are crucial for the establishment of spontaneous vegetation, as the interaction between plants and soil properties is well documented (Frouz et al. 2008; Alday et al. 2012). However, the spoil material is excavated from great depths (from as deep as 200 m) as much as the fly ash contains low amounts of organic matter and exhibits very low biological activity (Frouz and Novakova 2005; Elhottova et al. 2006; Baldrian et al. 2008). These characteristics are typical of low soil quality, presenting difficulties in plant growth. Formation of stable and productive soils on disposed anthropogenic substrates can only be achieved if soil functionality is restored. The latter consists of a basic precondition for the recovery of ecologically sensitive ecosystems such as the spoil heaps (Bradshaw 1997). Several gross microbial properties such as the amount of microbial biomass, soil respiration rate, and enzyme activities (Graham and Haynes 2004; Frouz and Novakova 2005; Sourkova et al. 2005) have been used as bioindicators to assess soil development in reclaimed post-mining lands.

The post-mining spoil heaps represent very simple habitats and allow the study of secondary ecological succession procedures relating to soil biochemical attributes, in terms of soil functions (soil enzymes) and microbial biomass. The succession that takes place in these new habitats could be considered secondary as they are rich in macronutrients (Yunusa et al. 2006), fossil C (Sourkova et al. 2005), and exhibit low toxic and heavy metal content (Monokrousos et al. 2009; Vlachodimos et al. 2013). The aim of this study was to identify which are the soil attributes of each material (limestone marlstones, red-grey formations, and fly ash) so as to predict the type of vegetation that could be established in each spoil heap. The study focused on the development of physicochemical and biological soil properties (soil enzymes related to C, N, and P cycle, microbial biomass, pH, organic C, organic N, extractable P, Ca^{2+}, Mg^{2+}) during the first year after the creation of the depositions. The types of deposition material and the time after deposition were the main factors considered in the present study. Our hypothesis is that all deposition sites, no matter the material they consisted of, present equal opportunity for the establishment of spontaneous vegetation.

Materials and methods

Study site

The study site is located south of the city of Ptolemais in North Greece, in the Kozani-Florina basin. The Kozani-Florina basin is situated between Vermio and Siniatsiko mountains (22″ latitude and 40″ 30′ longitude) at a mean average altitude of 667.5 m. The valley is rich in lignite with six active lignite mines in the area.

The climate of the area is subhumid Mediterranean with a cool winter. Average annual precipitation is 550.76 mm, with a large peak in November (68.19 mm mean monthly precipitation) and a smaller one in May (54.47 mm). The average annual air temperature is 12.26 °C. The dry period is relatively short and the distribution of precipitation even more than in other regions of Greece producing good moisture conditions for vegetation. During winter, snow as well as early and late frosts are common. Average annual relative humidity is 62.5 % with average monthly values varying between 48 and 76 % (data from the Ptolemaida meteorological station over a 5-year period).

The overburden that covers the lignite layers in the valley consists of marlstones, sediments, redsoils, alluvials, peatmould, peat, fossils, and others (Kolovos et al. 2000). Normally, after the mining of lignite, overburden layers are mixed together with bad quality lignite and lignite ashes. The new soils that arise from this mixture are rich in carbonate, heterogeneous, unstable, unconsolidated, and of low compaction (Panagopoulos and Hatzistathis 1995).

