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Phytomanagement with grassy species, compost and dolomitic limestone rehabilitates a meadow at a wood preservation site

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Abstract

Brownfield surface is expanding in Europe, but as often abandoned or underused, these areas become refuge for microbial, faunal and floral biodiversity. However, brownfield sites are generally contaminated, likely posing severe environmental risks. At a former wood preservation site contaminated with Cu, we evaluated the efficiency of compost and dolomitic limestone incorporation into the soil, followed by revegetation with Cu-tolerant grassy species, as a phytomanagement option to increase vegetation cover and plant diversity while reducing pollutant linkages. 7 years of phytomanagement enhanced natural revegetation through the improvement of soil physicochemical properties, particularly with compost-based amendments. The compost incorporation increased soil Cu solubility; however, no increment in Cu availability and a reduction in Cu-induced phytotoxicity were observed with the compost. The improved soil nutrient availability and the soil phytotoxicity mitigation in compost-amended soils facilitated over the 7 years the growth of beneficial plant colonists, including leguminous species, which can potentially promote essential soil functions. Soil treatments did not affect Cu uptake and translocation by plants and shoot Cu levels indicated no risk for the food chain. Overall, a long-term phytomanagement combining an initial amendment of compost and dolomitic limestone with the cultivation of Cu-tolerant grassy populations can ameliorate such Cu-contaminated soils, by mitigating risks induced by Cu excess, ultimately allowing the development of a meadow that can provide ecological and economic benefits in terms of ecosystem services.

Keywords: *Agrostis capillaris*; *Agrostis gigantea*; metal pollution; organic amendments; phytotoxicity; phytoremediation

1. Introduction

Since the 1970's, a vast land anthropization in Europe has led to the depletion of entire natural and semi-natural habitats and, ultimately, to a loss of biodiversity (Desrousseaux et al., 2019). In parallel, the increasing brownfield surface area, often abandoned or underused, has eventually become refuge for numerous fauna or flora species (Connop et al., 2016). Among them, current and former wood preservation sites are generally large areas that present Cu-contaminated soils derived from the use of Cu-based salts as wood preservatives and patches of mixed soil contamination including polycyclic aromatic hydrocarbons (PAH) (Mench et al., 2018; Frick et al., 2019). These sites are likely secondary sources of Cu contamination with potential deleterious effects on ecosystems and human health. Dispersion of metal(loid)s in these sites can be prevented by phytomanagement based on the implementation of a vegetation cover assisted with soil amendments (Lagomarsino et al., 2011; Marchand et al., 2011; Kidd et al., 2015; Frick et al., 2019).

Selection of plant species is crucial for the long-term success of phytomanagement of metal-contaminated soils. An appropriate mixed stand of plant species tolerant to metal excess and other adverse environmental conditions prevailing in these sites, i.e. low soil organic matter (SOM), poor soil structure and texture, low water holding capacity and nutrient contents, disturbed soil microbial communities, etc., would be needed for effective revegetation on the site, while keeping metal translocation to aerial parts as low as possible to avoid food chain contamination (Gómez-Sagasti et al., 2012; Burges et al., 2016).

In this respect, grassy species are known colonisers of metal-contaminated soils that can establish themselves and develop an extensive root system due to their ability to overcome the restraints of growth under the extreme ecological circumstances from these soils (Bleeker et al., 2002). At a former wood preservation site (hereinafter referred to as "Biogeco site", St. Médard d'Eyrans, France), Cu-tolerant populations of *Agrostis capillaris* and other grassy species have demonstrated their fitness and a Cu-excluder phenotype, i.e. limiting Cu translocation to aerial plant parts, useful for phytomanaging Cu-contaminated soils, particularly in combination with soil amendments able to minimize Cu exposure (Bes et al., 2010; Hego et al., 2014). This demonstrates also the importance of directing efforts to the conservation of the unique biodiversity existing in these polluted sites (Garbisu et al., 2020).

The application of mineral and organic amendments can facilitate the establishment of a plant cover by improving the *in situ* stabilization of metals and reducing their mobility and bioavailability in soil, via adsorption, sorption and/or precipitation processes (Hattab et al., 2014; Tiberg et al., 2016), but also by adding nutrients for plant growth, increasing SOM content, raising soil pH and water holding capacity, and enhancing soil biological activity (Alvarenga et al., 2009; Burges et al., 2020; Menzies Pluer et al., 2020). Bes and Mench (2008) have tested several inorganic and organic amendments for reducing available soil Cu and its accumulation in plants using potted Cu-contaminated soils from the Biogeco site. At this site, compost and dolomitic limestone, alone and combined, have demonstrated their efficiency to facilitate revegetation with various bioenergy crops, e.g. sunflower, tobacco, poplar and willow short-rotation coppice (Kolbas et al., 2011, 2020; Marchand et al., 2011; Hattab et al., 2014; Hattab-Hambli et al., 2016; Oustriere et al., 2016; Quintela-Sabaris et al., 2017; Mench et al., 2018; Xue et al., 2018).

Here, we hypothesized that the combination of organic amendment (e.g. compost) with dolomitic limestone followed by cultivation of a mixed stand of grassy species from Cu-contaminated sites could be one relevant feasible phytomanagement option to promote soil properties and natural revegetation and increase biodiversity, while reducing Cu-induced environmental risks. This will eventually lead to the rehabilitation of the soil ecosystem, enhancing its multiple ecological functions and maximizing the ecological and economic benefits provided by soil ecosystem services.

This study aimed at assessing in the field the effectiveness of compost and dolomitic limestone incorporation into the soil, alone and combined, and the use of Cu-tolerant grassy species for initiating a meadow as a potential phytomanaging option for Cu-contaminated soils.

