

Comparison of revegetation techniques on mineral clay soil: analysis of quantitative response of vegetation cover

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Abstract

Revegetation of mineral-clay soils is a notably complex ecological and technically challenging undertaking that depends on substrate profile and local micro-environmental conditions, factors making it a particularly long procedure as well. This study compared and assessed the medium-term effectiveness of four treatments employed to promote stable pedogenesis and herbaceous recolonisation of abandoned clay quarries in the Apennine foothills of northern Italy's Emilia-Romagna region. The treatments included: slow-release N organic fertiliser, phosphate fertiliser, organic amendment and topsoil [the soil top layer (0-0.2 m) of a local natural meadow]. The state of the vegetative cover was monitored monthly from 1994 through 2004, until problems of slope stability at the site compromised the integrity of the trial plots. Significant effects were achieved by the recycled topsoil through 8 years and by organic amendment through 6 years; the effects of slow-release nitrogen were notably limited over time and phosphorous delivered a medium-term response but of notable year-toyear swings. No interactions among factors emerged in the mediumterm. After 11 years, treatments did not induce effects statistical appreciable. Our results suggest that the tested agronomic strategies on mineral clay soil did not trigger, in the medium-term, secondary succession processes able to potentially alter the spontaneous revegetation course.

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Introduction

Mining is the human activity with the highest impact on the environment. Not only the biological activity is completely annihilated, but even all the physic, chemical and micro-climatic characteristics of the site are strongly modified (Haigh, 2000). In Italy there are 21,367 censed quarries, 5592 of which are still in use (Legambiente, 2014). Most of the 16,045 inactive quarries are completely abandoned to spontaneous re-naturation processes. This abandonment generates pollution, erosion and landslides, which are big issues for landscape, environment and territory (Legambiente, 2014). Among all the quarries, clay ones presents the highest difficulty in reclamation, due to the long periods necessary to re-activate the biological processes. Most of these clay quarries are located in the Emilia-Romagna region, in the provinces of Reggio Emilia, Modena and Bologna (Northern Italy).

The years of the post-World War II in Emilia-Romagna region saw the growth of a thriving ceramic tile industry based in the foothills of the North Apennines in several provinces. The industry's success relied on quarrying supplies of mineral clays from widespread local deposits. Millions of cubic meters had been extracted up to the 1990s, but by then, innovations in manufacturing and shifting market demands led to the importing of raw materials from other domestic districts and foreign countries. Consequently, many local abandoned quarries need to be reclaimed (Montanari, 2012). Clay soil properties like micro-porosity, low air and water permeability, low seepage rate and strong compaction make vegetation establishment or succession and pedogenesis particularly long-term processes, even when only the uppermost soil layers are targeted.

These soil properties obviously constrain the range of applicable problem-solving techniques. On one hand, they have led researchers and industry consultants alike to a recycling of substrates present at the sites themselves. Indeed, pains-taking collecting, handling and reuse of original, naturally occurring horizons, including both top and subsoil layers, are now even being incorporated in the planning stages of new quarries (Schuman *et al.*, 2000). This approach is based on the hard-won realisation that stewardship of a highly complex element like soil is the most effective means of promoting the rapid onset of the biological cycles needed for the dynamic processes of post-extraction reclamation and for the self-sustainability of the processes themselves (Haigh, 2000).

On the other hand, however, it is more often the case of having to address already compromised quarry areas whose original surface soil cannot be recovered either on site or even in sufficient volume from adjacent areas. There are also areas whose morphological conditions, like steep slopes, or former uses, like farming or pasture, have led to severe erosion and stripped soil profiles. It is thus neither possible nor conceivable in any of these conditions to procure surface horizons - up to 50 cm of topsoil and to 100 cm of subsoil - of adequate composition (Schuman *et al.*, 2000). The absence of a topsoil layer, by affecting the physico-chemical soil properties, may promote soil compaction, structural instability, accelerated runoff, erosion, and may lead to soil toxi-





city, weakened and depleted soil biological system, and infertility (Haigh, 2000).

Reclamation in these foothill areas has nearly always focused on *reconstituting* the environment (Baines, 1989; Muzzi and Rossi, 2003), *i.e.*, reconstructing topsoil layers, fertility (Marrs and Gough, 1995) and vegetative cover (Hodgson, 1995) directly upon the mineral-clay substrate.