Sampling

In September 2010, three types of new deposition sites of different deposition material were created within the existing mine spoil heap area. Each type consisted exclusively from one of the main overburden excavation material such as marl, red-grey formations (RGF), or lignite fly ashes (FA). For each deposition type, three separate rather homogeneous flat depositions (30×30 m each) were created. All deposits were close to each other ensuring same weather conditions. The red–grey formations originated from excavations up to 50 m in depth and were characterized as clay loam (average contents of clay, silt, and sand were 30.8 %, 29.6 %, and 39.6 %, respectively); the marl came from deeper layers, up to 200 m in depth (55 % $CaCO_3$), while the lignite fly ashes originated from the burn of lignite, and their properties are described in Kolovos (2002) study. Five soil samples were taken randomly from each spoil heap with a cylindrical auger (12 cm depth and 7.5 cm diameter). The samples were immediately intermingled so that a single composite sample was formed for each spoil heap. For each spoil type, three replicate samples were collected at each sampling occasion. Samples were taken in September 2010, and January and May 2011. The overall experimental design consists of 3 replicate samples × 3 sampling areas × 3 sampling periods. Samples were transported to the laboratory and stored at 4 °C until analyzed.

Biochemical analyses

Soil pH was determined using an electrode pH-meter in a 1:2 soil/water suspension (McLean 1982). Soil organic C (Corg) was determined by a wet oxidation titration procedure using an acid dichromate system (Allen 1974). Soil organic N (Norg) was measured by the Kjeldahl method. Soil microbial biomass C (MBC) was measured by the fumigation incubation method of Jenkinson and Powlson (1976) as modified by Ross (1990) in samples adjusted to 60 % of their water holding capacity. The Kc factor equals 0.45 (Jenkinson and Powlson 1976). For inorganic P (Pextr), the method of Olsen et al. (1954) as specified by Allen (1974) was used. The concentrations of Ca^{2+} and Mg^{2+} were determined by atomic absorption spectrophotometer (Perkin-Elmer 2380). The activities of urease and alkaline phosphatase were determined by the method of Tabatabai (1994). Briefly, the determination of urease activity is based on the determination of NH_4^+ released when soil is incubated at 37 °C for 2 h with 0.1 M Tris (hydroxymethyl) aminomethane buffer, toluene, and urea. The determination of alkaline phosphatase involves extraction and quantitative determination of p-nitrophenol released, when soil is incubated with p-nitrophenyl phosphate in modified universal buffer adjusted to pH 11. Dehydrogenase activity (DHG) (formazan release method) was determined by measuring spectrophotometrically the amount of triphenyl formazan (TPF) when soil is incubated at 37 °C for 24 h with an aqueous solution of the 2,3,5-triphenyltetrazolium chloride and $CaCO_3$. The activities of urease and alkaline phosphatase were assayed on <2 mm samples at optimal pH values in duplicates and one control sample.

Statistical analyses

To determine the effect of sampling time and deposition material (independent variables) and the effect of their interaction on the soil physical, chemical, and biological variables (dependent variables), a two-way analysis of variance (ANOVA) was used followed by a Bonferroni post hoc test. To meet the assumptions of ANOVA, the data were logarithmically transformed when judged necessary.

To explore whether time or deposition material induced variability is more important for the variability shown by the data, a principal components analysis (PCA) was conducted. A new combined variable, termed time-deposition material, was created, and the corresponding samples were ordinated in accordance to values of soil variables.

Results

In Table 1, we present the effect of sampling time, deposition material, and their interactive effect on the variability of soil physical, chemical, and biological variables. The effect of sampling time was not significant for all estimated soil physicochemical variables (pH, organic C, organic N, Pextr, Ca^{2+}, Mg^{2+}) (Table 1). In addition, almost all estimated biological variables (MBC and the activities of urease and dehydrogenase) were significantly affected by sampling time, presenting similar values in the first two samplings (September and January), whereas the values of these variables were significantly higher in the third sampling (May) (Table 2). The alkaline phosphatase activity values remained constant during the whole experiment.