2. Materials and methods

2.1 Site and field trial

The studied area is located in a wood preservation site at Saint-Médard d'Eyrans, Gironde, SW France (N 44°43.353´, W 000°30.938´) with a temperate Atlantic climate (variable mean rainfall and temperature; in 2012: 841 mm, 13.8°C). Wood preservatives have been successively used for over a century, i.e. initially creosote and thereafter various Cu-salts, mainly Cu sulphates (Mench et al., 2018). The site consists in about 10 ha of derelict areas with patches of natural attenuation and plant communities dominated by *Agrostis capillaris* L., *Rumex acetosella* L., *Senecio inaequidens* D.C., *Populus nigra* L., *Salix caprea* L., and *Cytisus scoparius* L. (Bes et al., 2010). Only 2 ha remain with a limited activity today. Anthropogenic topsoils are developed on an alluvial sandy soil (Fluvisol – Eutric Gleysols, World Reference Base for soil resources). A site survey indicated a high spatial variability (65-2600 mg Cu kg^{-1}) of total topsoil Cu, whereas As, Zn, Cr and other metal(loid)s were at their background levels (Mench and Bes, 2009; Bes et al., 2010). Some polycyclic aromatic hydrocarbons (PAH) reached high concentrations at sub-sites (in mg kg^{-1} soil dry weight - DW):

fluoranthene (1.9), indeno[1,2,3-cd]pyrene (0.95), benzo[g,h,i]perylene (0.8), and benzo[b]fluoranthene (0.8) (Mench and Bes, 2009). More data on PAH in Jones et al. (2016). Soil texture is sandy, i.e. 85.8% sand, 5.9% clay, and 8.3% silt, with 1.6% SOM, C/N 17.2, soil pH 7, and a low cation exchange capacity (CEC, 3.5 cmol kg^{-1}) (Mench and Bes, 2009). For detailed description on site history, soil characterization, and zoning of soil ecotoxicity, see Mench and Bes (2009), Bes et al. (2010) and Kolbas et al. (2020). Plant communities were characterized in Bes et al. (2010).

The field trial (hereinafter referred to as “the PG sub-site”, PG meaning Grassy Plots) consists in 4 blocks containing 4 plots each (1m x 3m). In April 2006, the 0 – 50 cm depth soil layer was loosened with a tiller, the integrity of the sub-soil being preserved. Then, four soil treatments were applied to the plots (n = 4) following a randomized block design with a 0.5 m space between plots: (1) DL: dolomitic limestone (0.2%, w/w); (2) OM: compost made from poultry manure and pine bark chips (5%, w/w); (3) OMDL: the combination of compost and dolomitic limestone; and (4) UNT: untreated soil. The amendments were incorporated into the topsoil (0-25 cm) and mixed with a stainless steel spade. The origin and composition of the soil amendments are detailed in Lagomarsino et al. (2011). In November 2006, five grassy species belonging to the *Poaceae* family were selected, based on their Cu-tolerant ecotype, and transplanted in all plots: *Agrostis capillaris* L. (AC), *Agrostis gigantea* Roth (AG), *Deschampsia cespitosa* (L.) P. Beauv. (DC), *Sporobolus indicus* (L.) R. Br. (SI) and *Vulpia myuros* (L.) C.C. Gmel (VM). Seeds of AG were collected at a Cu/Ni contaminated site in Sudbury, Canada (Bagatto and Shorthouse, 1999), while seeds of DC came from a contaminated site in Katowice, Poland (Dr. R. Kucharski et al, IETU, Katowice). Seeds of Cu-tolerant AC, SI and VM populations were collected from mature plants on the Biogeco site (summer 2006). The seeds were germinated on an uncontaminated sandy soil and 3-month-old plantlets were transplanted to the plots. Plants were weekly watered using individual plastic reservoirs (1.2 L) during the first summer to avoid early mortality due to drought effect. In the spring of 2008, mortality and shoot biomass of the transplanted plant species were monitored after a 2-year growth period, and thereafter shoot ionomes were determined as described below.

2.2 Soil and plant parameters

In early 2012, six topsoil samples (0-25 cm) were randomly collected within each plot and in an uncontaminated kitchen garden (CTRL) with the similar soil type located 17 km away of the site, Gradignan, France, using a steel spade, and combined to form a composite sample (2 kg fresh weight). Soil samples were air-dried and sieved to 2 mm prior to analysis. In parallel, fresh aliquots of topsoil samples for each plot were transferred to 1 L plastic pots where Rhizon moisture samplers (Eijkelkamp, the Netherlands), type MOM (used to collect soil pore water for analysis of macro and micro elements), were inserted (45° angle) and maintained one month at 75% of the water holding capacity. Subsequently, soil pore water was sampled (two times 10 mL, 5 days apart). Fresh aliquots of topsoil samples were also used for the phytotoxicity assay described in section 2.3. All soil analysis, including total metal(loid) concentrations, were performed at the INRA Laboratoire d'Analyses des Sols (LAS), Arras (France), using standard methods (INRA LAS, 2019). Soil pH was measured according to the French norm (Afnor X31-103, 1994). The negative of the base 10 logarithm of the molar concentration of free Cu ions in the solution (pCu) was computed based on the equation proposed by Sauvé (2003):

$$pCu^{2+} = 3.20 + 1.47 *\cdot pH - 1.84 *\cdot \log_{10}(\text{Total soil Cu})$$

where pCu^{2+} is free Cu activity and Total soil Cu corresponds to the total Cu concentration in the topsoil (in mg Cu kg^{-1}). Soil pore water solution was analysed for pH (Hanna instruments, pH 210, combined electrode Ag/AgCl - 34), electrical conductivity, i.e. EC, (WTW Multiline P4 metre, Germany) and element concentrations using ICP-AES (Varian Liberty 200).

Diffusive fluxes of Cu were measured in soil samples using diffusive gradients in thin films (DGT) fitted with chelex-100-resin-impregnated gels, in triplicate. Soil subsamples were incubated at 25°C for 24 h for equilibration, subsequently placed on the top of DGT sample devices, and kept in plastic boxes with water-saturated atmosphere at 25°C for a period of 24 h. Then, DGT devices were retrieved and opened, and the Chelex resins were eluted with 1 mL of 1M HNO_3 following Zhang et al. (2001). Soil solutions were obtained after centrifugation of soil slurries. Cu concentration for both soil solution (Cu_{sol}) and resin gel eluates were analysed using inductively coupled plasma-mass spectrometry (ICP-MS; Elan 9000 DRCe, Perkin Elmer). Time-averaged concentrations of Cu at the DGT-soil interface (DGT-Cu) and R (ratio between DGT-Cu and Cu_{sol}) were calculated according to Ernstberger et al. (2002).

In 2013, the occupancy of the plant cover and plant species richness were assessed per plot. Then, for each plot, aerial parts of all existing plants, except tree species, were harvested, washed thoroughly with deionized water, and oven-dried at 65°C for 48 h to calculate dry weights. For determining shoot ionome, samples were ground (<1.0 mm particle size, Fritsch Pulverisette 19) and subsamples (0.5 g dry weight) were wet-digested under microwaves (CEM Marsxpress 1200W) with 5mL suprapure 14M HNO_3, 2mL 30% (v/v) H_2O_2 not stabilized by phosphates and 1mL

MilliQ water, according to Bes and Mench (2008). Elements in digests were determined by ICP-AES (Varian Liberty 200).