This includes site preparation to boost soil nutrient availability and biological activity with inputs of mineral elements (Koide and Mooney, 1987; Hume and August, 1988; Reeder and McGinnies, 1989; Mays et al., 2000), organic matter (Pesant and Vigneux, 1992; Schoenholtz et al., 1992) and mycorrhizal inoculum (Tate, 1985; Cockrell et al., 1993). These applications are designed to make the grass cover *stable*, to foster natural dynamics like vegetative succession (Redente and Depuit, 1988) and to defend the site against erosion (Morgan, 1986). This reconstructive approach has been applied in Emilia-Romagna region for many years, through agronomic practices such as fertilisations, tillage and amendments. Nitrogen inputs elicit a prompt response by the vegetative cover, but since they become depleted over time, pernicious weed species may take over in a natural colonisation processes (Claassen and Carey, 2006; Pedrol et al., 2010; Borden and Black, 2011). Evidence of positive nitrate effects in the medium period on clay spoils has been reported by Bagnaresi et al. (1992).

This study was aimed to evaluate the effect of several strategies (alone or mixed) on soil reclamation and revegetation of abandoned mineral clay quarry sites. In detail, we tested the effect of nitrogen and phosphorous supply, the addition of organic matter and the effect of a clay topsoil blend. The latter represents an original strategy that, for as we know, was never tested on clay soils. The hypothesis was that the inclusion of topsoil to the superior clay substrate may: i) generate a sufficiently deep root-explorable layer, adequate to support a stable vegetation cover and to limit erosion. The detrimental consequences of a shallow root-explorable layer have been described in Schoenholtz *et al.* (1992); ii) ameliorate the physico-chemical features of the layer explored by roots; iii) bring a microbiological inoculum useful to sustain vegetation cover (Claassen and Zasoski, 1993).

The experimental procedure entailed three linked steps: to prepare the site with inputs of nutrients, organic amendments and limited volumes of recycled on-site soils, to establish a stable grass cover and to assess quantitatively the interaction and effectiveness of the inputs and cover in a reconstructive framework (Sawatsky *et al.*, 2000). In the given time window, such a cover should thus be able to prevent run-off erosion and to elicit the onset of an ecological succession capable of restoring the biological, environmental and landscape functions of these degraded sites. The results of this study might contribute to define reclamation strategies for clay spoils with deficient topsoil layer.

Materials and methods

Experimental site

The experimental site is a quarry owned by Sibelco Italia s.p.a. and located at Colombara, a hamlet on the Lavino winterbourne (44°24'22" N, 11°10'18" E) near the town of Monte San Pietro, Bologna province, facing West-Northwest at an elevation of 150 m a.s.l. The mean long-term yearly rainfall does not exceed 870 mm (Table 1). The mean across-trial rainfall rate per annum was 863.27 mm with yearly fluctuations ranging from respective minimums of 571 and 576 mm in 1998 and 2000 and maximums of 1078 and 994 mm in 1994 and 1996.

The microclimate of area surrounding the trial is favourable to mixed broadleaf woodland of oak (*Quercus pubescens* Wild.), along with ash (*Fraxinus ornus* L.), hornbeam (*Ostrya carpinifolia* Scop.) and maple (*Acer campestre* L.).

The test plots were established in the abandoned pit by re-terracing several strips of previously dumped mineral-clay soil (Table 2). The substrate was a mixture of red beds and greenish-gray clays.

Experimental design

The trial layout consisted of randomised blocks with three replicates; each block was placed on one of the re-terraced horizontal strips. Four input treatments to establish the grass cover directly on the clay soil were tested: slow-release nitrogen (N) organic fertiliser (Biosol®, 6% N), phosphate fertiliser (perphosphate, $21\% P_2O_5$), organic amendment such as dried pellets of cattle manure (3% N), and shallow topsoil. The top 20 cm of the latter was excavated with a power shovel from a meadow across the quarry that had not been farmed in twenty years. The soil was sieved (mesh 20×20 mm) to eliminate rocky and bulky plant mate-

Table 1. Pluviometric data of Monte San Pietro station (246 m a.s.l.).