The effect of mine spoil heap material proved significant for all estimated variables (Table 1). Among the estimated chemical variables, soil macronutrients (extractable P, Ca^{2+}, Mg^{2+}) presented constantly higher values in the fly ash sites compared with the other two

Table 1 The effects of sampling period and spoil heap material and their interaction as shown by two-way ANOVA analysis

	Sampling		Deposition site		Sampling × site	
	F value	*p* value	*F* value	*p* value	*F* value	*p* value
Water content	9.69	**	10.81	***	1.36	ns
pH	0.45	ns	32.9	***	0.12	ns
MBC	49.142	***	143.54	***	4.04	*
Organic C	0.958	ns	66.625	***	0.08	ns
Organic N	1.847	ns	24.48	***	0.25	ns
Alk. phosphatase	1.46	ns	62.17	***	1.21	ns
Urease	21.4	***	106.2	***	4.11	*
DHG	120.6	***	15.8	***	1.72	ns
Pextr	2.024	ns	4.136	*	0.91	ns
Ca^{2+}	2.3	ns	251.1	***	2.71	ns
Mg^{2+}	1.24	ns	33.63	***	0.19	ns

ns non-significant

*$p<0.05$,**$p<0.01$, ***$p<0.001$

sites during all sampling periods. The red–grey formation and marl site exhibited higher values of all biological variables and organic C and organic N, in all three sampling periods (Fig. 1). Finally, the interactive effect of sampling period × deposit spoil site was found to be significant for MBC and urease (Table 1).

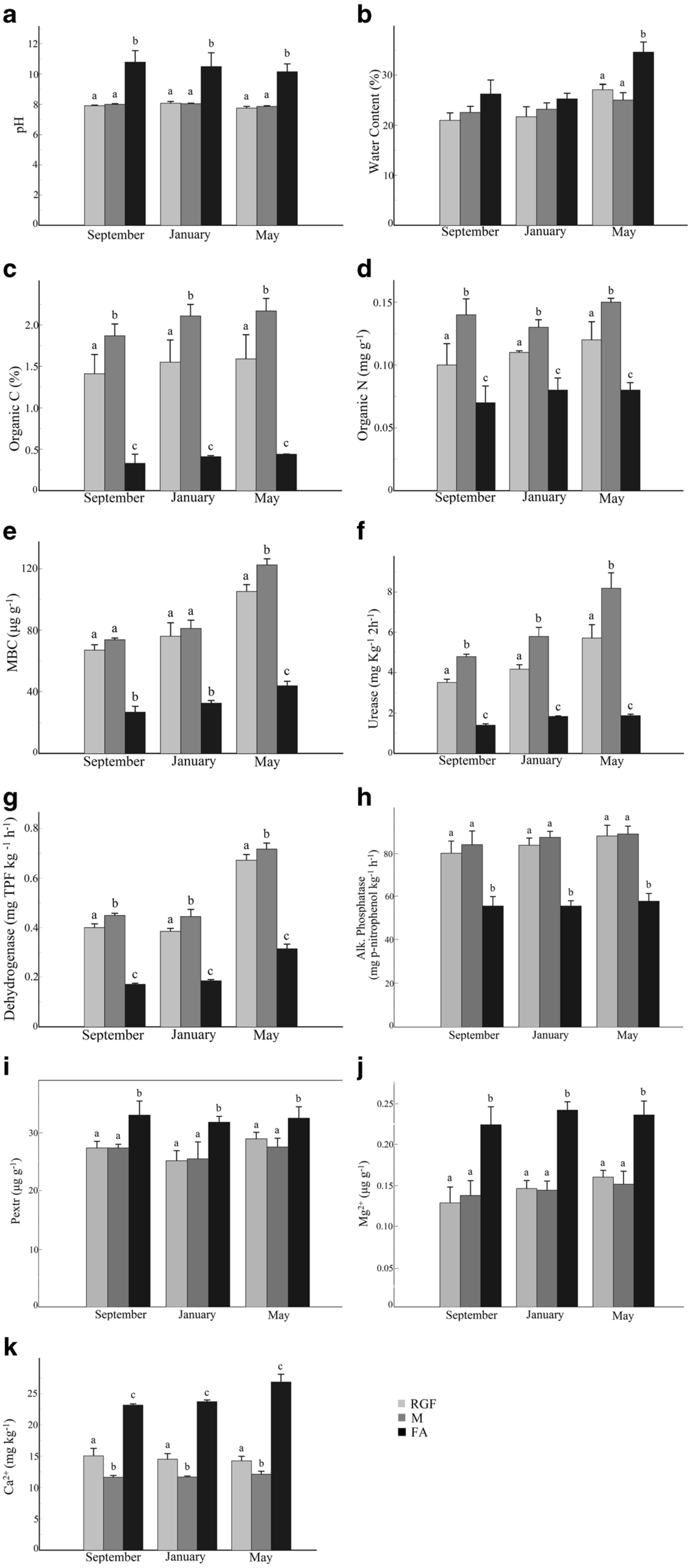
The ordination of samples and variables on the PCA biplot is depicted in Fig. 2. The first two axes of the PCA accounted for 78 % of the data variability (the variability explained by the first axis was 55.78 %, while that explained by the second was 23.2 %). Samples from the FA depositions were ordinated within the left side of the biplot, no matter the time of their collection, while the right side was occupied by samples from the other two sites, collected at the third sapling period. Ca^{+2} and Mg^{+2} concentrations, primarily, and Pextr, secondarily, determine positively the distribution of the FA samples along the horizontal axis. Similarly, DHG, urease, alkaline phosphatase activity, Norg, and MBC determine the distribution of the RGF and the marl samples from the third period. The rest of the RGF and marl samples rest together sharing probably the same moderate values of all the estimated soil biological variables.