2.3 Phytotoxicity bioassay

A Plantox test using dwarf beans (*Phaseolus vulgaris*, cv. Mangetout) was performed to evaluate soil phytotoxicity, as described in Bes and Mench (2008). Beans were sown in potted (1.3 L) fresh soil samples (4 plants per pot, 4 pots per treatment). Plants were grown under controlled conditions (14h/10h light/dark cycle, 25/22°C day/night temperature, 65% relative humidity, and a photosynthetic photon flux of 150 µmol m^{-2} s^{-1}) in a growth chamber and were watered daily with deionised water at 75% water holding capacity. Fifteen days after sowing, plants were harvested and fresh weights of the primary leaves and total shoots were determined for each plant.

2.4 Statistical analysis

The effect of soil treatments on soil physicochemical properties, plant properties and dwarf beans biomass was assessed by means of one-way analysis of variance (ANOVA) using R (version 3.6.0, R Foundation for Statistical Computing, Vienna, Austria). When significant differences occurred between soil treatments, multiple comparisons of mean values were made using post-hoc Tukey's HSD test. Linear Pearson regression was used to assess significant correlations between soil physicochemical properties. Multivariate analyses were performed by means of redundancy analysis (RDA) and variation partitioning analysis, using Canoco 5 (ter Braak and Šmilauer, 2012), to further evaluate the influence of soil treatments on the biomass, species richness and occupancy of the plant vegetation cover through changes in soil properties.

3. Results

3.1 Soil physicochemical and phytotoxicity parameters

Both compost treatments, *i.e.* OM and OMDL, significantly increased the soil CEC and contents of organic C, SOM and total N (Table 1). Levels of these soil properties in the DL plots were similar to those of the UNT plots. The DL and OMDL treatments slightly increased pH values in both soil and soil pore water, albeit differences were not significant as compared to the UNT soil.

Regarding the concentration and availability of Cu in soil, the soil treatments did not influence total soil Cu and free Cu activity (pCu) (Table 1). Total Cu concentration in the soil pore water (SPW-Cu) of the OM and OMDL soils, however, was roughly 2-fold higher than that of the UNT soils. Cu concentration measured with DGT (DGT-Cu) showed no significant influence of the soil treatments, whereas R values, which indicate the Cu resupply capacity from the solid phase to the soil solution, were lower in the OMDL soils. Pearson correlation values between soil physicochemical parameters indicated a positive relationship between SPW-Cu and soil properties improved by the OM and OMDL treatments (Supplementary table 1): values of SPW-Cu were more influenced by increasing levels of organic C ($R = 0.67$; $p<0.01$), total N ($R = 0.68$; $p<0.01$) and CEC ($R = 0.62$; $p<0.01$) in soil than by the total soil Cu.

The phytotoxicity bioassay showed, based on both primary leaf and shoot biomass (Figure 1), that all studied soil treatments promoted the growth and development of dwarf beans, as compared to beans cultivated in the UNT soils. Nonetheless, beans growth peaked in the OMDL soils, indicating that this soil treatment resulted in the highest mitigation of Cu-induced phytotoxicity in year 6.

3.2 Plant properties

After a 2-year growth period, the survival of the transplanted plant species was monitored, showing that all plant species managed to establish in the plots, except *S. indicus* that did not survive (Figure 2). Remaining plant species established in the field trial with varying success, with *A. gigantea* and *V. myuros* presenting the lowest mortality, followed by *D. caespitosa*, and, lastly, *A. capillaris*. However, identification of plant species in the field trial in year 7 (Table 2) indicates that changes in the population dynamics of the transplanted plants through the following years have occurred: *V. myuros* and *D. caespitosa* disappeared from all the plots whereas *A. gigantea* and *A. capillaris* were still present. In year 7, plots have been colonised by other herbaceous species, among which the dominant species were *Rumex acetosella* and members of the *Asteraceae*, i.e. *Senecio inaequidens*, *Hypochaeris radicata*, *Omalotheca sylvatica*, and *Fabaceae* families, i.e. *Ornithopus perpusillus*, *Medicago arabica*, and *Vicia sativa*. Colonising species also included shrubs and woody species, i.e. *Cytisus scoparius*, *Populus nigra* and *Quercus robur*.

Differences in the presence and occurrence of the identified plant species among soil treatments were also registered (Table 2). Generally, soil treatments increased the number of plant species, particularly in the OM and OMDL plots, as compared to the UNT ones. This is corroborated by the estimations of plant species richness (Table 3), which were significantly higher in the OMDL plots. Total shoot biomass and the occupancy percentage of the plant cover were also higher in the compost-amended plots (OM and OMDL), although these differences were not statistically significant. The similarity in species composition of plant communities between different plots was higher among amended plots (Supplementary table 2). The DL, OM and OMDL plots sheltered plant species not present in the UNT plots, i.e. *Erigeron sumatrensis* (also known as *Conyza albida*), *Cirsium vulgare* and *Crepis biennis* (Table 2). Other species, e.g. *Daucus carota*, *Ornithopus perpusillus, Medicago sativa* and *Crepis praemorsa*, grew only in the OM and OMDL soils. Figure 3 demonstrates the effect of compost-based amendments on the growth and development of the plant cover through the improvement of soil properties. The RDA shows soil parameters related to soil organic matter and nutrient contents being positively correlated with plant parameters, and species richness in particular, while soil parameters indicating the Cu contamination level showed little influence.

Regarding the shoot ionomes (Table 4), no significant effect of the soil treatments was identified for the majority of elements. Although not statistically significant, values of shoot Cu concentration showed a decreasing trend in the OMDL plots. Moreover, values of shoot Cu concentration in year 7 were considerably lower than those found in year 2 in the transplanted species (Supplementary table 3). Values of shoot P concentration, on the other hand, were significantly higher in both compost-amended plots (OM and OMDL). Shoot Mg, K, and B concentrations showed increasing levels in amended plots, but these differences were not significant.

4. Discussion

4.1 Effect of soil treatments on Cu availability and phytotoxicity

The incorporation of organic amendments into metal(loid)-contaminated soils can improve soil physicochemical properties, e.g. by reducing metal availability, incorporating organic matter and available nutrients, and modifying pH and other physicochemical parameters, and can promote soil microbial properties (Alvarenga et al., 2009; Epelde et al., 2009; Kidd et al., 2015; Mench et al., 2018; Burges et al., 2020). Here, both soil treatments containing compost, i.e. OM and OMDL, increased the levels of several soil properties, particularly those related to organic matter and nutrient contents, corroborating the lasting positive effect of this compost after 6 years of phytomanagement.

