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Ecology of Alluvial Arable Land Polluted by Copper Mine Tailings: New Insights for Restoration

Dissertation

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To the memory of my father

Table of Contents

List of tables

Supplements

List of figures

1 General introduction

1.1 The context of the study

1.1.1 Challenges of post-mining legacy

Mining activities have despoiled about 1% of the Earth’s surface (Walker, 1999). Economically most important mineral ores occur as metallic sulphides; most copper (Cu) deposits worldwide are mined in open pits, what generates huge amounts of waste and tailings, more than mining of any other metal (Dudka and Adriano, 1997). In the EU, waste from the extractive industries forms about 30% of the total solid waste generated every year - approximately 400 million tonnes; in Serbia this share is about 78% (EUROSTAT, 2013). The environmental impacts of such large volumes of waste range from physical effects on ecosystems, such as smothering of riverbeds, to pervasive acid drainage and leaching of heavy metals and other dangerous substances used for mineral processing, to ecological accidents like tailing dam failures with unforeseeable environmental consequences; finally, mining waste results in considerable area of barren land which is ecologically extremely fragile, economically unproductive, and aesthetically unacceptable (Dudka and Adriano, 1997; Lottermoser, 2003; Wolkersdorfer and Bowel, 2004, 2005).

Although tailings waste (a mixture of water and finely ground ore rock which remains after removal of mineral concentrate) of sulphidic metal ores, which combines high soil acidity and high concentrations of plant available fractions of heavy metals, are the most intractable issue on post-mining land (Bradshaw, 1997; Shu et al., 2005), their environmental effects have been rather well studied. Acid drainage from both active and closed mines is the single greatest challenge in the mining sector today (Wolkersdorfer and Bowell, 2004). In nature, acid sulphate soils are considered “the nastiest soils in the world” (Ljung et al., 2009). Sulphidic (commonly containing pyrite, FeS_2) tailings material has an extremely high acid generating potential, which can, in a series of cascade reactions, cause pH as low as 2-3 (Banks et al., 1997):

$2FeS_2(s) + 7O_2(g) + 2H_2O \rightarrow 2Fe_2+(aq) + 4SO_4^{2-}(aq) + 4H^+(aq)$

$4Fe^{2+(}aq) + O_2(g) + 4H^+(aq) \rightarrow Fe^{3+}(aq) + 2H_2O$

$Fe^{3+}(aq) + 3H_2O \rightarrow Fe(OH)_3 + 3H^+(aq)$

$FeS_2(s) + 14Fe^{3+}(aq) + 8H_2O \rightarrow 15Fe^{2+}(aq) + 2SO_4^{2-}(aq) + 16H^+(aq)$

Beside the direct ecotoxic effect, low pH increases mobility of residual metals in mine waste, and facilitates the entering of metals further in food chains (Banks et al., 1997; Clemente et al., 2003; Peplow and Edmonds, 2005; Stjernman Forsberg and Ledin, 2006, 2008; Hinojosa et al., 2010; Sarmiento et al., 2011). Weathering of sulphidic mining wastes also interferes with nutrient (primarily P) availability (Golez and Kyuma, 1997; Domínguez et al., 2010). Likewise, such low pH further affects the dynamics of soil minerals (e.g. Dorronsoro et al., 2002; Simon et al., 2002; Néel et al., 2003). Overall, post-mining soils pose a series of constraints for subsequent land uses. Plant growth on these substrates is limited by different factors (Bradshaw, 1983): a) physical soil constraints (e.g. structure, stability, moisture); b) deficiency of macro- and micronutrients; and c) toxicity problems (excessive concentrations of metals, salinity, low pH).

1.1.1.1 "Polluter pays" via technical reclamation

European mining policies have been shaped by the historical importance of mining for industrial development and the relatively recent introduction of environmental concerns in public policy (Wolkersdorfer and Bowel, 2004). In fact, the relaxed legislation favoured mining industry over other industrial sectors (e.g. Hámor, 2002). However, in the second half of the XX century, in particular after the mining-related accidents in Aberfan (Wales, 1966), and in Stava (Trento, Italy, 1985), the two major disasters (Azñalcóllar, Spain, 1998, and Baia Mare, Romania, 2000; Bird et al., 2008) have increased public awareness of environmental and safety hazards of mining activities and prompted the European action. Nowadays EU legislation aims at preventing water and soil pollution by mining activities (Water Framework Directive 2000/60/EC, 2455/2001/EC and 2005/646/EC; Groundwater Directive 80/68/EEC; Directive on Discharges of Dangerous Substances 67/548/EEC; Mining Waste Directive, 2006/21/EC; BAT amendment and Seveso II, Directive 2003/105/EC). Finally, the Environmental Liability Directive (2004/35/EC) introduces the "polluter pays" principle which obliges the mining industry to "remedy the environmental damage" and bring the barren post-mining land to some kind of acceptable use (Brans, 2005; Apitz et al., 2006).

The terms "reclamation", "rehabilitation", "remediation" or "restoration" are used to describe the different levels of reparation of post mining sites as compared to the original, pre-mining conditions (for review see Bradshaw, 1997). So far, the prevailing reclamation strategies of post-mining landscapes still rely primarily on technical approaches (Allen, 1997; Prach et al., 2011). These commonly include terrain levelling, correction of physical and chemical soil constraints (organic amendments, soil conditioners, irrigation/drainage), and correction of nutrient imbalances by fertilization and liming; finally, plantation forests or sown grasslands are commonly established, what leads to large-scale standardization of post-mining landscapes (Tordoff et al., 2000). Moreover, “phytoremediation” concept in reclamation of post-mining sites received quite some attention (e.g. Wong, 2003; Adriano et al., 2004). By and large, technical reclamation is a well-established and successful approach (e.g. Schaller and Sutton, 2000), yet failures still do occur due to insufficient understanding of the natural processes involved (Bradshaw and Hüttl, 2001).

1.1.1.2 Research gaps: long term impacts of tailings spills

While excavation and processing sites (tailing ponds, smelters) can be planned and managed in accordance with principles of “best available technique”, consequences of accidents involving uncontrolled spill of highly toxic tailings waste are a grave mining legacy whose environmental implications are difficult to predict. In the last four decades, major failures of tailings dams reported worldwide, followed by a discharge of metal mining waste into the local river systems, have severely degraded large areas of agricultural floodplain soils (reviews in Macklin et al., 2006; Bird et al., 2008; Rico et al., 2008). Almost half of the sediment-associated contaminant input in the river could be deposited in the floodplain soils of the river basin annually (Walling and Owens, 2003). Yet, the long term fate of these contaminants and their actual impact on soil quality and crop production, particularly when no remediation is undertaken, has surprisingly rarely been studied. One of the underlying reasons is certainly the legal obligation to immediately repair the environmental damage. It is thus seldom feasible to study the direct long-term effects of complex soil pollution (as for example by sulphidic metal tailings) on crops under field conditions and even less so to investigate the major trends on these soils by means of gradient analysis. For instance, in the largest reported and most thoroughly studied (more than 250 papers published so far) accident in Europe (Azñalcóllar disaster in 1998; a spill of pyrite tailings with high concentrations of metals into the local river system), the layer of toxic sludge was physically removed, soil amendments applied immediately following the accident, and the further agricultural use of the affected land was officially banned. Another underlying reason has perhaps been a worldwide trend to focus the ecological risk assessments on the toxicity issue of contaminated soils (Suter, 2000). It has commonly been looked either at its chemical properties or at single bioassay indicators (e.g. soil organisms or spontaneously growing “bioindicator” plants), and thus a full enough picture of soil quality has often remained elusive (Bone et al., 2010).

1.1.2 Ecological restoration: a new paradigm

1.1.2.1 An alternative focused on “naturalness”

Ecological restoration, an alternative approach to technical reclamation, is recently gaining an increasingly important role in environmental protection and management of post-mining sites. Essentially, this approach relies on thorough understanding of natural regeneration by spontaneous succession to sustainably restore the desired function and appearance of post-mining landscapes, including naturalness and socio-economic benefits (e.g. Wali, 1999; Prach and Pyšek, 2001; Prach et al., 2001a; Choi, 2007). Primary succession is a complex process of ecosystem development on barren surfaces (which are a common post-mining legacy), where severe disturbances have removed most vestiges of biological activity (Walker and del Moral, 2003). The emergence of ecological restoration was fostered by issues such as conservation of ecological integrity of degraded land (including structure and ecological processes), the acceptance of the “nature conservation” as a legitimate post-mining land use, and the question of marginal costs of reclamation and economic efficacy (e.g. Hobbs and Norton, 1996; Choi, 2007; Prach and Hobbs, 2008). So far, ecological restoration is primarily being accepted in the developed countries as UK, The Netherlands, USA and Germany (Prach and Pyšek, 2001); in the Lusatian post-mining region in Germany, for example, 15% of land is reserved as a priority area for nature conservation and left to spontaneous succession (Wiegleb and Felinks, 2001a). There is a growing evidence that ecological restoration can yield a much higher conservation value of post mining sites for much a lower price compared to technical reclamation, with increased landscape amenity, created refugia for rare and endangered species, and preserved biodiversity of vascular plants and other organisms (Hodacová and Prach, 2003; Prach and Hobbs, 2008; Tropek et al., 2010; Mudrak et al., 2010; Prach et al., 2011; Doležalová et al., 2012; Tropek et al., 2012).

1.1.2.2 “Comeback” of vegetation succession research

Thorough understanding of vegetation succession (a species change over time in the broadest sense) on post-mining land is a key concept of ecological restoration; the integration of fundamental ecological research in restoration practice is crucial (Prach et al., 2001a; Prach, 2003). Stable seral stages are commonly the target of restoration projects (Parker, 1997). Knowledge of dynamics and patterns in spontaneous vegetation development can determine the success of restoration measures, the likely time frame, and the key intervention points to achieve the desired vegetation cover (Prach, 2003; Walker et al., 2007; Prach et al., 2007; Walker and del Moral, 2009; Prach and Walker, 2011). A large body of literature on plant successions is available to rely upon; it underpins the necessity for sufficient knowledge on environmental factors and processes like hydrological factors, nutrient cycling, dispersal and competition (Pickett et al., 1987; Wali, 1999; Walker and Chapin, 1987; Glenn-Lewin et al., 1992; Prach et al., 2001a; Walker and del Moral, 2003; and the references therein). Moreover, the growing popularity of the ecological approach in managing the barren post-mining sites has contributed to a recent “comeback” of broader research interest for primary successions; a great progress is made in linking successional theory with practical restoration (e.g. Walker and del Moral, 2003, 2009; Walker et al., 2007; and the references therein).