Table 2 Mean values (±SE) of soil physicochemical and biological variables in all deposition sites recorded on the three sampling periods

Biochemical variables	September	January	May
Water content (%)	23.27±1.25a	23.62±0.77a	28.72±1.81b
pH	8.90±0.52	8.88±0.49	8.59±0.42
MBC (μg g^{-1})	0.56±0.08a	0.63±0.08a	0.90±0.12b
Organic C (%)	1.21±0.24	1.36±0.27	1.40±0.27
Organic N (mg g^{-1})	0.10±0.013	0.11±0.012	0.12±0.011
Alk. phosphatase (mg PNP $kg^{-1}h^{-1}$)	72.32±5.31	74.66±2.83	77.31±4.02
Urease (mg $kg^{-1}2h^{-1}$)	3.23±0.49a	3.93±0.59a	5.26±0.97b
DHG (mg TPF $kg^{-1}h^{-1}$)	0.33±0.01a	0.34±0.02a	0.56±0.02b
Pextr (μg g^{-1})	28.85±1.13	27.44±1.52	29.40±1.97
Ca^{2+} (mg kg^{-1})	16.62±1.75	16.72±1.84	17.74±2.34
Mg^{2+} (mg g^{-1})	0.164±0.013	0.177±0.017	0.183±0.015

Different letters correspond to statistically significant differences among the sampling periods (Bonferroni's post hoc test)

Fig. 1 Mean values (±SE) of soil physicochemical and biological variables in the three deposition sites (*RGF*: red–grey formations, *M*: marl, *FA*: fly ash) per sampling period. Different *letters* correspond to statistically significant differences among deposition sites of the same sampling period (Bonferroni's post hoc test)