The incorporation of soil amendments with the same chemical composition (OM, DL and OMDL) in other phytomanaged field trials at this site has generally resulted in an overall decrease in the available soil Cu fraction assessed by single chemical extractions (notably salt solutions, e.g. 1 M NH_4NO_3) (Kolbas et al., 2011; Hattab-Hambli et al., 2016; Mench et al., 2018). In these studies, total Cu concentrations in soil pore water can however increase in the compost-amended soils, but the percentage of free Cu ions was low (i.e. < 5%). Here, Cu availability in soil estimated in terms of total Cu concentration in the soil pore water (SPW-Cu) was significantly higher in compost-amended plots, owing to the higher organic matter content in topsoil (Table 1) and its biogeochemical cycle (mineralisation / humification). This input of degradable organic matter may come from the decay of senescent plant shoots and rhizodeposition, containing cellulose, lignin and other polysaccharides, and from the compost incorporated into the OM and OMDL soils. In comparison, in a short-rotation coppice with poplar and willows at this site, Xue et al. (2018) reported an ongoing C decomposition 6 years after the application of similar soil amendments, suggesting the slow organic matter decomposition of this compost and carbon inputs via the leaf and herbaceous litter. The (bio)degradation of the organic matter may not only release metals bound to the remaining compost and soil organic matter but also result in a flush of dissolved organic matter that is known to promote Cu solubilisation, increasing total Cu concentration in the soil pore water (Brandt et al., 2010; Burges et al., 2015).

Total metal concentration in the soil pore water is believed to mirror root exposure to metals (Sauvé, 2003; Hattab et al., 2014). However, soluble metals include metals bound to colloids and organic ligands, that are not all directly bioavailable, and estimations of soluble Cu do not account for the depletion at the root-soil interface and depletion-induced resupply from the solid phase. DGT is a passive sampling method that excludes strongly bound, organically complexed metals and colloidal species, measuring the labile metal fraction in soil that can be taken up by biota and, accordingly, can contribute to toxicity (Zhang et al., 2001; Ernstberger et al., 2002; Paller et al., 2019). Here, DGT-Cu values showed no influence of the soil treatments (Table 1), suggesting that much of the soluble Cu in compost-amended soils was associated with organic ligands that are unable to dissociate and, hence, was not available. This was similar in the OMDL plots under a sunflower – tobacco rotation in another field trial (Supplementary table 4, Mench et al., 2018). In contrast, R values, indicating the depletion extent of soil solution concentration at the DGT interface (Ernstberger et al., 2002), decreased in compost-amended soils, notably in the OMDL plots under grassy crops (Table 1) and high yielding crops (sunflower / tobacco) as well (Supplementary table 4). Accordingly, despite the increment of soluble Cu, Cu bioavailability in soil was limited, as well as the Cu resupply capacity from the solid-phase to the soil solution, with the incorporation of compost and the subsequent increase in DOM.

In any case, the potential Cu toxicity must be assessed not only through the interpretation of soil physicochemical parameters, but also by relating such data with the ecotoxicological responses in bioassays. Soil phytotoxicity bioassays are frequently used to assess the influence of soil organic amendments on the mitigation of metal-induced phytotoxicity (Bes and Mench, 2008; Marchand et al., 2011; Galende et al., 2014; Kumpiene et al., 2014; Lacalle et al., 2018). Although this often results from a reduction in metal availability, Marchand et al. (2011) reported a decrease in phytotoxicity in the leachates of compost-amended soils, despite finding no changes in free Cu. Here, the ecotoxicity bioassay using dwarf beans showed that soil treatments, and OMDL in particular, allowed a higher shoot biomass, indicating a better mitigation of Cu-induced soil phytotoxicity in these soils amended with compost and dolomitic limestone in combination (Figure 1). This suggests that the compost may have contributed with forms of dissolved organic matter that complex with Cu, e.g. fulvic acids, resulting in much of the soluble Cu not being available and not contributing to Cu phytotoxicity. Increase in soil CEC, total soil N, and soil organic matter (and likely water holding capacity) (Table 1), would also contribute to promote the growth of bean plants.

4.2 Effect of soil treatments on the development of the plant cover

Here we focused on the potential of this mixed stand of 5 grassy species, *A. capillaris* and *V. myuros* populations being present and their seeds collected at this site, to initiate a meadow, as well as the potential beneficial influence of soil treatments on its development. Despite successful colonisation of most of the transplanted plant species in the first 2 years, *D. caespitosa* and *V. myuros* disappeared from the plots in the following years after repeated heat waves and long periods of drought in the summer (Table 2). It is worth mentioning that sampling time has proved to be a relevant factor in explaining differences in plant diversity and composition in the site (Bes et al., 2010). *Vulpia myuros* has a short annual life cycle in spring and tends to disappear in summer, possibly indicating a seasonal absence at the sampling time here (end of summer) rather than a complete loss of this specie. Both *A. capillaris* and *A. gigantea*, however, were widely present in the plots in year 7, being likely more resilient to progressive climate change conditions. *Agrostis capillaris*, in particular, was a dominant species across all the plots. This goes in line with previous findings that demonstrated the fitness and the excluder phenotype of the Cu-tolerant populations of *A. capillaris* from this site (Bes et al., 2010; Hego et al., 2014). Then, as a typical succession, there was a progressive colonization in the plots with other plant species (Table 2), dominated mainly by members of the *Poaceae* and *Asteraceae* families, which are plant groups commonly found in areas contaminated with industrial waste (Desjardins et al., 2014), followed by *R. acetosella* (*Polygonaceae*), which is also known to include Cu-tolerant ecotypes (Bagatto and Shorthouse, 1999). This plant succession likely occurred from natural spreading and germination of seeds from plant species from the site, the majority of colonising species being already present in this site before this study (Bes et al. 2010), and the surroundings. Heavy colonization by *C. scoparius* has made it necessary to carry out an annual cut to maintain the grassland option. Nevertheless, the colonisation by trees, i.e. *Q. robur* and *P. nigra*, over the period of 7 years could be gradually turning this grassland into a mixed woody-herbaceous system.