On the other hand, large scale surface mining offered a unique chance to observe primary succession in, for instance, central Europe, over longer periods of time, since otherwise large-scale disturbances have become very rare (Kirmer et al., 2008). Succession provides an excellent framework both for seeking common patterns in the operation of ecosystem variables, and for deciphering principles that are plausible and widely applicable (Wali, 1999). Landscapes degraded by mining activities provide an exciting opportunity to study the effect of severe environmental filters on spontaneous vegetation, and thus to understand some important ecological principles which might not be apparent under “normal” conditions (e.g. Wiegleb and Felinks, 2001a; Kirmer et al., 2008; Walker and del Moral, 2003, 2009).

1.1.2.3 Research gaps: spontaneous revegetation process of severely and multiply constrained post-mining land

After a century of successional research, primary succession on man-made barren land is nevertheless still insufficiently understood (Johnson et al., 1994; Wiegleb and Felinks, 2001a). Considerable efforts are devoted to a search for environmental gradients best correlated with vegetation pattern, yet clear and generally valid correlations are difficult to find (Prach et al., 2001a, 2007b). The low possibility for extrapolations of the findings to different sites is actually a reflection of a broader failure to find general principles in community ecology (McGill et al., 2006). For instance, a need to identify more predictable ecosystem responses and general ecological principles is bringing about a major shift in plant community ecology approach, from a "nomenclatural" one, centered on species identities, towards a focus on explicit environmental gradients and ecophysilogical plant traits (McGill et al., 2006).

Process of spontaneous vegetation development on barren land seems so far to be highly site-specific; comparative studies over a gradient of contrasting environmental conditions are required to reach some generalizable conclusions (Prach et al., 2001; Walker and del Moral, 2003). For instance, recent studies have demonstrated that substratum pH and climate (Prach et al., 2007b), and regional species pool (Kirmer et al., 2008) significantly affect vegetation patterns in the course of succession on non-toxic post mining soils of Central Europe. Mining of sulphidic metal ores however creates multiple constraints for plant establishment: metal toxicity, low pH and nutrient deficiency. There is a pronounced scarcity of data concerning the principles of spontaneous vegetation development on toxic post-mining substrates, in environmental conditions which considerably differ from the Central European (Prach et al., 2001; Prach, 2003). Moreover, on severely degraded post-mining sites spontaneous restoration might not be feasible (Prach and Hobbs, 2008); although this is increasingly recognised in the concept of "novel ecosystems" (e.g. Hobbs et al., 2006, 2009), case-studies from such localities are still scarce.

Studies dealing with ecological restoration of formerly agricultural flood-plains degraded by mining effluents are lacking (Carreira et al., 2008). Furthermore, mechanisms of Cu tolerance in spontaneous vegetation which develops on substrates other than serpentine soils are rarely studied and still insufficiently understood (e.g. Poschenrieder et al., 2001). Majority of studies published so far on spontaneous revegetation of barren land degraded by metal tailings were carried out in applied frameworks (focused on management issues for reclamation, or, more commonly, on phytoremediation possibilities). Moreover, the strict environmental legislation apparently decreased the number of suitable research sites. Though describing species responses to well defined environmental gradients is fundamental to developing and testing ecological theory (e.g. Austin et al., 1994), studies focused on gradients in spontaneous vegetation as induced by gradients in the properties of soils complexly damaged by mining activities, are extremely rare. A few studies explicitly focused on vegetation gradients on metal (mostly Cu) polluted soils (Lepp et al., 1997; Ginocchio, 2000; Salemaa et al., 2001; Strandberg et al., 2006; Becker and Brändel, 2007; Dazy et al., 2009) did agree about trends of species richness and diversity, but the patterns in spontaneous vegetation were not consistently related to the patterns of soil constraints which would include both excessive metal concentrations and nutrient imbalances.

1.1.3 Research area

1.1.3.1 Environmental hotspot with long tradition

The Bor copper mines (Bor, Veliki Krivelj and Cerovo) in Eastern Serbia belong to the Timok magmatic complex, which is a part of the South-West Carpathian magmatic belt, famous for copper ore deposits (Karamata et al., 1997). The Timok magmatic complex is of Upper Cretaceous-Paleogene age, dominated by subduction-related magmatic rocks of calc-alkaline character (mostly medium- to high-potassic andesites with abundance of hornblende). Copper ore in the Timok magmatic complex occurs as massive Cu deposits (Bor mines), or porphyry Cu (Majdanpek mines); ore deposits are characterized by high epithermal sulphidation. Cu is regularly accompanied by Mo (occasionally also Au) and As; Zn and Pb also occur (Karamata et al., 1997). The major ore minerals are enargite, pyrite, chalcopyrite, bornite, chalcocite, covellite, molybdenite, magnetite, pyrrhotite, galena, sphalerite, and occasionally grey copper. The archaeological evidence of the historic copper mining in the Bor metallogenic zone can be traced back to the early Neolithic age (about 5000 B.C.); the Rudna Glava mine (of the Majdanpek complex) is one of the earliest evidence of copper mining in Europe (Jovanović, 1985).

Contemporary mining production started in 1903 with the exploitation of only the underground mine, followed by exploitation of three open pits in the Bor area. The mining activities have left a remarkable scar on the surrounding landscape; the three huge opencast mines (in total accounting for some 1,800 ha) are the most prominent landmark of the area. The city’s centre is less than one kilometre away from pits and smelter (Fig. 1.1). Interestingly, the Cu processing in the Bor mines provoked the first environmental uprising in Europe already in 1935. The problems were caused by acidic rains (from sulphidic impurities which occur with the ore), which destroyed crops in several nearby villages; demonstrations lasted for about a month, and were eventually broken by the army; yet, the farmers succeeded in their demands (Bubnjević and Majdin, 2010).

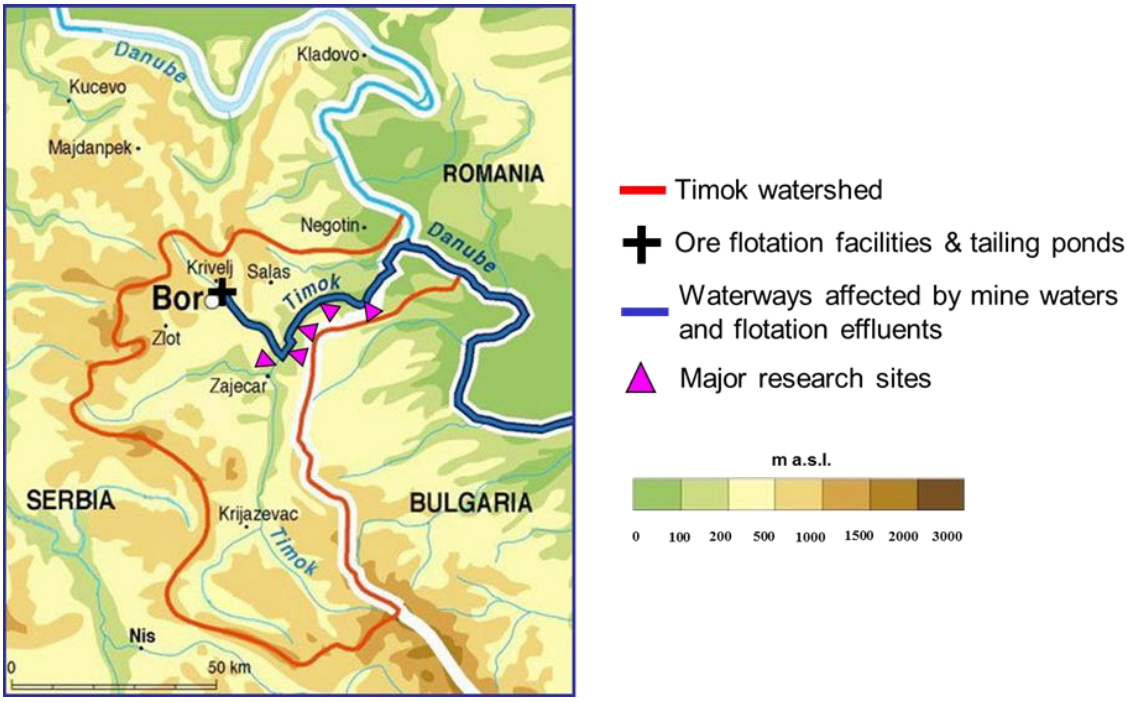

Fig. 1.1: Research area and the major research sites.

The industrial complex of the Bor Cu mines nowadays includes: Cu mining; concentration; smelting and refining of Cu and noble and rare metals; and production of sulphuric acid, Cu billets and blocks, Cu alloys and alloy-based casts. Environmental risks are quite numerous: toxic/acidic effluents; uncontained waste rock; dust emissions; poorly contained and/unstable tailings waste; poorly contained smelter residues; toxic solid waste; airborne toxics and SO_2 (Peck, 2004).

The level of pollution and associated environmental risks make the Bor area one of the four environmental hotspots in Serbia; cross-border pollution also affects the neighbouring countries Bulgaria and Romania (UNEP and UNCHS, 1999). The mines are estimated to release about 22 million m^3 of mine slurry directly into the local river system annually; consequently, acid drainage, As, Pb and Zn are reported to be the main polluting agents (Wolkersdorfer and Bowel, 2005). Beside the localized pollution in the city of Bor, the Cu mines have thus actually degraded a far larger area of the Timok watershed, all the way down to the Danube river (Fig. 1.1) by direct release of tailings waste (highly sulphidic, with still high concentrations of Cu) into the rivers. Tailings waste was further deposited over the alluvial fields in the Timok floodplain via regular and uncontrolled floods twice a year. Although this pollution has actually lasted for at least 40 years (from the mid 1940's to the beginning of the 1990's; allegedly, since there are no official data available) there is, as yet, no published assessment of the actual damage. The underlying reasons of this curious phenomenon are beyond the scope of this thesis.

The research area was the floodplain of the Timok river affected by long-term discharge of tailings waste from the two mines of the Bor mine complex (Bor and Krivelj; see Fig. 1.1). The major research sites were at the meander position of the Timok flow, where the largest tracts of barren, fluvially deposited sediments of mine tailings occur (pales of five villages: Čokonjar, Trnavac, Braćevac, Tamnič and Rajac), while one "control" locality was taken immediately before the two local rivers, directly carrying the pollution from the tailing lakes, join the Timok (Fig. 1.1).