Year	Jan	Feb	Mar	Apr	May	June	July	Aug	Sept	Oct	Nov	Dec	Tot
1994	52	29	1	163	78	224	32	18	292	75	72	22	1078
1995	10	75	33	25	104	227	48	120	57	46	81	84	910
1996	68	60	56	104	80	69	6	57	101	212	78	103	994
1997	73	18	42	57	20	78	41	60	35	34	102	106	666
1998	29	27	46	30	61	48	59	21	94	61	16	79	571
1999	37	58	27	82	51	83	5	95	38	77	240	70	863
2000	9	2	47	56	12	82	79	26	22	105	82	54	576
2001	72	36	75	101	83	46	60	40	93	38	86	13	743
2002	17	70	5	125	76	29	109	128	99	69	67	137	931
2003	55	9	40	132	15	37	0	71	55	105	190	55	764
2004	70	150	74	93	46	33	34	8	49	108	141	114	920
Mean	44.7	48.6	40.6	88.0	56.9	88.7	43.0	58.6	85.0	84.6	105.0	76.1	819.6
Mean 1926-04	56.9	57.5	68.8	82.8	80.5	67.6	46.5	54.6	75.1	100.6	97.4	75.1	863.3





rial and then spread in a layer of 5 cm, a notably shallower depth than 30-100 cm topsoil range reported elsewhere (Halvorson and Doll, 1985; Schuman *et al.*, 2000; Bowen *et al.*, 2005). Yet the purpose of this study was to spur the onset of the biological activity normally present in topsoil layers (Bellgard, 1993) and not to recreate a new topsoil horizon for plant life. Compared to previous trials (Bagnaresi *et al.*, 1992; Muzzi *et al.*, 1997), the amount of agronomic inputs was restricted in order to limit the impact on the topsoil properties.

The trial presented a 2^4 layout with each treatment in two distinct levels: i) slow-release N: Biosol® (N) 0-100 g m $^{-2}$ of N (60 kg ha $^{-1}$); ii) phosphorous: triple perphosphate (P) 0-20 g m $^{-2}$ of P_2O_5 (200 kg ha $^{-1}$); iii) organic amendment: manure pellets (OM) 0-200 g m $^{-2}$ of organic matter (2000 kg ha $^{-1}$); iv) on-site topsoil: (S) 0-0.05 m (500 m 3 ha $^{-1}$). Each block contained 16 plots and covered 20 m 2 (4×5 m).

Experiment setup and follow-up

Spring 1993: after experimental area was delimitated, topsoil and fertilisers were spread on the plots and the blocks were harrowed to aerate the substrate and provide a minimal connection between topsoil and mineral clay. By superficial tilling (0.20 m), the topsoil and the nutrients were then mixed with the mineral clay. A final soil milling improved the seedbed.

Grasses and legumes (26 g m⁻²) in a mix of matching proportions were sown: i) legumes: *Vicia villosa* Roth. (1.00%); *Medicago lupulina* L. (7.00%); *Hedysarum coronarium* L. (7.00%); *Lotus corniculatus* L. (25.00%); *Melilotus alba* L. (5.00%); *Melilotus officinalis* L. (5.00%); grasses: *Alopecurus myosuroides* Huds. (3.00%); *Lolium perenne* L. (2.00%); *Dactylis glomerata* L. (11.00%); *Cynodon dactylon* L. (25.00%); *Agropyron repens* (L.) P. Beauv. (4.00%); *Schedonorus arundinaceous* (Schreb.) Dumort (5.00%).

Sowing occurred on 12 September 1993 by manual broadcasting and the seeds were manually raked into the soil.

Daily microclimatic data (air temperatures and rainfall) were recorded for the whole trial duration by a weather station placed at the Monte S. Pietro, within 2 km from the experimental site.

Starting from January 1994 until 2004, when slope-instability compromised plot integrity, the three blocks percentage of the vegetative cover (Braun-Blanquet, 1964; Di Tommaso, 1992) was visually estimated monthly by the same person.

Statistical analysis

The daily rainfall recorded by the weather station were reprocessed per quarter and correlated to the mean percentage of the grass coverage of June and December (Table 3).

June percentage of the fractions of vegetation cover, for all available years, were initially used to analyse the trial layout via Proc Glimmix (The SAS System for Windows, Release 9.0.; 2002. SAS Institute Inc., Cary, NC, USA) for binomial data repeated over years as follows: i) years as repeated factor, after autoregressive order 1 covariance correction; ii) blocks as random factor; iii) treatments (2^4 =16) as fixed factor.

The same June percentage of the vegetation cover data, were then transformed using the Bliss formula (Steel and Torrie, 1980) and the results analysed using Proc Mixed (The SAS System for Windows, Release 9.0.; 2002. SAS Institute Inc., Cary, NC, USA) while retaining the same structure of Repeated Measures. The results of both analyses were compared against the Akaike's information criterion (AIC) index (Table 4). The statistic significance of treatments and their interactions (Table 5) were evaluated through contrasts of Proc Mixed (Davidian, 2005). Standardised effects of the treatments were calculated using the inverse transformation of means (Montgomery, 1997) (Table 5).