The application of soil amendments, and compost-based amendments in particular, facilitated the colonisation of a higher number of species, resulting in an increase in plant diversity (Table 2; Table 3). This spontaneous vegetation promoted by soil amendments, represented by members of the *Asteraceae* and *Fabaceae* families, and *Daucus carota* (*Apiaceae*), corresponded mostly to herbaceous species with an annual life cycle, usual colonisers in contaminated sites as annual species respond more quickly to environmental changes (Rich et al., 2008; Shutcha et al., 2015). Untreated soils, however, remained colonised mainly by perennial grasses (Supplementary table 5), which are often regarded as pioneer species in highly contaminated sites due to their tolerance to metal-induced stress (Bagatto and Shorthouse, 1999; Bes et al., 2010). Interestingly, the majority of the new colonising species, i.e. not previously identified in this site (Bes et al., 2010), belonged to the *Asteraceae* family. Compost-amended plots sheltered fodder species from the N-fixing *Fabaceae* family, i.e. *M. arabica*, *O. perpusillus* and *V. sativa*, that in this site were known to grow in the less contaminated areas (Bes et al., 2010). Other studies in this site have also reported that the incorporation of greenwaste compost, in combination with dolomitic limestone, promoted the growth of leguminous species, i.e. white clover, following a reduction in soil Cu bioavailability (Mench et al., 2018). This was accompanied by structural changes in soil microbial communities, reflected in increments of N-fixing bacterial groups, such as *Rhizobiales* or *Rhodospirillales* (Burges et al., 2020). Considering that *Fabaceae* members tend to grow in less phytotoxic soils (Wang et al., 2004; Shu et al., 2005), our results suggest that the compost incorporation may have prompted better soil conditions for the growth and development of these plant species (Figure 3).

Limited nutrient availability can be a factor for increasing phytotoxicity (Bes and Mench, 2008), which explains that Cu-induced phytotoxicity reduction here, along with plant species diversity, was paralleled with increasing levels of soil organic matter and nutrients (Table 1; Figure 3), and shoot P concentration in plants (Table 4). Moreover, despite both compost-based treatments equally improved soil properties, the OMDL treatment allowed both the most pronounced Cu-induced toxicity reduction and the highest plant species richness (Figure 1; Table 3). This suggests an additional synergic effect, potentially due to the nutrients supplied by the incorporation of dolomitic limestone that are not sufficiently present in the compost alone, i.e. content in Ca, Mg, etc. Overall, the application of compost, notably in combination with dolomitic limestone, not only promoted plant growth and development of this grassland, in line with previous findings (Bes and Mench, 2008; Marchand et al., 2011; Hattab-Hambli et al., 2016; Mench et al., 2018), but it also allowed a plant diversity that can potentially enhance soil key functions, such as N cycling and SOM accumulation, contributing to C sequestration and improving ecosystem services (Teixeira et al., 2015).

No influence of the soil amendments was identified on the uptake and accumulation of Cu by plants (Table 4). However, the shoot Cu concentrations found here in any of the plots did not exceed the upper critical threshold levels, i.e. the lowest tissue concentration of an element above which the plant yield is reduced by 10%, reported by Macnicol and Beckett (1985) for *Agrostis* spp. and other grassy species (30-35 mg Cu kg^{-1} DW). Comparing with plants grown in plots with similar topsoil Cu levels from this site, our values of shoot Cu concentration were similar to those obtained by Mench et al. (2018) in sunflower in amended soils (26 mg Cu kg^{-1}) and considerably lower than those obtained in year 2 in the transplanted plant species, including *Agrostis* (59-137 mg Cu kg^{-1}; Supplementary table 3). This could indicate that the plants composing the grassland are able to control the uptake and translocation of Cu, even in the absence of amendments, limiting the entrance of Cu into the food chain. Moreover, shoot Cu values here were slightly above the minimum average value of 10 mg Cu kg^{-1} considered adequate in pasture for both plant nutrition and animal health for New Zealand conditions (Longhurst et al., 2004). European Union directives do not have any particular restrictions for Cu in animal fodder, while guidelines in Switzerland state a limit of 40 mg Cu kg^{-1} in fodder for cattle (Directive, 2002). Therefore, the shoot Cu concentrations here fell within the optimum range for maintaining both food chain safety and an adequate plant nutritional status, suggesting also the suitability of this grassland as a grazing system. However as some PAH are present in the soils at this site

(Jones et al., 2016), exposure through soil ingestion by herbivores during grazing should be investigated.

5. Conclusions

Soil amendments containing a compost made of pine bark chips and poultry manure improved soil physicochemical properties, including an increment in soil organic matter and nutrient content, at a Cu-contaminated site phytomanaged with grassy species, 6 years after their application. The compost incorporation increased Cu solubility. However, most of this soluble Cu was likely complexed with dissolved organic matter and, hence, was not available, as reflected in the reduced Cu resupply capacity from the solid-phase to the soil solution and the mitigation of Cu-induced phytotoxicity. After 7 years of phytomanagement, soil amendments, and notably compost combined with dolomitic limestone, increased plant diversity, facilitating the colonization of several plant species. The improvement in nutrient availability and the reduction in Cu-induced toxicity with the incorporation of compost contributed to promote the colonization of species from the *Fabaceae* family involved in N-fixation. Shoot Cu concentrations remained adequate regarding the food chain safety and plant nutrient status of this grassland. Results demonstrate the persistent beneficial influence to combine compost and dolomitic limestone on the growth and development of this grassland, promoting the diversity of plant species that enhances soil key functions and provides ecological benefits. Further monitoring, including also soil microbial communities and plant cover, is needed to assess the effectiveness of these soil treatments and grassland system in the longer-term, as a relevant long-term phytomanagement option for such Cu-contaminated soils.

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Table 1. Effect of soil treatments on physico-chemical properties of soil and soil pore water in year 6 (Mean values ± standard deviation). Values followed by different letters are significantly different ($P<0.05$) according to Tukey's test. No letter in a row indicates no significant difference. UNT: untreated soil; DL: dolomitic limestone; OM: compost; OMDL: compost and dolomitic limestone; CTRL: uncontaminated control soil Eutric gleysol from a kitchen garden nearby (17 km) the site, Gironde, France (Mench et al. 2018).