1.1.3.2 Exceptional model locality

Though the research locality addressed by this thesis has caused grave and largely still not quantified environmental damage, it has also provided a unique chance to observe and study some processes and phenomena which are otherwise rarely encountered. In particular, the following characteristics can make this area an exceptional model locality:

1. Large-scale, long-term pollution without any remediation/restoration measures undertaken. This primarily makes possible to observe the "nature's way" of recovery, i.e. to study spontaneous development of vegetation and possible change of soil properties. The large extent of this mine-induced land degradation overcomes the common problem of small, isolated, "island" habitats typical for metal tailings, which can severely bias the revegetation process (e.g. Becker and Brändel, 2007). The long development (several decades) is likely to bring forward more stable patterns of vegetation and soil dynamics, and thus increase the chance to observe meaningful trends.

2. Agricultural soils severely polluted, but still used for cropping (Fig. 1.2). Arable alluvial soils constitute an important global resource, estimated to provide food for as much as 25% of the world's population (Gerrard, 1987); these soils are under increasing cropping pressure worldwide (Tockner and Stanford, 2002). It is very rarely possible to have a chance to study direct long-term effects of complex soil pollution (as

for example by sulphidic metal tailings) on crops under field conditions and even less so to investigate the major trends on these soils by means of gradient analysis.

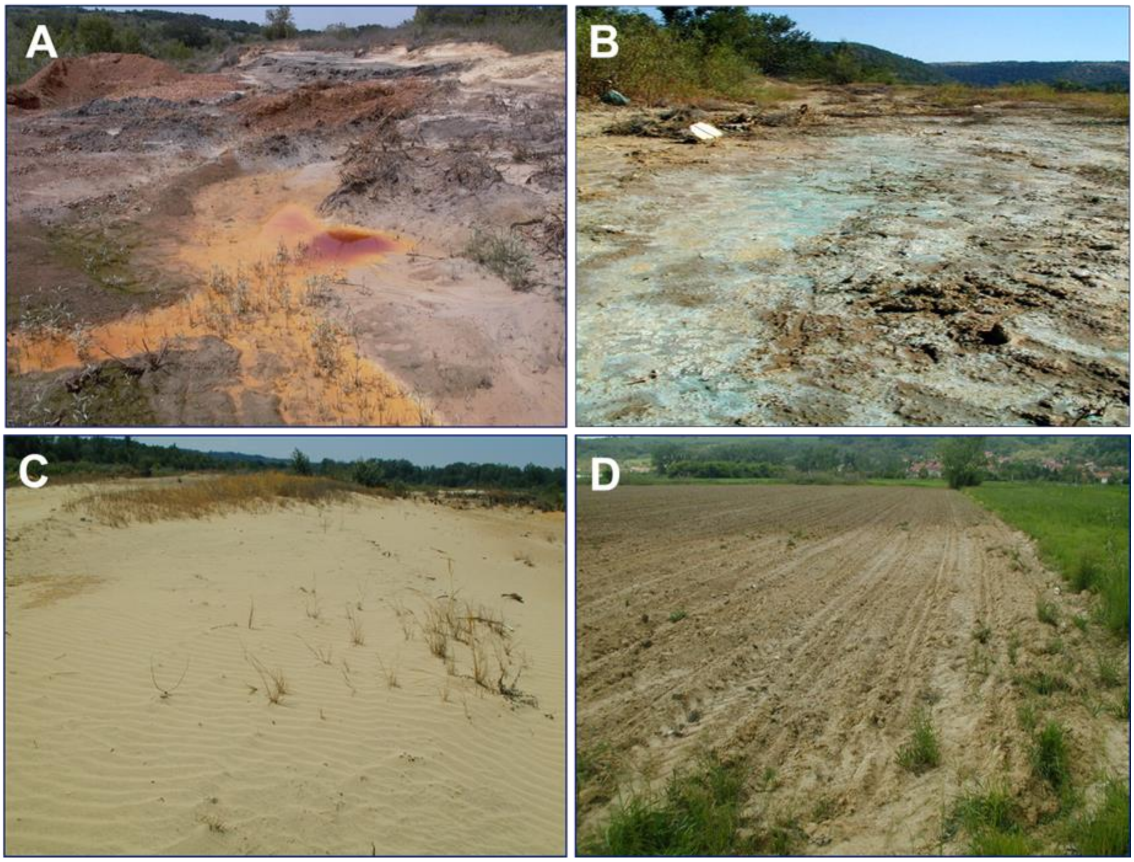

Fig. 1.2: Long-term and large-scale fluvial deposition of sulphidic Cu tailings over the alluvial fields has created a mosaic of soil constraints. A – “fresh” tailings sediments; B – Cu efflorescense; C – highly weathered, acidic and nutrient deficient tailings sediments; D – moderately affected field at the outer edge of the polluted area. Pictures were taken at least 40 km downstream from the tailings lakes.

3. Multiple soil constraints. Because of the unregulated Timok flow with pronounced fluvial dynamics, it is possible to study the effect of very different soil constraints on spontaneous revegetation process (Fig. 1.2). These constraints are a consequence of the weathering process of tailings deposits, and normally they are found separated in time.

4. Acidic soils amidst calcareous surrounding. The environmental filtering is further increased by the climatic context of the research locality (including natural spontaneous vegetation) which is very different from the context of the majority of the published studies on spontaneous revegetation of post-mining sites from Central Europe.

5. Fluvial pollution mode. Finally, we hypothesize that the chance to observe meaningful gradients and clear patterns in vegetation and soil variations will be increased compared to other studies published so far because of the regularities in fluvial mode of pollution deposition. Sediments deposition during floods regularly decreases in the lateral direction (Asselman and Middelkoop, 1995) and follows the lateral hydraulic sorting of particles (Miller, 1997).

1.2 Objectives

This explorative study aims to contribute to better understanding of the two main issues relevant for ecology and restoration of land complexly polluted by mining waste (combination of high concentrations of metals and sulphides), which have so far been very poorly understood: Firstly, the long-term fate of contaminants and their actual impact on soil quality and crop production under true field conditions (research questions 1 and 4). Secondly, the process of spontaneous revegetation of barren post-mining land under the extreme soil conditions and the environmental setup (including the regional species pool) considerably different from the well-studied Central European (research questions 2 and 3).

The major research questions addressed by this study are:

1. What are the main limiting factors for cereal production (the dominant pre-pollution land use) on the alluvial soils polluted by mine tailings? i.e., how does this multiple pollution actually affect crop growth under field conditions? (Chapter 1).

2. How does "natural recovery" of spontaneous vegetation proceed? What are the characteristics of the primary succession? (Chapter 2).

3. How do weeds respond to the sole and drastic change of soil if all the other factors are held constant? And, can ecophysiological adaptations explain the effect of severe environmental filtering on species assemblages in a simple system as cereal weeds? (Chapter 3).

4. What would be the consequences for the agronomic soil quality and for the environment if these severely polluted fields were used for cropping anyway? How would pollution intensity gradient and land use intensity gradient affect soil parameters and crop growth? (Chapter 4).

In the gradient approach framework, a combination of detailed analyses of soil parameters with vegetation surveys (species abundance, biomass, foliar analyses) is expected to bring forward some new ecological patterns relevant for the restoration science and practice, and also to provide and insight into a “would be scenario” on similar mining-affected sites.

Chapter 1

2 Phosphorus deficiency is the major limiting factor for wheat on alluvium polluted by the copper mine pyrite tailings: a black box approach

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Abstract

Failures of tailings dams have degraded large areas of agricultural alluvial soils worldwide, and concomitant soil pollution studies are abundant. Yet, the data on the actual effects of thereby imposed stresses on major crops are scarce. This work analyses the effect of pyrite tailings from a copper mine, deposited over crop fields by long-term flooding, on wheat (*Triticum aestivum* L.) under field conditions. The major previously reported polluting agents were Cu, As, Zn, Pb and acidity generated by sulphide oxidation. Flexible systematic sampling, based on visual symptoms in wheat, included transects through partially damaged fields (from calcareous to acid soils). Multivariate analysis of soil properties, leaf mineral composition and growth parameters revealed a consistent underlying soil gradient of decreasing available P and increasing S_{tot}. Phosphorus was shown to have the highest unique contribution to predicting wheat yield, consistent correlation with growth and visual symptoms, and concentrations in the range of severe deficiency. In P deficient plants N deficiency, decrease of available micronutrients and increase of As occur irrespectively of their soil concentrations, and the competition with superior “pyrite” weeds increases. Different sorption of P and possible rhizotoxic effects of other pollutants imply that fertilization can hardly be a solution.

Keywords: pyrite; copper; wheat; abiotic stress; phosphorus deficiency; gradient analysis.

2.1 Introduction

Abiotic stress is the primary cause of crop loss worldwide, reducing average yields of major crops by more than 50% (Bray et al., 2000). Marginal conditions for crop growth are increasingly being created anthropogenically, mostly as a consequence of industrial activities (Adriano et al., 1997; Chen et al., 2002). In the last four decades, 59 major failures of tailings dams reported worldwide have severely degraded large areas of agricultural floodplain soils (Macklin et al., 2006). The largest reported accident in Europe and second largest in the world was the Azñalcóllar (Spain) disaster in 1998, a spill of pyrite tailings with high concentrations of metals into the local river systems (López-Pamo et al., 1999). The Azñalcóllar case has been thoroughly researched (more than 250 articles published so far), with major focus on the characterization of soil pollution. The two commonest reported toxicity problems: high concentrations of trace metals and As, and low pH due to weathering and oxidation of sulphide minerals (e.g. Álvarez-Ayuso et al., 2008) are the most intractable issues in post-mining reclamations worldwide (Bradshaw, 1997). Although the Azñalcóllar with other recent accidents has increased public awareness of environmental issues and safety hazards (e.g. prompting the EU Directive 2000/60/EC), field studies of the actual effect of the abiotic stress thereby imposed upon major crop plants, are, as yet, extremely scarce.