Results and discussion

Comparing the mean vegetation cover fractions of June and December, *i.e.*, respective maximum and minimum windows of biological activity, against yearly rainfall evinces a positive correlation: +0.562 (r)/0.473 (ρ) for June and +0.631 (r)/0.542 (ρ) for December (Table 3). A maximum positive correlation was also found between June and December vegetation cover data and rainfall in the second quarter [0.766 (r)/0.638 (ρ) and 0.750 (r)/0.776 (ρ) respectively].

Vegetation cover composition was dominated by grass species, namely *Lolium perenne* L. in the first year, then replaced by *Elymus*

Table 2. Chemical composition of tested clay substrates.

	Clay s			
		Mineral	Topsoil	
Sand	%	36.0	24.0	
Loam	%	37.0	51.0	
Clay	%	27.0	25.0	
pH (H ₂ O)		9.69	8.21	
Total lime	g 100g ⁻¹	2.00	12.0	
Active lime	g 100g ⁻¹	1.60	8.80	
CEC	Meq 100g ⁻¹	23.7	26.2	
Ca	mg kg ⁻¹	2048	4406	
Mg	mg kg ⁻¹	734	353	
K	mg kg ⁻¹	411	280	
Na	mg kg ⁻¹	1472	134	
OM	$g \ 100 g^{-1}$	0.44	2.15	
Total N	$\rm g~kg^{-1}$	0.33	1.70	
C/N		7.73	7.34	

CEC, cation exchange capacity; Ca, calcium; Mg, magnesium; K, potassium; Na, sodium; OM, organic matter; N, nitrogen; C, carbon.

Table 3. Rainfall-Grass cover correlation.

	June gras	ss cover	December	grass cover
Coefficient correlation	r Pearson	ρ Spearman	r Pearson	ρ Spearman
Average yearly rainfall	0.562	0.473	0.631	0.542
1st quarter rainfall	0.090	-0.100	0.098	0.196
2 nd quarter rainfall	0.766	0.638	0.750	0.776
3 rd quarter rainfall	-	-	0.218	0.310
4 th quarter rainfall	-	-	0.055	0.050





repens (L.) Gould and Schedonorus arundinaceous (Schreb.) Dumort.

The June vegetation cover, after a maximum mean value of 75% in the second year, was evaluated of 60% for all following years (Figure 1). This trend is in agreement with a previous study (Roberts *et al.*, 1988), and may be dependent on the progressive mineralisation of the added organic matter, which occurs in absence of other N sources (*e.g.*, N fixation). A clear effect of treatments on herbaceous cover is apparent for the first seven years (Figure 1); thereafter, the gap between *control* and *treatments* is reduced by the spread of a native pioneer species (*e.g.*, *Dittrichia viscosa* (L.) Greuter).

Analysis of variance for vegetation cover shows a clearly lower AIC vis- \dot{a} -vis Glimmix's, the reason for having proceeded with the subsequent comparisons. The Table of variance in Table 4 shows a significant interaction of all treatments with time. All interactions between treatments were not significant, except for N × OM [p(F)=0.04]. A pos-

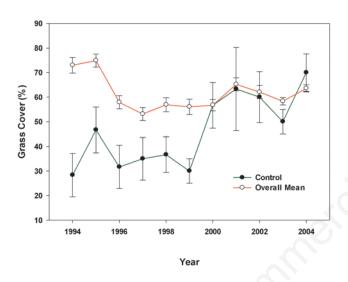


Figure 1. Mean of total grass cover and of control over Years (mean ± standard error of the mean).

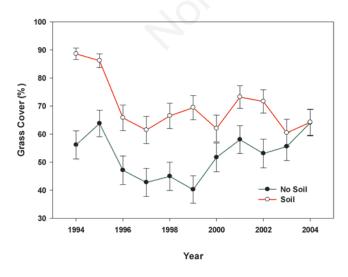


Figure 2. Plot of grass cover as Year \times Soil interaction (mean \pm standard error of the mean).

itive interaction between topsoil and chemical fertiliser (NPK) was found by Richardson and Evans (1986); by contrast, no significance were detected by McGinnies and Nicholas (1980), and by Sydnor and Redente (2000).