	UNT	**DL**	**OM**	**OMDL**	**CTRL**
pH	7.3 ± 0.8	7.7 ± 0.2	7.3 ± 0.2	7.6 ± 0	7.5
CEC (cmol kg^{-1})	2.92 ± 0.8^{b}	3.17 ± 0.13^{b}	4.84 ± 0.79^{a}	5.64 ± 0.58^{a}	16.1
SOM (g kg^{-1})	11.9 ± 2.5^{b}	11.6 ± 1.4^{b}	26.9 ± 7.2^{a}	29.2 ± 6.8^{a}	71.4
Organic C (g kg^{-1})	6.8 ± 1.5^{b}	6.7 ± 0.8^{b}	15.6 ± 4.2^{a}	16.9 ± 3.9^{a}	41.3
Total N (g kg^{-1})	0.44 ± 0.06^{b}	0.45 ± 0.06^{b}	0.91 ± 0.22^{a}	0.99 ± 0.21^{a}	2.91
C/N	15.5 ± 1.4^{a}	15.1 ± 0.8^{a}	17 ± 0.8^{a}	17.1 ± 0.4^{a}	14.2
SPW-pH	7.1 ± 0.6	7.4 ± 0.1	7.1 ± 0.2	7.4 ± 0.1	
SPW-CE (µS cm^{-1})	495 ± 139	584 ± 108	401 ± 76	538 ± 59	
SPW-Cu (µg L^{-1})	310 ± 138^{b}	223 ± 36^{b}	627 ± 150^{a}	698 ± 195^{a}	49 ± 14
Total Cu (mg kg^{-1})	848 ± 160	844 ± 147	846 ± 194	1028 ± 132	22
DGT-Cu (µg L^{-1})	424 ± 298	410 ± 247	494 ± 274	658 ± 423	16
R ratio	0.36 ± 0.04^{a}	0.33 ± 0.09^{ab}	0.26 ± 0.05^{ab}	0.22 ± 0.06^{b}	0.13
pCu	8.8 ± 0.7	9.2 ± 0.5	8.5 ± 0.2	8.9 ± 0.1	

SOM: soil organic matter; SPW-pH: pH in the soil pore water; SPW-CE: soil electrical conductivity in the soil pore water; SPW-Cu: Cu concentration in the soil pore water; DGT-Cu: Cu concentration using DGT; R ratio: ratio between DGT-Cu and Cu concentration in the soil solution; pCu: - Log10 [potential concentration of free Cu ions].

Table 2. Identified plant species and their occurrence (n.: number of plots per soil treatment where that plant species is present) in year 7. UNT: untreated soil; DL: dolomitic limestone; OM: compost; OMDL: compost and dolomitic limestone.

UNT		DL		OM		OMDL	
Species	**n.**	**Species**	**n.**	**Species**	**n.**	**Species**	**n.**
Rumex acetosella	4	*Rumex acetosella*	4	*Cytisus scoparius*	4	*Cytisus scoparius*	4
Agrostis capillaris	4	*Agrostis capillaris*	4	*Rumex acetosella*	4	*Rumex acetosella*	4
Quercus robur	2	*Cytisus scoparius*	3	*Agrostis capillaris*	4	*Agrostis capillaris*	4
Cytisus scoparius	2	*Erigeron sumatrensis*[2]	3	*Populus nigra*	3	*Erigeron sumatrensis*[2]	4
Senecio inaequidens	2	*Senecio inaequidens*	2	*Senecio inaequidens*	3	*Quercus robur*	3
Gnaphalium sylvaticum[1]	2	*Agrostis gigantea*	2	*Erigeron sumatrensis*[2]	3	*Senecio inaequidens*	3
Populus nigra	1	*Crepis* sp.	2	*Quercus robur*	2	*Daucus carota*	3
Agrostis gigantea	1	*Cirsium vulgare*	1	*Vicia sativa*	2	*Cirsium vulgare*	3
Hypochaeris radicata	1	*Hypochaeris radicata*	1	*Crepis* sp.	2	*Crepis* sp.	3
Crepis sp.	1	*Gnaphalium sylvaticum*[1]	1	*Ornithopus perpusillus*	2	*Ornithopus perpusillus*	2
Asteraceae sp.	1	*Asteraceae* sp.	1	*Agrostis gigantea*	1	*Populus nigra*	1
		Crepis biennis	1	*Daucus carota*	1	*Agrostis gigantea*	1
		Pilosella tardans[3]	1	*Cirsium vulgare*	1	*Hypochaeris radicata*	1
				Hypochaeris radicata	1	*Gnaphalium sylvaticum*[1]	1
				Gnaphalium sylvaticum[1]	1	*Medicago arabica*	1
				Medicago arabica	1	*Crepis praemorsa*	1
				Crepis praemorsa	1	*Crepis biennis*	1
				Crepis biennis	1	*Conyza canadiensis*	1
						Hypericum perforatum	1

1, also known as (a.k.a.) *Omalotheca sylvatica*; 2, a.k.a. *Conyza albida*; 3, a.k.a. *Hieracium tardans*.

Table 3. Effect of soil treatments on plant parameters in year 7 (Mean values ± standard deviation). Values followed by different letters are significantly different (P<0.05) according to Tukey's test. No letter in a row indicates no significant difference. UNT: untreated soil; DL: dolomitic limestone; OM: compost; OMDL: compost and dolomitic limestone.

	UNT	DL	OM	OMDL
Total shoot biomass (g DW plot^{-1})	219 ± 152	165 ± 91	371 ± 164	361 ± 204
Species richness	5.3 ± 2.2^{b}	6.8 ± 1.7^{ab}	9.3 ± 1.7^{ab}	10.8 ± 2.9^{a}
Plant cover occupancy (%)	46 ± 32	59 ± 42	68 ± 27	76 ± 28

Table 4. Effect of the soil treatments on the shoot ionomes of plants in year 7 (Mean values ± standard deviation, in mg kg DW^{-1}). Values followed by different letters are significantly different ($P<0.05$) according to Tukey's test. No letter in a row indicates no significant difference. UNT: untreated soil; DL: dolomitic limestone; OM: compost; OMDL: compost and dolomitic limestone.

	UNT	**DL**	**OM**	**OMDL**
Al	76 ± 59	44 ± 8	34 ± 12	39 ± 15
B	7.3 ± 5.1	8.8 ± 3.5	10.5 ± 1.9	11.2 ± 3.4
Ca	5575 ± 1543	5826 ± 1678	5310 ± 614	5519 ± 439
Cu	26 ± 15	23 ± 10	26 ± 4	18 ± 7
Fe	99 ± 58	76 ± 12	67 ± 7	70 ± 23
Mg	881 ± 380	1432 ± 387	1236 ± 179	1262 ± 81
Mn	152 ± 67	141 ± 18	111 ± 20	144 ± 40
P	992 ± 289^{b}	1006 ± 176^{b}	1695 ± 64^{a}	1491 ± 212^{a}
K	4304 ± 238	5700 ± 2327	5203 ± 1007	6841 ± 1714
Na	141 ± 65	159 ± 116	199 ± 111	298 ± 102
Zn	68 ± 35	48 ± 14	49 ± 19	43 ± 13

Supplementary table 1. Pearson's correlation values between physicochemical parameters of soil and soil pore water in year 6.