Research on the effects of pyrite tailings from Azñalcóllar on wild plant species has been limited to monitoring the concentration of trace elements in plants for prospective phytoremediation (Del Rio et al., 2002; Madejón et al., 2002; Madejón et al., 2004; Walker et al., 2004; Madejón et al., 2007; Domínguez et al., 2008). However, these studies lacked the concomitant analysis of the status of major nutrient elements in soil and plants, although the importance of available nutrients for plant growth on mining-affected sites might be overriding (e.g. Thompson and Proctor, 1983). Furthermore, as the layer of toxic sludge was physically removed immediately following the accident, and the regional authorities banned previous agricultural uses of the affected land, it was difficult to study the true effects on agricultural production. The available results for few crops (sorghum, sunflower, mustard and olives: Murillo et al., 1999; Madejón et al., 2003; Clemente et al., 2005; Domínguez et al., 2010, respectively) grown after remediation on this polluted soil have shown no phytotoxic concentrations of elements in plant tissues. Phosphorus deficiency in wild olives has been the only nutrient disorder reported (Domínguez et al., 2010); it has thus remained unclear which factors limit the growth of crops on the pyrite tailings affected land.

On the other hand, true field conditions with multiple environmental stresses are very valuable for the research of stress tolerance in crop plants (Wang et al., 2003). However, the soil pollution by mining wastes has been shown to have an immanently irregular patchy distribution over very short distances (less 10 m; e.g. Hüttl and Weber, 2001; Néel et al., 2003), which makes a gradient analysis very difficult. In addition, natural river flooding dynamics on damaged alluvial soils also affects this spatial heterogeneity (Gallart et al., 1999; Burgos et al., 2006). The other issue of working in field conditions is the nature of the imposed multiple stresses: it is usually not possible to distinguish separate effect of each factor, but rather only the sum effect of all the stress factors and their myriad interactions can be demonstrated (the so-called "black box" approach).

The aim of this work has been to study the effect of pyrite tailings from a copper mine on wheat crop in field trials under real stress conditions. In the research area (Timok watershed, Serbia) about 10,000 ha of arable soil have been permanently lost for agriculture and far larger area has been damaged, while the majority of rural population is dependent on extensive agriculture for daily subsistence. The objective of this study was to investigate the limiting factors for grain production on these soils, where severe multiple stresses have been caused by decades of flooding the grain fields by tailings. Besides the direct relevance for the regional land management, this research should offer an insight into a "would-be scenario" on similar post-accident spots as for instance Azñalcóllar, had the land been left to continue in agricultural use without any rehabilitation measures.

2.2 Materials and methods

2.1.1 Research locality

Copper mines of the Bor metallogenic zone in the Eastern Serbia are one of the four environmental hotspots in the country and the key environmental issue in the Timok (a tributary to the river Danube) watershed, affecting not only Serbia, but also the neighbouring countries, Bulgaria and Romania. Copper ore occurs as massive sulphide (predominantly pyrite) deposits; due to out-of-date processing technology, copper, acid mine drainage, As, Pb and Zn appear as the main polluting agents (Wolkersdorfer and Bowel, 2005).

In the alluvial flatland of the Timok watershed the climate has both subcontinental and submediterranean characteristics, with average annual rainfall under 600 mm and temperature of 11.6 °C (annual amplitudes of 24.6 °C). The bimodal rainfall distribution and the mountainous surrounding landscape with lasting snow cover have commonly caused spring and autumn floods of the cropped fields, particularly at the meander locations where the river flow slows down and seasonal riverbeds occur. Since the mid 1940's, the two tributaries of the Timok river began to receive copper ore flotation waste - pyrite tailings spilled twice a year (after snow melting and heavy rainfalls) from the overfilled sedimentation ponds of the Bor copper mines. Pollution was further carried downstream to the Timok and finally to the Danube. The majority of soils of the flooded fields in the lower course of the Timok, traditionally used for grain production, were originally of loamy texture, calcareous, and poor in available N, P and soil organic matter; these soils were concomitantly most severely affected by pyrite tailings (Antonović, 1974). Over decades of regular annual flooding, a layer of pyrite tailings sediments, few centimetres to over a metre thick, has been deposited over the fields. About 10,000 ha of agricultural land has been destroyed, and far more damaged. Due to an interplay of historical and political circumstances, no rehabilitation measures have been undertaken. The pollution ceased about 15 years ago, when the mining activities drastically decreased, and the problematic sedimentation ponds were taken out of use.

2.1.2 Field trials

The survey included cropped fields partially damaged by the sediments of pyrite tailings, at the outer edge of the affected area. Despite the very visible delineation of the damaged regions in each field (Fig. 2.1), the whole field area is uniformly cropped (local cultural issue). The fields were within the 3 km radius from the point N 44º 05′50″, E 22º33′30″ (about 70 km from the pollution source), in the pale of the Rajac village (satellite image of the area available at: http://maps.google.com/maps?t=h&hl=en&ie=UTF8&ll=44.082899,22.550297&spn=0.05438,0.11673&z=13).

The representative fields were selected on the basis of the participatory survey with local farmers, with major criteria being similar land use histories, loamy soil texture, extensive crop management and the absence of waterlogging in the year of the trial. The trials were conducted in the fields of the rainfed winter wheat crop (*Triticum* *aestivum* L. cv Evropa-90), the major crop species and variety in the area, which, in the predominant low-input system, yields between 2 and 4 t ha^{-1} on non-polluted soils. The surveyed fields were fertilized with 50-75 kg ha^{-1} of NPK (15:15:15) at sowing, followed by about 50 kg N ha^{-1} early in spring.

2.1.3 Sampling design

Sampling was carried out according to the flexible systematic model (Smartt 1978), a form of stratified sampling where samples were allocated on the basis of the observed difference in the appearance of the wheat crop. In each field at least two physiognomically uniform "band" areas (zones) were visually distinguished (Table 2.1). From a total of 29 samples selected for this survey, 6 were designated to Zones 1 and 2 each, 7 to Zone 3, and 10 to Zone 4. In each zone three 1m × 1m quadrates (replications) were laid for the measurements, and mean values of these replications were used for further analyses.

Table 2.1: Visual symptoms in wheat crop along the gradients of soil pollution by pyrite tailings.

Zones	Visual symptoms				
	Growth reduction	Chlorosis	Translucent ears	Fungal shoot infestation	Weed infestation
1	−	−	−	−	−
2	−	−	+	+	+
3	++	++	++	++	+++
4	+++	+++	+++	+++	+++

−, no symptom; +, low; ++, moderate; +++, severe

2.1.4 Vegetation and tissue sampling and analyses

Vegetation and plant tissues sampling was done in middle to late milky ripeness of the wheat crop (Zadoks growth stage Z75-79, Feekes 11.1). This is the phase when about two thirds of the final plant biomass i.e. about half of the final grain yield is achieved, and photosynthesis in the upper leaves is still high (Knowles and Watkin, 1931; Bollons and Barraclough, 1999). Only for the generative parameters (total grain yield, harvest index, 1000 grains weight and number of grains per ear) measurements were taken in the full maturity of the wheat crop. In each zone (sample), three 1 m x 1 m quadrats (replications) were laid for destructive sampling of aboveground biomass. Clipped vegetation was sorted by species, air dried and weighed; mean values of the 3 replications were calculated per sample. For elemental analyses, flag (youngest fully emerged) leaves from 20 wheat plants were taken for a composite sample per quadrat. Ten leaves from each weed species in each replication were taken for tissue P analysis. P content in weed vegetation in each sample was calculated on the basis of the measured dry weight (DW) of each species per m^2.

Plant tissue samples were thoroughly washed with deionised water, dried at 70 ^oC and digested with conc. HNO_3 in a microwave oven. Following digestion, the concentrations of nutrients (P, K, S, C, Mg, Fe, Cu, Zn, Mn, B, Mo, and Ni) and other elements (Al, As, Pb, Cd, and Cr) were determined by inductively coupled plasma optical emission spectrometry (SpectroGenesis EOP II, Spectro Analytical Instruments GmbH, Kleve, Germany). The concentrations of C and N in leaf samples were determined by the CHNOS Elemental Analyzer Vario ELIII (Elementar Analysensysteme GmbH, Hanau, Germany).

2.1.5 Soil sampling and analyses

Soil sampling was done together with plant tissue and vegetation sampling (middle to late milky ripeness). A composite soil sample was obtained by mixing the subsamples from the rhizosphere depth (0-30 cm) taken in each of the three 1m x 1m quadrats where the vegetation was sampled. After drying and sieving the soil samples, mechanical composition was determined by pipette method and pH was measured in water (soil : water = 1:2.5). Water soluble salts were determined conductometrically, soluble $CaCO_3$ by calcimetry, and the concentrations of total C, N and S by the CHNOS Elemental Analyzer Vario ELIII (Elementar Analysensysteme GmbH, Hanau, Germany). Organic carbon was calculated from total C and $CaCO_3$. Potential CEC was determined by ammonium acetate extraction buffered at pH 7 (with ethanol treatment adjusted for salty and carbonate samples). Different extraction procedures were applied to determine plant available concentrations of elements (Allen, 1974): ammonium acetate - ammonium lactate for K, ammonium acetate for Mg and Ca, DTPA for As and metals (Fe, Cu, Zn, Pb, Cd, Cr, Ni and Mn), KCl extraction for available Al, and hot water (70 °C) for B. For plant available P two extractions were used: AL (ammonium acetate – ammonium lactate, pH 3.7; Egner et al., 1960), and Bray2 (soil: extractant 1:10, 5 min extraction; Bray and Kurtz, 1945). Both methods are applicable over a range of soil pH values, but slightly preferentially extract certain fractions. The concentrations of mineral elements in soil samples subjected to different extraction procedures were determined by inductively coupled plasma optical emission spectrometry (SpectroGenesis EOP II, Spectro Analytical Instruments GmbH, Kleve, Germany) with the exception of the P concentrations which were determined colorimetrically at 882 nm.

2.1.6 Statistical analyses

After discarding outliers, analyses were performed with 29 samples. Regression analyses (ANOVA and multiple linear regression) were done by STATISTICA 6 (StatSoft Inc., Tulsa, USA). *A posteriori* comparison of means was done by Tukey's test, at p=0.05. Multivariate gradient analyses were performed by Principal Component Analysis (PCA) by PC-ORD5 software (MjM Software Design, Gleneden Beach, USA). Correlation coefficients (centered and standardized by SD) were used for the PCA cross-product matrix. Scores for attributes were calculated as distance-based biplots, and axes were scaled in proportion to the longest axis. Ordination solutions were not rotated.