Richardson and Evans (1986) and Beauchamp $et\ al.$ (2006) determined a significant N × OM interaction (or chemical fertiliser × organic matter), while no significant results were reported in Heeraman $et\ al.$ (2001). In the present study, the absence of interactions between treatments may be partially attributed to the vegetation cover values expressed in percentage: they have a high (Beauchamp $et\ al.$, 2006) minimum detectable difference (Zar, 1984), which can mask weak effects. The standardised effect of all treatments in Table 5 confirmed the temporal trends supra and provided a precise quantification over time of the individual treatment effects and of their interactions.

A significant contribution of topsoil to vegetation cover was deter-

Table 4. Analysis of variance for both analysis methods employed.

A	Anova repeated measures analysis* Glimmix Mixed									
Effect	F value	Pr > F	F value	Pr > F						
Year	25.40	< 0.0001	24.81	< 0.0001						
Soil	65.90	< 0.0001	58.46	< 0.0001						
$\overline{Y \times S}$	11.17	< 0.0001	9.80	< 0.0001						
N	0.80	0.37	0.56	0.46						
Y × N	2.10	0.02	2.14	0.02						
$S \times N$	0.33	0.57	0.20	0.66						
$Y \times S \times N$	0.60	0.81	0.67	0.75						
Phosphorus	16.58	< 0.0001	15.89	< 0.0001						
$\overline{Y \times P}$	2.07	0.03	2.21	0.02						
$S \times P$	0.85	0.36	0.31	0.58						
$Y \times S \times P$	0.83	0.60	0.73	0.69						
$N \times P$	0.24	0.62	0.22	0.64						
$Y \times N \times P$	1.38	0.19	1.33	0.21						
$S \times N \times P$	0.04	0.84	0.15	0.69						
$Y \times S \times N \times P$	1.11	0.36	1.22	0.27						
Organic matter	21.97	< 0.0001	18.78	< 0.0001						
$\overline{Y \times OM}$	5.25	< 0.0001	4.44	< 0.0001						
$S \times OM$	0.13	0.72	0.61	0.44						
$Y \times S \times OM$	0.68	0.74	0.76	0.66						
$N \times OM$	3.95	0.05	4.34	0.04						
$Y \times N \times OM$	1.23	0.27	1.56	0.12						
$S \times N \times OM$	3.06	0.08	3.28	0.07						
$Y \times S \times N \times OM$	0.99	0.45	1.03	0.42						
$P \times OM$	1.11	0.29	1.20	0.28						
$Y \times P \times OM$	1.47	0.15	1.46	0.15						
$S \times P \times OM$	0.17	0.68	0.28	0.59						
$Y \times S \times P \times OM$	1.16	0.32	1.00	0.45						
$N \times P \times OM$	0.82	0.37	1.16	0.28						
$Y \times N \times P \times OM$	1.13	0.34	1.13	0.34						
$S \times N \times P \times OM$	2.29	0.13	2.40	0.12						
$\overline{Y \times S \times N \times P \times O}$	M 1.32	0.22	1.26	0.25						
AIC	630).4	-392	.7						
*Significant P-values are	*Significant Publice are reported in italice V year: S. coil: N. nitrogen: P. perphocobate: OM. organic									

*Significant P-values are reported in italics. Y, year; S, soil; N, nitrogen; P, perphosphate; OM, organic matter; AIC, Akaike's information criterion index.





mined over approximately 10 years from sowing (Figure 2 and Table 5). This result agrees with Richardson and Evans (1986), and Schwenke *et al.* (1999), where topsoil was mixed with the mineral substrate; however, both cited studies were of short duration (1-2 years) and could not provide the observation over time. In the present survey, the effect of topsoil tended to decline over time, from 31.4% in 1994 to 0.10% in 2004 (Table 5).

N did not explicate a significant effect, with values ranging from 8.16% in 1994 to -0.17% in 2004 (Figure 3 and Table 5). Also Richardson and Evans (1986) did not determine substantial effects of chemical fertilisation, even using a triple NPK fertiliser (12-18-12); *vice versa*, McGinnies and Nicholas (1980) found a significant effect of N at higher doses (112 kg ha⁻¹), compared with the present experiment, and in a short period (2 year).

P see-sawed over time: its effect was notable the first year, then vanished for about 5 years and notably resurged towards the end of the trial (Figure 4 and Table 5). P effect varied from 13.2% in 1994 to 6.1% in 2004, and reached its maximum of 14.1% in 2001 (Table 5). Richardson and Evans (1986) and Heereaman *et al.* (2001) did not find significant effects using P in short-term experiments.