	Tot Cu	**SPW-Cu**	**DGT-Cu**	**R**	**pCu**	**CEC**	**OC**	**SOM**	**pH**	**Tot N**
SPW-Cu	N.S.									
DGT-Cu	N.S.	0.53*								
R	N.S.	N.S.	N.S.							
pCu	N.S.	-0.53*	N.S.	N.S.						
CEC	0.51*	0.62**	N.S.	-0.62**	N.S.					
OC	N.S.	0.67**	N.S.	N.S.	N.S.	0.95***				
SOM	N.S.	0.67**	N.S.	N.S.	N.S.	0.95***	0.99***			
pH	N.S.	N.S.	N.S.	N.S.	0.94***	N.S.	N.S.	N.S.		
Tot N	N.S.	0.68**	N.S.	N.S.	N.S.	0.94***	0.99***	0.99***	N.S.	
C/N	N.S.	N.S.	N.S.	N.S.	N.S.	0.79***	0.79***	0.79***	N.S.	N.S.

Tot Cu: total soil Cu; SPW-Cu: Cu concentration in the soil pore water; DGT-Cu: Cu concentration using DGT; R: ratio between DGT-Cu and Cu concentration in the soil solution; pCu: - Log_{10} [potential concentration of free Cu ions]; CEC: cationic exchange capacity of soil; OC: soil organic C; SOM: soil organic matter; Tot N: total soil N.

Supplementary table 2. Sørensen index values showing the similarity coefficient in plant species composition between plots in year 7 (Mean values ± standard deviation). UNT: untreated soil; DL: dolomitic limestone; OM: compost; OMDL: compost and dolomitic limestone.

UNT-DL	0.52 ± 0.14
UNT-OM	0.49 ± 0.13
UNT-OMDL	0.47 ± 0.14
DL-OM	0.55 ± 0.13
DL-OMDL	0.56 ± 0.12
OM-OMDL	0.63 ± 0.11

Supplementary table 3. Shoot ionomes (mg kg^{-1}) in year 2 depending on soil treatment and plant species (Mean values ± standard deviation).

UNT: untreated soil; DL: dolomitic limestone; OM: compost; OMDL: compost and dolomitic limestone.

	Al	Ca	Cu	Fe	K	Mg	Mn	Na	P	Zn
Agrostis gigantea										
UNT	454 ± 254	4400 ± 978	91 ± 66	742 ± 402	5559 ± 1677	1287 ± 341	217 ± 195	141 ± 29	828 ± 502	42 ± 3
DL	354 ± 22	6359 ± 2050	59 ± 11	632 ± 238	8708 ± 2712	1582 ± 352	96 ± 18	152 ± 14	1000 ± 252	36 ± 10
OM	515 ± 41	3844 ± 345	65 ± 22	974 ± 271	7116 ± 249	1204 ± 202	190 ± 104	194 ± 57	992 ± 40	38 ± 5
OMDL	554 ± 343	5524 ± 1144	83 ± 57	785 ± 336	7553 ± 1760	1641 ± 127	130 ± 9	163 ± 8	1124 ± 65	46 ± 1
Agrostis capillaris										
UNT	616 ± 60	4548 ± 1237	137 ± 1	1018 ± 46	9298 ± 3359	915 ± 141	102 ± 28	178 ± 103	1248 ± 288	54 ± 30
DL	nm	nm	nm	nm	nm	nm	nm	nm	nm	nm
OM	634	2840	104	994	9220	766	172	210	1352	64
OMDL	678 ± 54	4950 ± 1287	98 ± 34	1036 ± 156	7710 ± 1485	903 ± 117	300 ± 261	380 ± 212	1295 ± 16	56 ± 9
Deschampsia caespitosa										
UNT	626 ± 178	3693 ± 951	71 ± 23	486 ± 132	5359 ± 853	1518 ± 36	51 ± 19	98 ± 2	575 ± 3	25 ± 6
DL	647 ± 9	5267 ± 877	65 ± 9	519 ± 24	5944 ± 80	2366 ± 11	80 ± 30	76 ± 11	788 ± 124	41 ± 15
OM	632 ± 252	3310 ± 1174	50 ± 19	504 ± 192	6690 ± 1457	1913 ± 180	63 ± 12	76 ± 0	2353 ± 1056	43 ± 9
OMDL	1004 ± 6	3643 ± 145	100 ± 18	783 ± 4	6749 ± 837	2671 ± 411	66 ± 4	99 ± 17	2702 ± 144	58 ± 9
Vulpia myuros										
UNT	1488 ± 84	2232 ± 365	499 ± 83	978 ± 42	1095 ± 35	576 ± 1	47 ± 9	191 ± 1	923 ± 92	36 ± 6
DL	689 ± 128	3879 ± 1379	150 ± 5	553 ± 48	1322 ± 438	663 ± 251	35 ± 13	191 ± 4	860 ± 152	28 ± 3
OM	837 ± 606	2130 ± 184	159 ± 123	515 ± 295	1097 ± 25	596 ± 57	49 ± 10	202 ± 15	1051 ± 202	39 ± 6
OMDL	646 ± 344	2410 ± 88	151 ± 123	472 ± 110	1223 ± 58	638 ± 71	33 ± 3	220 ± 6	1169 ± 70	43 ± 6

1, n = 1; 2, n = 2; 3, n = 3; nm, not measured.

Supplementary table 4. Soil and soil pore water physicochemical properties in year 9 from a field trial phytomanaged with compost and dolomitic limestone (OMDL) and a sunflower-tobacco rotation crop (Mean values ± standard deviation; n = 1 for UNT). (Mench et al. 2018; Kolbas et al. 2020). UNT: untreated soil; OMDL: compost and dolomitic limestone.

	UNT	**OMDL**
pH	6.3	7.4 ± 0.2
CEC (cmol kg^{-1})	3.1	7.0 ± 3.3
SOM (g kg^{-1})	17	33 ± 14
Organic C (g kg^{-1})	10	19 ± 8
Total N (g kg^{-1})	0.7	1.4 ± 0.6
C/N	14	14 ± 0.3
SPW-pH	5.4	6.9 ± 0.2
SPW-Cu (µg L^{-1})	547	752 ± 68
Total Cu (mg kg^{-1})	760	842 ± 69
DGT-Cu (µg L^{-1})	269 ± 9	293 ± 79
R ratio	0.50 ± 0.01	0.27 ± 0.02
pCu	5.4	6.4 ± 0.2

CEC: cationic exchange capacity of soil; SOM: soil organic matter; SPW-pH: pH values in the soil pore water; SPW-Cu: Cu concentration in the soil pore water; DGT-Cu: Cu concentration using DGT; R: ratio between DGT-Cu and Cu concentration in the soil solution; pCu: - Log_{10} [potential concentration of free Cu ions];

Supplementary table 5. Additional information on the plant species found in year 7. For species number, values were total number of plant species per treatment. For plant type, plant life cycle and reproduction method, values were percentages based on the total number of plant species per treatment. ND: not determined. UNT: untreated soil; DL: dolomitic limestone; OM: compost; OMDL: compost and dolomitic limestone.