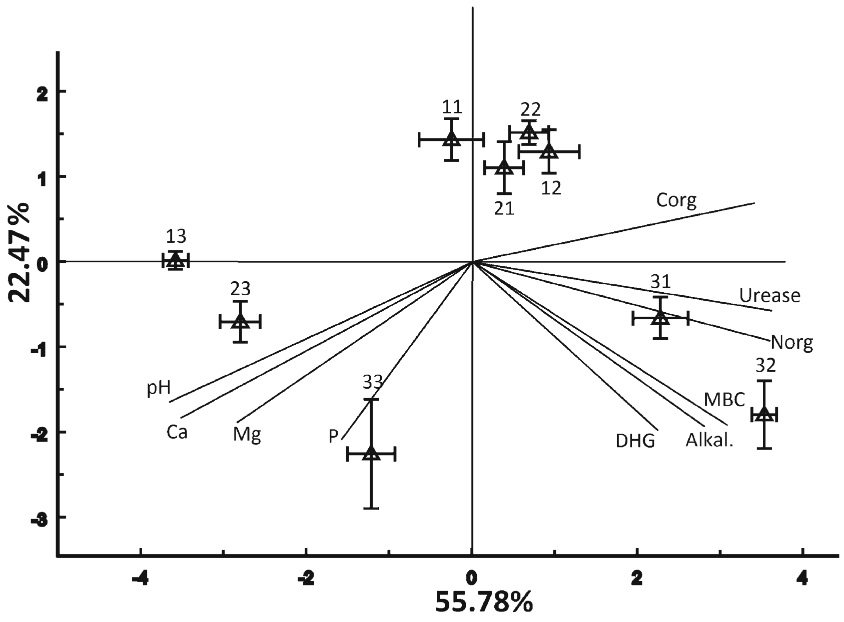


Fig. 2 Ordination of the soil samples and the soil physicochemical and biological variables on a PCA biplot. Each point corresponds to a sample collected from a specific site at a specific time [the first symbol corresponds to time (*1*: September, *2*: January, *3*: May) and the second symbol corresponds to site (*1*: red–grey formations, *2*: marl, *3*: fly ash)]. Error *bars* indicate standard errors (*n*=3)

Discussion

Significant differences were recorded among the estimated physicochemical and biological variables among the sampling sites. FA sites presented higher water content values, especially in the third sampling period. Fly ash is known to react with water to cement soil particles under wet conditions due to the podzolonic reaction of fly ash (Adriano et al. 1980). This latter, in combination to the fact that fly ashes originated from the combustion of Greek lignites, present high CaO content that reflects the ash composition of the parent lignite (Kolovos 2002), resulting in large quantities of calcium deposition and creating a very stiff and impermeable layer in the FA sites.

The pH values of RGF and marl deposits were found to be slightly alkaline in all three samplings (7.76–8.08), while the pH value of the FA sites had a constant and significantly higher value resulting to an alkaline environment (10.3–11), within the range of 8.5–12.5 reported in other studies (Carlson and Adriano 1993; Singh and Yunus 2000). Almost a year after the creation of the depositions, pH values in all sampling sites decreased slightly but not significantly. Thus, the initial conditions of three types of depositions differed significantly, and any subsequent difference in soil properties could be ascribed to these differences.

All three mine spoil sites showed considerable differences in their chemical properties in all three sampling periods. Organic C and organic N content in the FA sites was lower, which was expected, since the C and N lignite content is likely to be oxidized and lost in gases during coal combustion and be present in small amounts in ashes (Hodgson and Holliday 1966). However, the difference was more pronounced in Corg as both the RGF and marl sites exhibited significantly higher values than FA. Higher values of Corg and Norg recorded in the marl deposits could be attributed to the fact that this spoil material originates from layers closer to the lignite ones, and therefore, they could contain large amounts of fossil C (Sourkova et al. 2005). The combination of higher values of Corg and Norg in the marl sites could imply that these sites have the potential for soil quality improvement. Almost a year later, Corg and Norg concentrations remained constant in all sites without any further increase, probably due to the lack of vegetation cover which could help the improvement of soil characteristics (Chodak et al. 2009).

No statistically important differences were found among the sampling periods for any of the estimated macronutrients, Pextr, Mg^{2+}, and Ca^{2+}. However, differences were recorded among the sampling sites as the FA area was much richer in extractable P, Ca^{2+}, and Mg^{2+}, while RGF and marl spoil heaps presented similar concentrations of the estimated macronutrients. The substantial presence of plant nutrients suitable for improving the fertility and growth of various crops and plant species in fly ash has been widely reported (Kalra et al. 2003; Garg et al. 2005; Kishor et al. 2010). This means that fly ash contains most elements required for plant growth and metabolism, with the exception of nitrogen and available phosphorus (Singh and Yunus 2000). All the above indicate that the FA areas have a sufficient nutrient pool in Ca^{2+}, Mg^{2+}, and Pextr to support spontaneous vegetation growth. As anticipated, calcium oxide dominates in fly ash obtained from Ptolemais power plants, due to the increased Ca^{2+} content of the raw lignite (Kolovos 2002).