2.3 Results

The gradual change of the physiognomy of wheat crop along the of soil pollution gradient is illustrated in Fig. 2.1. The visible zones of wheat growth are accompanied by the gradual change of soil properties (Table 2.2). In general, the gradient of soil pollution from the Zone 1 (almost unaffected carbonate alluvial soils) towards the Zone 4 (acidic, highly degraded soil and severe growth reduction) comprises directional change of major measured soil properties: pH, CEC, carbonates, concentrations of available P, Ca, Mg, K and B decrease, while concentrations of total S, available Fe, Cu and Al increase along the gradient. Elements found in trace concentrations, far beyond the critical toxicity levels for plants (As, Co, Cr, Cd, Ni, and Pb) are not shown. In general, soils are low in B, C_{org} and N, and particularly low in plant available P.

As all the transects investigated match the distance gradient from the river, it would be expected that the percent of the lightest particles (clay) increases from the area nearest to the river (Zone 4) outwards towards the Zone 1, and for the heaviest particles (sand) to have an opposite trend. While this holds for the Zones 1 to 3 (Table 2.2) in the Zone 4 (most severely affected soils) the inversion (increase of silt and decrease of sand) is caused by the deposition of the mine tailings (fresh pyrite sediments consist of about 50% sand and 38% silt, not shown).

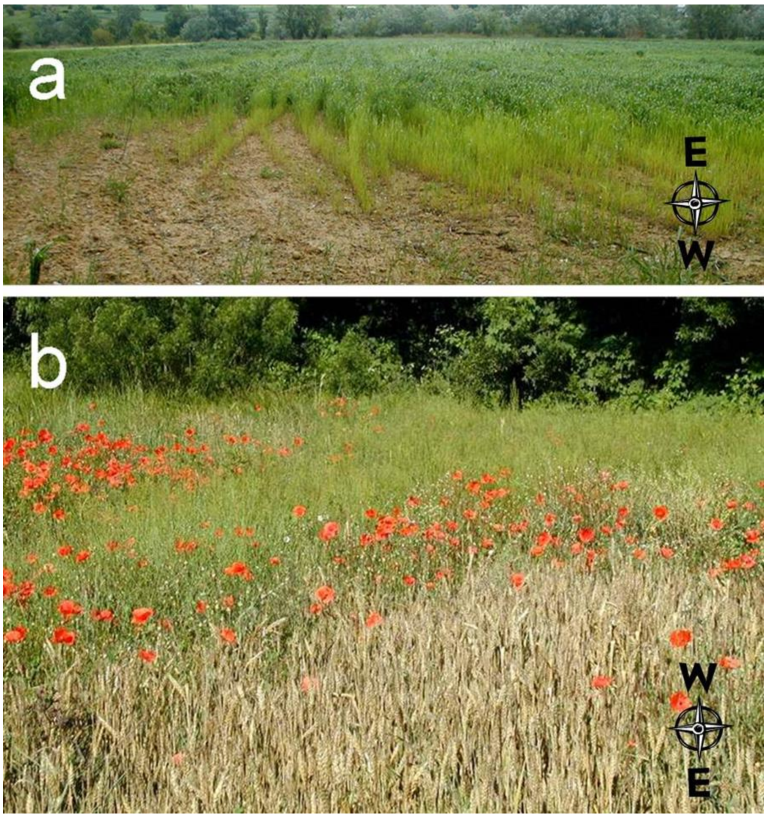

Fig. 2.1: Zones in wheat crop on soils polluted by pyrite tailings. A – stem elongation phase; growth reduction, chlorosis and low plant density occur with severe soil pollution (pollution increases from top to bottom). B – hard dough phase; three successive “bands” along the contour lines, reflecting the gradient of increasing soil pollution (from bottom to top): Zone 2, Zone 3 (conspicuous “non-specific” weeds) and Zone 4 (conspicuous “pyrite weeds”).

The PCA ordination of soil samples (Fig. 2.2A) shows that only two of 31 soil parameters analysed have a correlation with the ordination matrix of soil samples greater than 85%: total S, and AL-extractable P. These two variables are highly correlated with Axis 1 (eigenvector values of 0.91 for S_{tot} and -0.97 for AL-P) and indicate the most important environmental gradient. That is, soil samples taken from the successive zones of wheat growth disorders (see Table 2.1 and Fig. 2.1) are ordered along the gradient of increasing total soil S, or, at the same time, along the gradient of decreasing AL-P concentrations in soil.

Table 2.2: Selected soil properties along the gradient of wheat growth disorders on soil polluted by pyrite tailings. Plant available concentrations of elements (obtained by different extractions: ammonium acetate-ammonium lactate for P and K; ammonium acetate for Mg and Ca; DTPA for As, Fe, Cu, Zn and Mn; KCl for Al, hot water for B) are shown. Mean values±SD followed by the same letter in a row are not different at P=0.05.

Parameter	Zones			
	1	2	3	4
Sand (%)	34 ± 9 a	51 ± 3 bc	61± 6 c	47 ± 14 ab
Silt (%)	42 ± 7 a	29 ± 4 b	23 ± 4 b	34 ± 11 a
Clay (%)	24 ± 3 a	20 ± 2 a	16 ± 3 b	19 ± 4 b
pH (in H_2O)	8.0 ± 0.3 a	7.5 ± 0.4 a	5.8 ± 0.7 b	4.7 ± 0.6 c
CEC (cmol kg^{-1})	19 ± 1 a	16.8 ± 0.6 a	13 ± 2 b	11 ± 2 b
$CaCO_3$ (%)	1.7 ± 0.9 a	0.7 ± 0.3 b	0	0
Soluble salts (%)	0.13 ± 0.17 a	0.08 ± 0.02 a	0.06 ± 0.02 a	0.14 ± 0.12 a
C_{org} (%)	1.2 ± 0.4 a	1.1 ± 0.3 a	1.4 ± 0.6 a	1.0 ± 0.3 a
N_{tot} (%)	0.13 ± 0.02 a	0.13 ± 0.03 a	0.14 ± 0.02 a	0.12 ± 0.03 a
S_{tot} (%)	0.18 ± 0.08 a	0.44 ± 0.08 b	0.87 ± 0.11 c	1.49 ± 0.21 d
Ca (mg kg^{-1})	364 ± 79 a	272 ± 16 b	164 ± 27 c	147 ± 50 c
Mg (mg kg^{-1})	22 ± 2 a	19 ± 3 b	16 ± 3 b	14 ± 6 b
K (mg kg^{-1})	207± 68 a	160 ± 26 b	167 ± 29 b	133 ± 31 b
P (mg kg^{-1})	88 ± 5 a	54±2 ab	28 ± 10 bc	8 ± 4 c
B (mg kg^{-1})	0.8 ± 0.1 a	0.5 ± 0.3 a	0.3 ± 0.2 b	0.2 ± 0.2 b
Fe (mg kg^{-1})	9 ± 6 a	13 ± 10 a	58 ± 27 b	54 ± 18 b
Cu (mg kg^{-1})	32± 17 a	49 ± 34 a	105 ± 45 b	86 ± 46 c
Zn (mg kg^{-1})	1.6 ± 0.5 a	1.7 ± 1.4 a	3.2 ± 2.7 a	1.8 ± 1.2 a
Mn (mg kg^{-1})	17 ± 7 a	11 ± 2 a	20 ± 10 a	17 ± 7 a
Al (mg kg^{-1})	2.0 ± 0.6 a	2 ± 1 a	10 ± 7 b	60 ± 30 c

A joint plot (Fig. 2.2B) produced by superimposing soil properties on the ordination scores of wheat aboveground biomass (including all the measured yield components, see Table 2.4) indicates the same environmental gradient of decreasing AL-P, i.e. increasing S_{tot}. The Kendall correlation (tau) of AL-P and S_{tot} with the ordination scores on the first PCA axis is very strong (-0.92 and 0.84, respectively). Furthermore, although the higher soil Cu concentrations coincide with drastic reduction of wheat growth with (Fig. 2.2A and B; see also Fig. 2.3), they are not reflected in the leaf mineral composition (Fig. 2.2C).

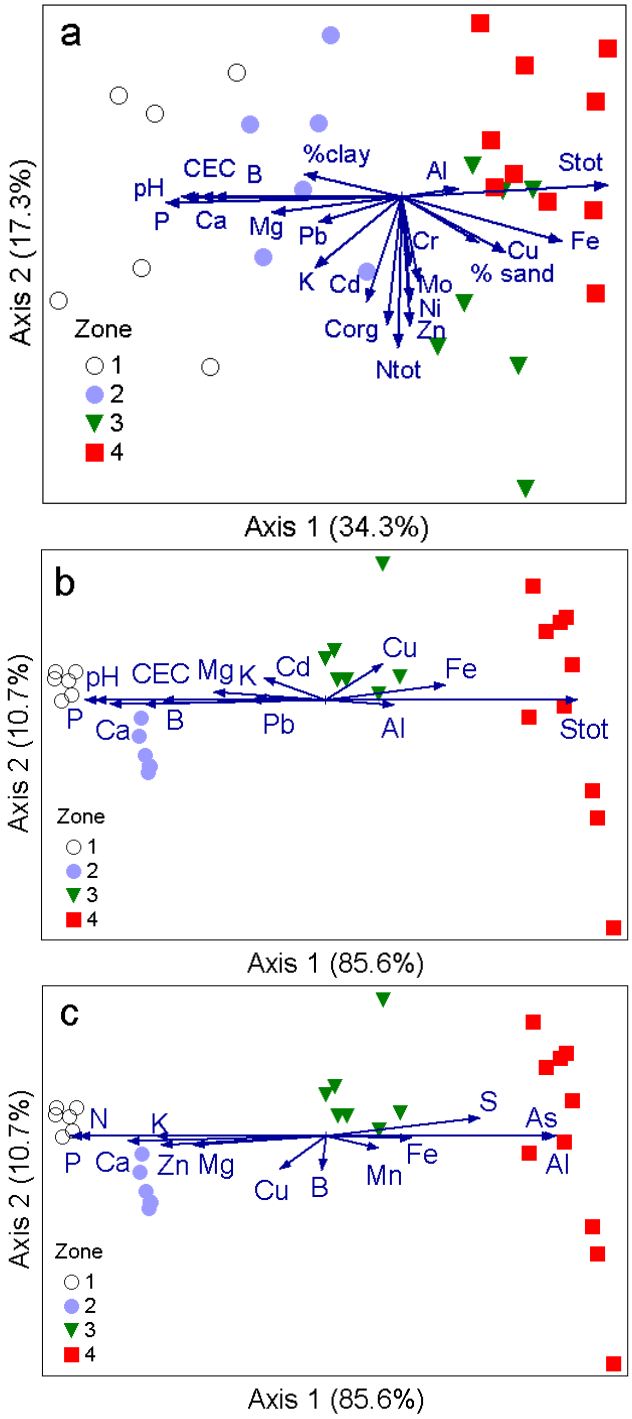

Fig. 2.2: PCA of environmental and plant growth variables on soils affected by pyrite tailings. The values in parenthesis denote the proportion of variance represented by each PCA axis. The angles and lengths of the radiating lines indicate the direction and strength of relationships of the superimposed variables with the ordination scores. A – gradients in soil properties; soil variables of correlation with ordination scores > 20% are plotted, omitting some severely correlated variables. B – ordination of wheat growth parameters, with soil variables superimposed. C – ordination of wheat growth parameters, with leaf mineral composition (correlated by more than 10% with ordination scores) superimposed. Plant available concentrations of elements (except N, C and S), and AL-P are included in the analysis.