	UNT	**DL**	**OM**	**OMDL**
Species number	**11**	**13**	**18**	**19**
Plant type				
Tree	18.2	0	11.1	10.5
Schrub	9.1	7.7	5.6	5.3
Creep	0	0	0	0
Herbaceous	63.6	84.6	77.8	84.2
ND	9.1	7.7	5.6	0
Life cycle				
Annual	0	23.1	38.9	36.8
Perennial	81.8	53.8	55.6	57.9
ND	18.2	23.1	5.6	5.3
Reproduction method				
Woody species[1]	18.2	0	11.1	10.5
Seeds	27.3	46.2	55.6	57.9
Tubers	0	0	0	0
Bulbs	0	0	0	0
Rhizomes	27.3	23.1	22.2	21.1
Runners	9.1	7.7	5.6	5.3
Tuft[2]	0	0	0	0
Rosettes[3]	0	0	0	0
ND	18.2	23.1	5.6	5.3

[1] Woody species represent all perennial species without reproduction by tuber, bulb, rhizome, runner, tiller, and rosette like trees and shrubs

[2] adventive or secondary tufts (collection of grasses growing together at the base) that separate from the parent to form genetically-identical offspring

[3] adventive or secondary rosettes (circular cluster of leaves growing from the base of a stem) that separate from the parent to form genetically-identical offspring

Figure captions

Figure 1. Effect of soil treatments on the shoot and primary leaf biomass of dwarf beans (g DW plant^{-1}). Mean values ($n = 16$) ± SD. Treatments with different letters are significantly ($P < 0.05$) different according to Tuckey's HSD-test. DL: dolomitic limestone; OM: compost amendment; OMDL: compost amendment with dolomitic limestone; UNT: unamended; CTRL: Uncontaminated soil Eutric gleysol from a kitchen garden nearby (17 km) the site, Gironde, France (Mench et al. 2018).

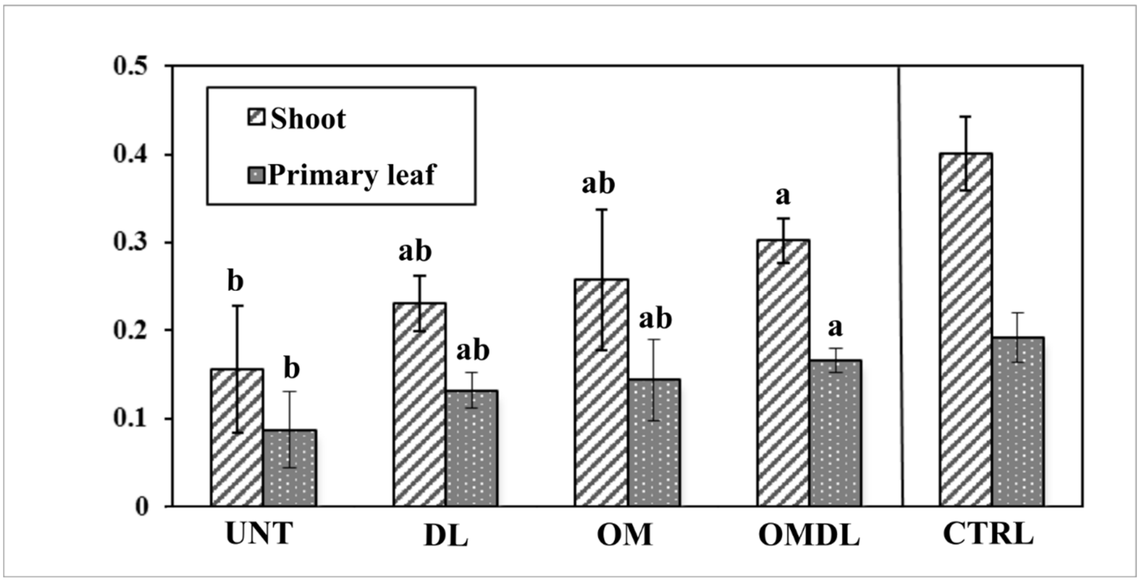

Figure 2. Percentage of mortality (a) and total shoot biomass (g DW plot^{-1}) (b) of the transplanted plant species in the field trial in year 2. AC: *A. capillaris*; AG: *A. gigantea*; DC: *D. caespitosa*; SI: *S. indicus*; VM: *V. myuros*.

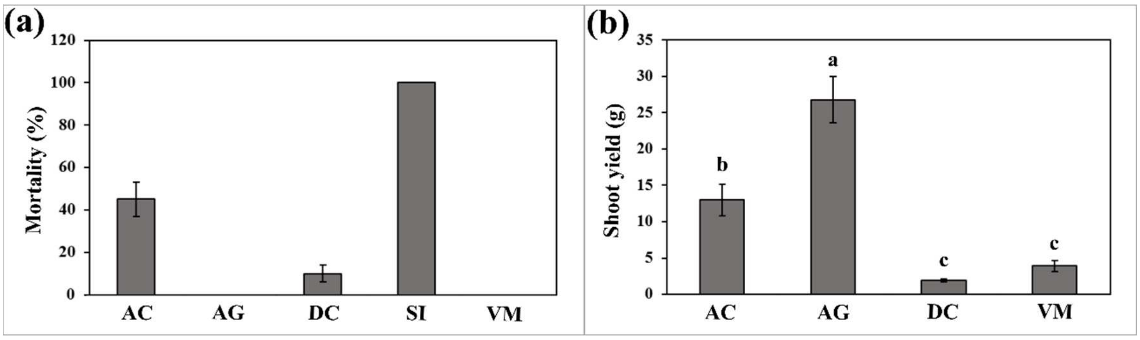

Figure 3. Biplot of redundancy analysis (RDA) showing the variation of plant parameters in year 7 explained by most relevant soil physicochemical properties (bald arrows) ($F = 2.5$; $P < 0.05$). RDA1 and RDA2 account for 56% and 17% of the total variance, respectively. Cu: total soil Cu; SPW-Cu: Cu concentration in the soil pore water; DGT-Cu: Cu concentration using DGT; R: ratio between DGT-Cu and Cu concentration in the soil solution; CEC: cationic exchange capacity of soil; OC: soil organic C; SOM: soil organic matter; N: total soil N.