Three months after heaps' creation, soil MBC values were not increased in any of the sampling sites. This could be attributed mostly to the low winter temperatures of the study area, mostly below zero, that could suppress the MBC growth (Wardle 1992). Nevertheless, in the third sampling, an important increase was observed in the RGF and marl sites while, in the FA depositions, the increase is slight and not that significant. MBC is a good early indicator of changes in soil biotic and abiotic environment, increases with increasing successional stage, and is positively correlated with organic C concentrations (Ohtonen et al. 1999; Baer et al. 2002; Vlachodimos et al. 2013). The relatively increased values of C found in the two spoil material sites compared with the low organic C values found in the FA could explain these differences. Moreover, much of the carbon in unweathered fly ash could be expected to be present in the form of charcoal-like substances with few characteristics of soil organic matter, and this could affect its accumulation from the soil microbial populations. Additionally, the high pH in fly ash has been found to be hazardous to the majority of microbes, mostly those conducting N fixation (Klubek et al. 1992).

In all sites, the activity of all three estimated enzymes followed the pattern of MBC, given the high correlation between the microbial community and the enzyme activity (Chodak and Niklinska 2010). Moreover, the positive correlation among enzymatic activities and amounts of organic C and organic N is frequently reported in literature. Thus, the high enzymatic activities in marl sites could be attributed to the high amount of organic C and organic N compared with the low concentrations in fly ash areas. This was further supported by the lack of temporal changes in enzymatic activities and amounts of organic C and N all over the sites. Dehydrogenase is an intracellular enzyme which is only present in viable cells and is involved in the initial breakdown of soil organic matter (Pascual et al. 2000). It has often been used as a measure of the overall soil microbial activity (Izquierdo et al. 2005; Tan et al. 2008) and as a measure of changes in the metabolic state of soil microorganisms, providing a good index of soil quality (Visser and Parkinson 1992). The RGF and marl sites presented higher values of dehydrogenase activity than the FA area. An active microflora has been found to depend on the availability of organic C and organic N (Wong and Wong 1986). The significant differences in dehydrogenase activity between sites were the result of deficiency in the availability of C and N at the FA study areas. Increases in dehydrogenase activity in the marl and RGF deposits shows signs of microbial activity improvement, and this is indicative of better biological activity. Moreover, the high pH presented in the FA areas may have reduced the cell growth and dehydrogenase activity. Beyer et al. (1992) and Wong and Lai (1996) observed that increasing the amount of fly ash in ash–sludge mixtures caused a significant drop in dehydrogenase activity which was attributed partially to pH increases.

Urease plays an important role in the soil–nitrogen cycle as it catalyzes the release of NH_4^+ from urea (Caldwell 2005). Urease is a constitutive–intracellular enzyme, and consequently, it increases with microbial biomass (Nannipieri et al. 1979). Similarly to dehydrogenase, urease activity is strongly correlated to the MBC concentrations. The availability of soil organic N in either of marl or RGF sites presented better opportunities for increased urease activity. Moreover, Wong and Wong (1986) and Cerevelli et al. (1986) reported that the studied adverse effects of alkaline fly ash on nitrification activity could be attributed to high alkalinity. The decline in urease activity at 50 % in ash–sludge amendment might be due to the high pH and salinity of fly ash which created unfavorable conditions for microbial growth and nutrient cycles. Additionally, the high pH in fly ash has been found to be hazardous to the majority of microbes, mostly nitrifying bacteria; Klubek et al. (1992) showed that the nitrifying bacteria showed a quadratic relationship with pH, indicating lower populations under acidic or alkaline conditions such those found in the FA sites.