In the field, the gradual increase of total soil S, which indicates the presence of the pyrite tailings but has no direct effect on plant growth, gives exactly a “mirrored image” of the effect of AL-P on wheat growth (Fig. 2.3). Decrease of wheat biomass by 90% along the soil pollution gradient has a sigmoid pattern and leads to a dwarf appearance of plants (nanism) in Zone 4. A group of only three species (the so-called "pyrite-specific" weeds: *Rumex acetosella* L., *Agrostis capillaris* L., and occasionally *Vulpia myuros* (L.) C.C. Gmel.; see Zone 4 on Fig. 2.1B) exclusively occurs on soils polluted by pyrite tailings in the study region (see Chapter 3). This group of weeds gradually increases the dominance along the gradient, replacing other common cereal weeds (the so called "non-specific weeds", association of Consolido-Polygonetum avicularis, see Zone 3 in Fig. 2.1B; phytocoenology is not shown).

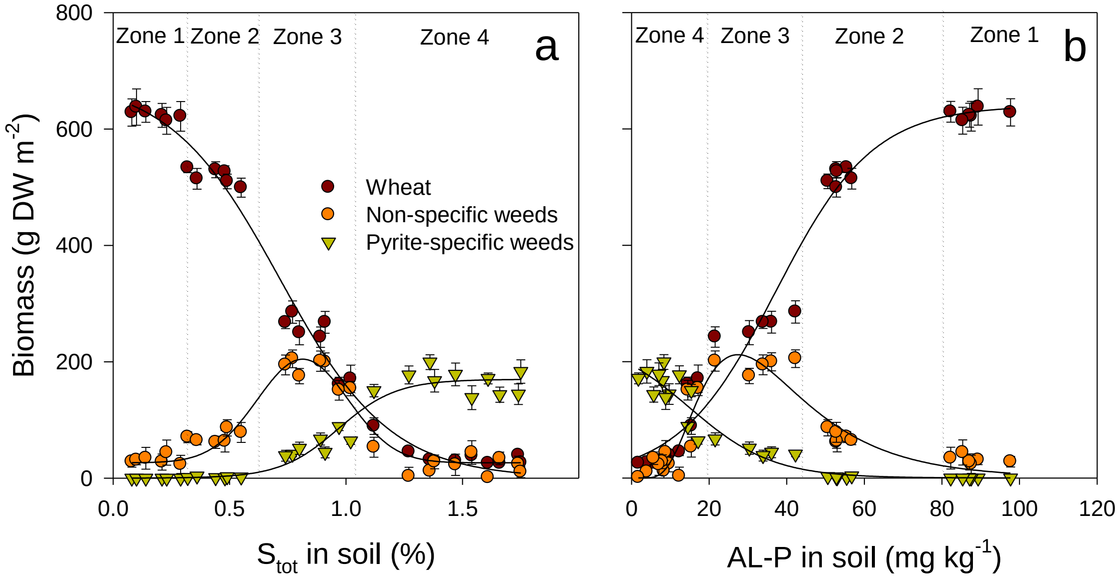

Fig. 2.3: Direct ordination of shoot biomass of wheat (milky ripeness, Z75-79) and the two groups of accompanying weeds along the major soil gradient: A – the increasing S_{tot} concentration. B – the decreasing concentration of available P. Data are means of 3 replications ±SD.

When wheat shoot biomass per m^2 is regressed on the measured soil properties, only two variables, AL-P and pH, are nominally significant predictors (Table 2.3). While AL-P explains only about 5% of all the variation in the model (squared semipartial correlation), it has the highest unique contribution to predicting wheat yield (squared partial correlation about 58%). Thus, though the contribution of AL-P to explaining wheat yield is rather redundant with other soil variables (also indicated by low values of tolerance), it does predict the significant part of variability in crop growth which is not accounted for by other soil parameters.

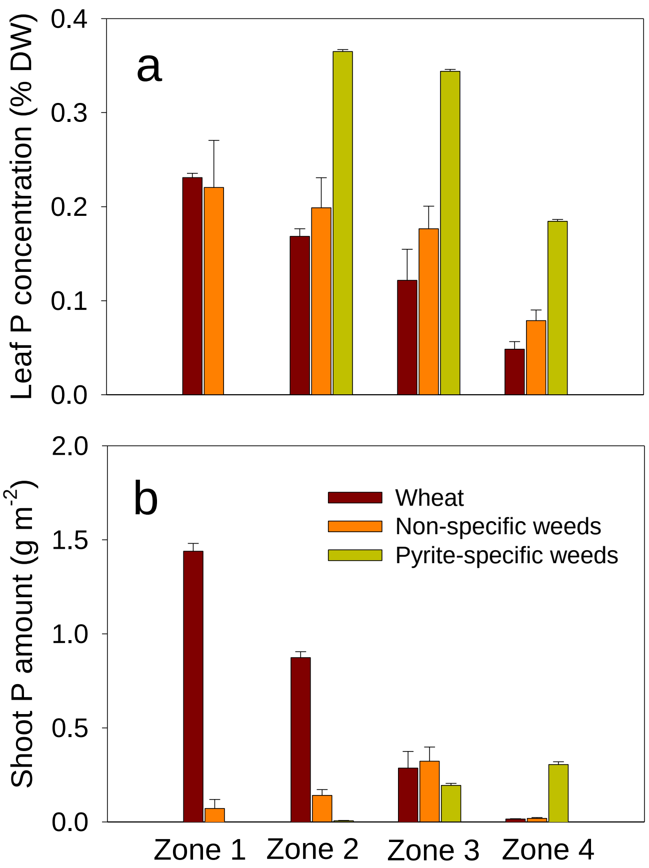

Fig. 2.4: Leaf concentration (A) and shoot amount (B) of P in wheat (milky ripeness, Z75-79) and the two groups of accompanying weeds along the gradients of soil pollution.

The grain yield decreases from the maximum of 4.1 t ha^{-1} in the non-affected zone, to the less than 100 kg (minimum only about 60 kg) per ha in the Zone 4 (Table 2.4). The first reduction of grain yield (Zone 2) is primarily caused by the reduced tillering (15% less ears per plant); tillering ceases completely in the Zone 3. The drastic reduction of the number of grains per ear abruptly occurs at the end of the gradient (Zone 4), while the 1000 grains weight rather regularly decreases after the Zone 2. Only one third of the plant density in Zone 1 remains vital at the end of the gradient. However, about 15% of the plants in the Zone 4 fail to produce any grain yield; this has caused the increase of variability of harvest index (ratio of grain yield to whole shoot DW), which otherwise changes relatively little over the soil degradation gradient.

Table 2.3: Multiple regression (stepwise forward) of wheat shoot biomass per m^2 (milky ripeness, Z75-79) on 23 selected soil parameters. The four most important soil explanatory variables are shown.

Soil variable	β	Partial correlation	Semipartial correlation	Tolerance	P value
AL-P (mg kg^{-1})	0.605	0.76	0.22	0.128	0.000009
pH (in H_2O)	0.362	0.62	0.14	0.159	0.0009
Silt (%)	-0.329	-0.30	-0.058	0.032	0.139
Cu (mg kg^{-1})	-0.77	-0.30	-0.058	0.581	0.138

β – standardised regression coefficient; R^2= 0.966; $F(5,23)$=131.59; $p<0.00000$

Table 2.4: Yield components of wheat along the gradient of soil pollution by pyrite tailings. Mean values ± SD followed by the same letter in a row are not different at P=0.05.

Yield component	Zones			
	1	2	3	4
Grains (t ha^{-1})	4.01 ± 0.09 a	3.19 ± 0.09 b	1.09 ± 0.23 c	0.11 ± 0.06 d
Straw (t ha^{-1})	5.43 ± 0.15 a	4.82 ± 0.08 a	1.56 ± 0.30 b	0.18 ± 0.20 c
Ears $plant^{-1}$	2.12 ± 0.03 a	1.82 ± 0.12 b	1.1 ± 0.1 c	0.9 ± 0.2 d
Grains ear^{-1}	35.1 ± 0.4 a	35.12 ± 0.5 a	30.5 ± 1.5 b	12.4 ± 0.8 c
1000 grains weight (g)	34.7 ± 0.6 a	33.0 ± 0.2 b	26.3 ± 1.5 c	17.9 ± 0.8 d
Harvest index (%)	42.5 ± 0.4 a	39.5 ± 0.7 b	41.3 ± 1.5 bc	38.7 ± 3.8 c
Plants m^{-2}	156.6 ± 2.1 a	151.2 ± 4.8 a	121.8 ± 12.6 b	57.7 ± 12.9 c

Leaf nutrient status (Table 2.5) shows that, in general, concentrations of bases, N and P decrease, and of Fe, Al, S and As increase along the gradient of soil pollution. The concentrations of trace elements (Mo, Co, Cr, Cd, Ni, and Pb) were in the range of normal values (not shown, according to Mordtvedt et al., 1991). The only element in leaf which significantly discriminates between all the zones of the observed crop growth disorders, and, at the same time, has the concentrations in the range of severe deficiency, is phosphorus. Multivariate analysis (Fig. 2.2C) demonstrates that leaf P status has the highest correlation (r^2=0.93) with the ordination of plant growth parameters. PCA (Fig. 2.2C) reveals a strong gradient in leaf elemental composition: decreased P and N concentrations, and at the same time increased concentrations of Al, As and S.