The effects of the organic amendment went from 26.2% in 1994 to 0.8% in 2004, resulting significant in early years and gradually tapering off after year 7 (Figure 5). These findings match the results gained from corresponding tests performed both in short (Heereman et~al., 2001; Beauchamp et~al., 2006; Claassen and Carey, 2006; Grigg et~al., 2006; O'Dell and Claassen, 2006; Curtis and Claassen, 2009) and medium periods (Roberts et~al., 1988).

Conclusions

First and foremost, overall data show the notably significant acrosstrial effectiveness of the topsoil, even in the minimal volume applied, on the grass cover. This input likely improved the physical and chemical properties of the mineral-clay substrate as well as to spur the onset of biological elements, from enzymes to seed, that primed the cover's prompt and lasting response. The other inputs (N, P and OM) showed

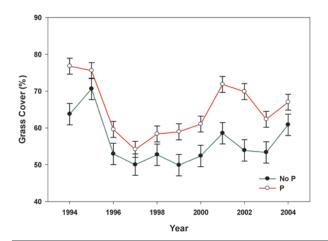


Figure 4. Plot of grass cover as Year × P (phosphorus) interaction (mean ± standard error of the mean).

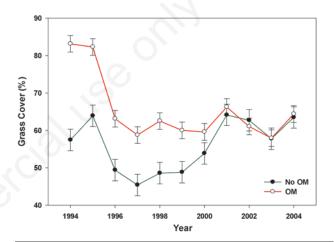


Figure 5. Plot of grass cover as of the Year × Organic matter (OM) interaction (mean ± standard error of the mean).

Table 5. Standardised effects of treatments and their interaction over time.

Treatments	1994	1995	1996	1997	1998	1999	2000	2001	2002	2003	2004
Soil	31.45	23.23	18.82	19.26	22.08	29.55	10.68	15.41	18.80	4.97	0.10
N	8.16	9.01	8.62	4.23	6.94	-0.55	-5.76	-3.97	-7.32	-1.12	-0.17
Phosphorus	13.20	4.73	7.01	4.77	6.39	10.60	9.53	14.09	17.26	9.27	6.08
Organic matter	26.21	18.82	14.54	14.33	14.74	12.66	6.29	2.25	-1.48	0.16	0.83
$P \times OM$	-11.85	-5.30	-3.93	-3.89	-1.65	-1.47	-4.33	3.12	-1.32	0.84	0.03
$N \times OM$	-3.70	2.59	4.75	6.12	3.50	4.71	11.67	8.55	3.65	3.53	6.43
$S \times OM$	-9.72	-7.44	-4.02	-3.02	-4.26	1.10	1.93	-1.79	-2.81	0.97	1.79
$N \times P$	-0.27	0.28	-2.89	-2.58	-2.40	-6.16	-1.50	0.35	3.66	-4.26	2.44
$S \times P$	-2.00	1.52	4.74	4.46	3.24	4.02	-1.04	-2.67	0.73	-2.71	-1.90
$S \times N$	-3.59	-6.37	-4.53	-0.89	-1.04	-2.29	0.57	0.18	2.24	-0.89	3.45
$S \times N \times P$	-4.01	1.70	2.55	0.68	0.12	1.65	-5.08	-4.35	-1.33	-3.50	1.55
$S \times N \times OM$	-1.89	-7.10	-1.24	-4.19	-6.65	-4.87	-6.39	-6.42	-2.43	-1.71	-3.68
$S \times P \times OM$	-3.22	0.49	-0.17	3.05	1.49	6.20	2.64	0.13	-0.26	2.75	1.68
$N \times OM \times P$	1.59	-3.86	-11.40	-6.93	-3.17	-5.04	-3.23	2.92	4.76	-4.58	1.85
$S \times N \times OM \times P$	-1.83	7.41	10.01	7.09	5.95	11.97	1.52	-0.70	1.85	1.57	-0.80

^{*}Significant P-values (P<0.05) are reported in italics. N, nitrogen; P, perphosphate; OM, organic matter; S, soil.





results limited over time.

In a more general view, the *reconstructive* approach we employed evinced its practical and biological limits over the 10 years trial. Treatment effects gradually faded, compromising in part the vegetative response as the cover's effectiveness in checking erosion from run-off of heavy rainfall considerably weakened over time. In fact, as the midterm trend of control corroborates, the cover's thinning laid bare swaths of the substrate after 6 years and allowed colonising species to invade and spread. A reappraisal of approach to this kind of reclamation is thus in order so as to make greater and better, more fundamental use of original site substrates.

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