Alkaline phosphatase also presented a similar pattern with the other enzymes, as the FA sites presented lower values compared with marl and RGF spoil heaps. Alkaline phosphatase is mainly extracellular and is released by microorganisms in response to increased substrates, particularly C and N (Qiu et al. 2010). Thus, similarly, to the other two enzymes, the profile distribution of this enzyme was influenced by organic C and total N in soils. However, no significant differentiation was observed between the sampling periods, like the other two enzymes. Pextr values in all sites were found to be relatively high, within the range of values observed in a typical Mediterranean system (Monokrousos et al. 2004), and thus, none of the sites was limited to phosphorus. It has been indicated that low soil phosphorus availability increases phosphatases activities (Bargaz et al. 2012) and that the production of phosphatase would be increased when the requirement for P in an ecosystems is increased (Clarholm 1993; Olander and Vitousek 2000). In our case, the increased values of Pextr did not alter the phosphatase activity as they were found to be relatively high.

In relation to the process of secondary succession, the first PCA axis could be used as a gradient of improvement in soil quality. Enzyme activities and MBC have been widely used as early indicators of soil quality improvement, because they reflect rapid changes in the soil environment of reclaimed mine areas (Baldrian et al. 2008; Chodak and Niklinska 2010; Vlachodimos et al. 2013). The higher amounts of organic C and organic N, the increased values of enzymatic activity, and microbial population abundance suggest an increase in maturity of the stage of soil development in these sites (Vitousek and Reiners 1975). Based on the results of the analysis, the closer the samples were ordinated towards the right end of the first axis; the better the soil conditions were based on the aforementioned parameters. For all three spoil deposition materials, the samples of the first two sampling periods (September and January) were classified closely, while the samples of the third sampling period (May) were classified separately. This makes clear the fact that soil processes that take place within the first 3 months of spoil creation are very slow. This could also be attributed to the recorded increase of soil moisture in the third sampling, as it is well documented to be strongly correlated with most of soil biochemical variables (Monokrousos et al. 2004). However, in the RGF and marl sites, from the first moment of deposition creation, the soil functionality is significantly better than in the FA sites. Nine months after the deposition creation, the soil conditions were improved faster in the marl deposits compared with the RGF sites, while slight improvement was also observed in the FA sites, reaching almost the levels of the initial conditions of the other two spoil materials. All the above are clear indications that, as regards the time needed for soil functionality to be restored, the three deposition sites are ordered as marl<RGF<FA. In the FA deposits, despite the increased nutrient concentrations that favor the growth of plants, the alkaline environment and the stiff surface layer pose a serious problem that seemed to delay significantly the improvement of soil biological parameters.

Conclusions

Based on our results, we had to reject our initial hypothesis that all deposition sites, regardless the material they consisted of, present equal chance for the establishment of the spontaneous vegetation. Although, the FA sites presented the highest soil macronutrients concentrations (Pextr, Ca^{2+}, and Mg^{2+}), they also showed low improvement in the estimated soil biological properties and increase of organic matter, which consist basic precondition for the recovery of ecologically sensitive ecosystems such as the spoil heaps. Therefore, we could hypothesize that the FA deposit sites are more likely in the future to be occupied only by N-fixing plants that can survive in alkaline environments. On the contrary, in the RGF and marl sites, soil microbial biomass and all enzyme activities increased significantly during the first year after the deposition's creation, presenting more favorable conditions for the growth of a larger variety of plants.

In terms of management, a mixture of excavated spoil materials that originate from deeper layers close to the lignite ones and fly ash seems to be the most appropriate approach. The percentage of the fly ash in the mixture should be low, sufficient enough to ensure the availability of macronutrients for the plants without increasing significantly soil alkalinity.

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