Table 2.5: The concentrations of selected elements in the flag leaf of wheat (milky ripeness, Z75-79) along the gradient of growth disorders on soils polluted by pyrite tailings. Mean values±SD followed by the same letter in a row are not different at P=0.05.

Concentration	Zones			
	1	2	3	4
Ca (%)	0.84 ± 0.04 a	0.79 ± 0.06 a	0.71 ± 0.03 b	0.70 ± 0.03 b
Mg (%)	0.21 ± 0.01 a	0.21 ± 0.01 a	0.17 ± 0.01 b	0.18 ± 0.02 b
K (%)	2.24 ± 0.16 a	2.16 ± 0.12 a	1.07 ± 0.12 b	1.07 ± 0.22 b
N (%)	1.69 ± 0.03 a	1.59 ± 0.04 a	1.04 ± 0.12 b	0.66 ± 0.10 c
S (%)	0.38 ± 0.14 a	0.39 ± 0.05 a	0.58 ± 0.10 ab	0.73 ± 0.15 b
P (%)	0.23 1± 0.004 a	0.156 ± 0.005 b	0.10 ± 0.01 c	0.048 ± 0.007 d
Fe (mg kg^{-1})	177 ± 53 a	280 ± 43 b	297 ± 18 b	295 ± 27 b
Cu (mg kg^{-1})	18.4 ± 0.7 a	18 ± 1 a	12.9 ± 0.5 b	16.5 ± 0.9 c
Zn (mg kg^{-1})	28.1 ± 1.5 a	27.8 ± 1.2 a	19.0 ± 0.7 b	21 ± 2 b
B (mg kg^{-1})	16.2 ± 0.5 a	14 ± 1 b	7.9 ± 0.6 c	15 ± 2 b
Mn (mg kg^{-1})	193 ± 53 a	179 ± 43 a	182 ± 18 a	229 ± 27 a
Al (mg kg^{-1})	132 ± 5 a	162±15 a	249 ± 44 b	312 ± 42 c
As (mg kg^{-1})	0.05 ± 0.06 a	0.15 ± 0.04 b	0.8 ± 0.1 c	1.5 ± 0.5 d

Fig. 2.5A shows that leaf P concentration consistently decreases with the decrease of plant growth along the gradient of soil pollution. The relation between the leaf P concentration and the “plant available” fraction of soil P greatly depends on the extraction method (Fig. 2.6). In this study, AL-P is shown to have significantly higher correlation with leaf P-status of wheat than Bray2-P. For the same leaf P concentration, Bray2 extraction gave higher values of the available soil P on acidic soils with higher Fe concentrations (Zones 3 and 4, see Table 2.2) i.e. somewhat lower values on soils rich in exchangeable Ca (Zones 1 and 2), as compared to the AL-P extraction.

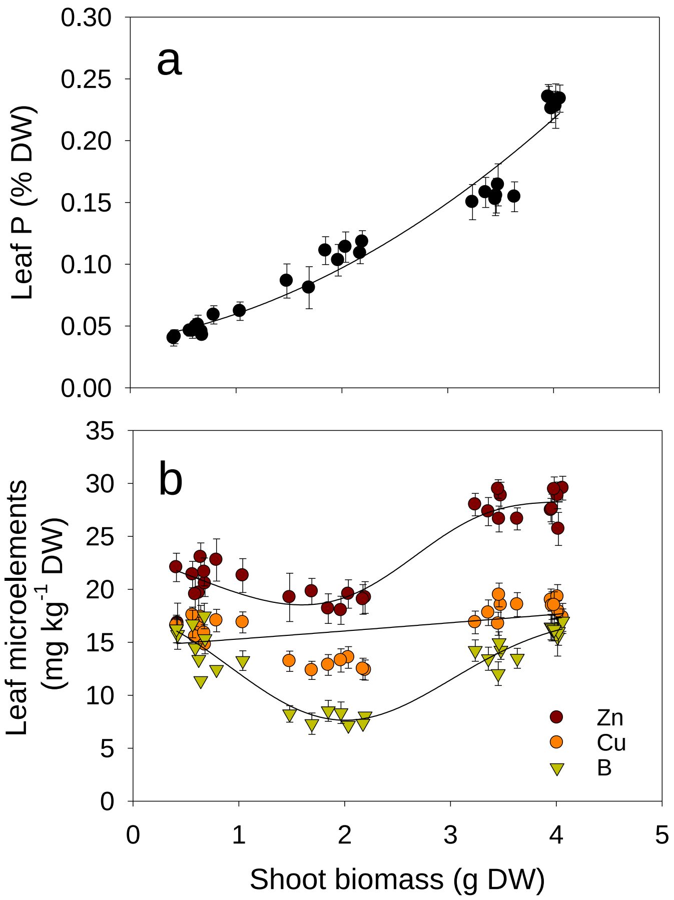

Fig. 2.5: Leaf concentrations of P (A) and microelements (B) as affected by wheat growth reduction along the gradient of soil pollution by pyritic Cu tailings.

The concentrations of three micronutrients: Cu, Zn and B, despite their different trends in soil, have a similar pattern in leaves over the gradient (Fig. 2.5B). The saddle shaped curves show the "concentration effect" of these elements with drastic reduction of plant growth (nanism) on the most severely polluted soils. Concentration effect precludes thus a stronger linear correlation between wheat growth and leaf micronutrient status (Fig. 2.2C).

Fig. 2.7A shows a decrease of leaf N along the major soil gradient of decreasing AL-P. Soil N at the same time does not change and is also not significantly correlated with ordination of samples neither on the basis of their soil properties (Fig. 2.2A) nor on the basis of plant growth parameters (Fig. 2.2B). On the other hand, the leaf concentration of As along the major soil gradient shows the opposite pattern (Fig. 2.7B). With diminishing concentrations of soil AL-P, the leaf As increases, despite the drop of As concentrations in soil.

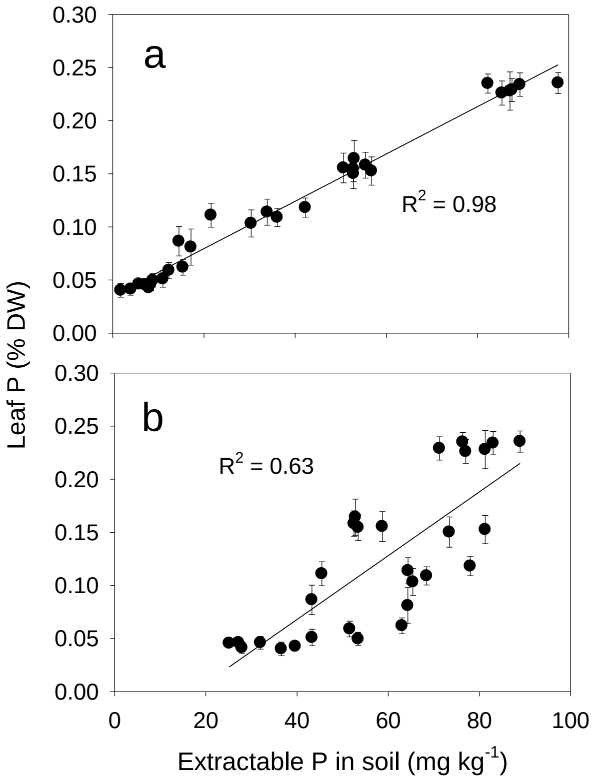

Fig. 2.6: Two extraction methods for available P in the polluted soils evaluated by P concentration in in wheat leaves; A – AL extraction; B – Bray2 extraction.

2.4 Discussion

The most striking feature of the studied locality is a remarkably regular change of plant growth over extremely short transects, with clear-cut boundaries between different zones, and very consistent underlying soil gradient of decreasing AL-P and increasing S_{tot} (Fig. 2.1; Fig. 2.2; and Fig. 2.3). Zonation of vegetation along water level gradients is one of the best studied phenomena in plant ecology; however, in the majority of studies concerned with soil gradients on post-accident polluted alluvial soils the research focus has been exclusively on As and metals (for review see Macklin et al., 2006; Bird et al., 2008). The immediate removal of the tailings deposits following the Azñalcóllar accident (see Gallart et al., 1999; Burgos et al., 2006) precluded the identification of unequivocal soil gradients.

On the other hand, on the thoroughly researched coal mine dumps in Lusatia (also high content of pyrite and low nutrients, but no fluvial influence nor visible zonation), the chronosequence studies showed a very low explanatory power of soil variables (e.g. Hüttl and Weber, 2001; Wiegleb and Felinks, 2001a). Hence, it seems that a unique combination of fluvial dynamics with a long-term sedimentation of mine tailings brought about very pronounced gradients demonstrated by this study. The gradients are the most obvious in the border area between polluted and unpolluted soils;

majority of the land affected by the tailings would, however, probably have similar properties as Zone 4. Miniscule differences in elevation (Fig. 2.1, up to maximum 0.5 m, impossible to detect with standard GPS devices), which normally have no visible effect on soil or plant growth, have an overwhelming importance in this polluted area and finally result in more than 30 times lower wheat biomass (Table 2.4).

Oxidation of sulphides is the major process on tailings sediments; soil acidification and subsequent leaching of metals is the common scenario (e.g. Néel et al., 2003; Álvarez-Ayuso et al., 2008). Leaching processes at low pH can explain the lack of phytotoxic concentrations of metals (Table 2.2). Only Cu is occasionally found in substantially elevated concentrations (Table 2.2) but no systematic variations of soil Cu are found to coincide with plant growth disorders (Fig. 2.2B). This study did not address any of the possible rhizotoxic effects which the oxidation of sulphides might have induced. Yet, the contribution of the multiple stresses imposed upon wheat roots to the restriction of nutrient uptake can neither be excluded. Restricted nutrient uptake in this case is likely to be most clearly visible in decreased leaf P status. In particular, the three stress factors, which remain in the domain of a “black box”, are likely to be a) direct toxicity of low pH (e.g. Marschner, 1995), b) root fungal infections promoted by severe P deficiency (e.g. by take-all, see Brennan, 1988), and c) rhizotoxicity of high available Cu (accompanied by growth inhibition and decrease of shoot P; e.g. Adalsteinsson, 1994).

No phytotoxic concentrations of elements, and deficiency of P and N were found on polluted soils (Table 2.5; evaluation criteria of Bergmann and Neubert, 1976; Mordtvedt et al., 1991; Bergmann, 1992; Marschner, 1995). Leaf concentrations of P and N are the only diagnosed nutrient disorders found to be significantly correlated with growth disorders (Fig. 2.2C). Adequate N concentration in upper wheat leaves in flowering is about 2.6-3% (Bergmann and Neubert, 1976), which is about 1.7-2.1% in milky ripeness (after Knowles and Watkin, 1931, as mineral contents decrease significantly after anthesis). Thus, the wheat crop suffers from N deficiency in the severely degraded soils (Zones 3 and 4). This coincides with the observed chlorosis symptoms (Table 2.1) and growth reduction (Fig. 2.2C; Fig. 2.3; Table 2.4). Adequate P concentration in upper leaves at anthesis is about 0.2-0.3% (Bergmann and Neubert, 1976). Bollons and Barraclough (1999) for example found that shoot P concentration of 0.2% at anthesis still enables 95% of the maximum grain yield in winter wheat, when all other nutrients are optimally supplied. This indicates that P-limited wheat growth starts from the Zone 2 (Table 2.5), i.e. with the beginning of the tailings sediments, and that low wheat yields (up to only 4 t ha^{-1}; Table 2.4) on the non- affected soils (Zone 1) are not due to P deficiency.

The levels of Ca, Mg and K are close to the upper limits considered adequate along the whole gradient, while S is very high (Bergmann and Neubert, 1976). Concentrations of Fe and Mn are considered high, Zn is on the lower, and Cu on the higher limit of adequate concentrations (Bergmann and Neubert, 1976; Bergmann, 1992; Marschner, 1995). Low B in the Zone 3 is within deficiency range for wheat (about 7 mg kg^{-1} at booting stage; Rerkasem and Jamjod, 2004). However, probable micronutrient deficiencies in the most severely affected Zone 4 are mimicked by the concentration effect (Fig. 2.5B) as a consequence of severe growth inhibition (see also Fig. 2.3). This concentration effect also brought about an artefact of significant correlation of leaf B, Cu and less so Zn with wheat growth ordination (Fig. 2.2C), as well as an apparent independence of leaf B and Cu concentrations from the main gradient of leaf mineral composition (Fig. 2.2C). On the other hand, as available soil B is the only micronutrient severely deficient along the soil gradient (Table 2.2; criterion after Rerkasem and Jamjod, 2004), and significantly correlated with wheat growth (Fig. 2.2B), we can not exclude a possibility, again within a “black box”, that low plant B might have contributed to decreased yield, although concentration effect precluded the diagnosis of B deficiency in leaves. The only observed effects that can be attributed to low B however are translucent appearance of ears and increased incidence of fungal shoot infestations (Table 2.1; Marschner, 1995).

In this survey, P deficiency was shown to be the only factor which has a) the same trend along the gradient, diagnosed both in soil and in plants (Table 2.2; Table 2.5; Fig. 2.2), b) soil concentrations consistently and significantly correlated with growth inhibition (Fig. 2.2B and Fig. 2.3B) and the highest unique contribution to predicting wheat yield (Table 2.3), and c) leaf concentrations significantly correlated with growth inhibition (Fig. 2.2C and Fig. 2.5A) and consistent with visual symptoms (Fig. 2.1 and Table 2.1) along the gradient (Fig. 2.4). This study thus identifies P as a master limiting soil factor for wheat growing under multiple abiotic stresses induced by soil pollution with pyrite tailings from a copper mine.

The only direct effect of P deficiency demonstrated here is stunted growth of wheat plants along the gradient of soil pollution (Fig. 2.2 and Fig. 2.3). Sigmoid growth response (Fig. 2.3) has been observed upon P application to very low P soils (see e.g.

Barrow and Mendoza, 1990). No other common visual symptom of P deficiency (e.g. Bergmann, 1992, Marschner, 1995) was observed. This is consistent with a thorough study of Elliot et al. (1997a), who found no unequivocal visual P deficiency symptoms in wheat besides pale leaf colour (but they did not discuss leaf N). The sequence of the observed decline of yield components along the soil pollution gradient: first reduction up to cessation of tillering, then decrease of 1000 grains weight, and finally of number of grains per ear, with little change of harvest index (Table 2.4) is consistent with experimental P deficit reported by Elliot et al. (1997a).

Phosphorus deficiency along the pollution gradient is accompanied by a decrease of leaf N (Fig. 2.7A), an increase of leaf As (Fig. 2.7B), and an increase of adapted weeds (Fig. 2.3). The possible causal relations among these observed phenomena are however yet to be proven. The major impact of P nutrition on N economy has been reported in several plants species (e.g. de Groot et al., 2003 and references therein). Nevertheless, how exactly P deficiency causes multiple alterations of the N assimilation pathway is still under debate. Elliot et al. (1997b) have shown that under severe P limitation, even extremely high application of N had a negligible effect on leaf N status of wheat. Moreover, at all N levels, severely P-deficient wheat accumulated significantly less N than plants of adequate P status. N deficiency in wheat on the pyrite tailings sediments reported here can not be alleviated by a further increase of N fertilization. On the contrary, on aerobic soils of light texture and very low C_{org} (Table 2.2) increased N fertilization is expected to yield only nitrate leaching.

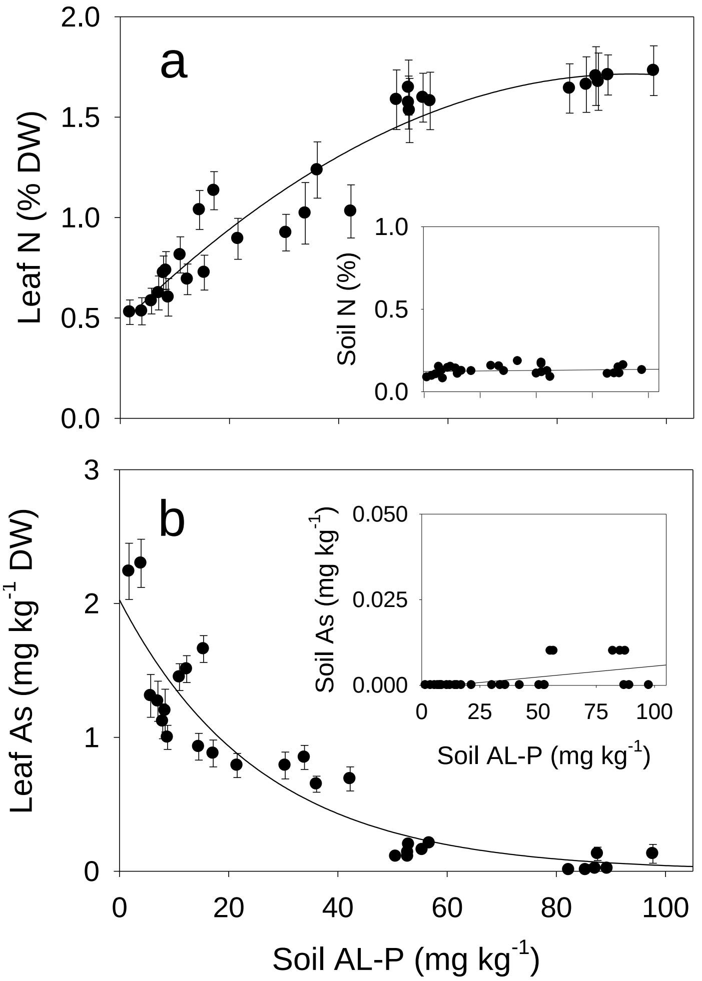

Fig. 2.7: Concentrations of N (A) and As (B) in wheat leaves as affected by soil P status. Respective concentrations of N and As in soils along the gradient of AL-P are shown in the boxes.

An increase of As concentration in wheat leaves irrespectively of very low soil As (Fig. 2.7B) also occurs with decreasing P (see also Fig. 2.6A). Arsenate (as a dominant form of As in aerobic soils) is a chemical analogue of phosphate, competing for the same transporters on the root plasma membrane (Meharg and Hartley-Whitaker, 2002); it has been demonstrated that low P status causes an increase of As concentration in rice (Wang and Duan, 2009). The decreased DTPA-extractable As along the pollution gradient Fig. 2.7B) might be attributed to neoformations of secondary minerals which scavenge As released in the process of pyrite sludge weathering (e.g. Álvarez-Ayuso et al., 2008). In fresh tailings sediment in the area, however, we found up to 740 mg kg^{-1} of total As (own unpublished results). Phytotoxic As levels range between 3 and 10 mg kg^{-1} while the health limit for dry human food is only 1 mg kg^{-1} (Naidu et al., 2006); under P deficiency, wheat grains achieve about 3 times lower As concentration than shoots (e.g. Pigna et al., 2009). It might be assumed that on younger tailings sediments, with higher soil As concentrations, such a low soil P status might easily bring about phytotoxic (or potentially toxic for humans and animals) As concentrations in leaves and grains.

A succession of the two functional groups of weeds along the gradient of soil pollution, i.e. the gradient of decreasing AL-P (Figures 2.1, 2.3 and 2.4) promotes the dominance of “pyrite weeds” which are superior to wheat in maintaining P homeostasis on extremely P deficient soils. *Rumex acetosella* and *Agrostis capillaris* do not occur in the predominant Consolido-Polygonetum avicularis association of cereal weeds in the alluvial lowlands of Serbia, but they do appear in the mountainous regions on more acidic and nutrient poorer soils (e.g. Kojić and Vrbničanin, 2002). Moreover, as they are hemicryptophytes which easily invade fields from the surrounding non-cropped successional matrix via stolones and rhizomes, it would be difficult to control them with commonly applied herbicides.

Finally, to understand the reasons underlying differences in success of the two compared soil P extractants (Fig. 2.6), more needs to be known about the adaptations of wheat to low P soils where high Fe replaces high Ca over very short distances. Whether wheat has different efficiency and probably even different mechanisms for accessing either Ca- or Fe/Al-bound P is still not clear (see Bhadoria et al., 2002; Pearse et al., 2007, Rose et al., 2010). For instance, Bhadoria et al. (2002) found that for the same yield, wheat needed about 6 times more AL-P on a Luvisol (predominantly Ca-P) than on an Oxisol (predominantly Al/Fe-P). In alluvial soils furthermore, P dynamics is intrinsically related to flooding and draining cycles (Sah and Mikkelsen, 1986). Rregularly flooded calcareous soils are found to offer favourable condition for P nutrition, presumably because soluble Fe-P also exists beside predominant Ca-P and sparingly soluble Fe-P (Weiss et al., 1991).

In conclusion, though statistical significance of other plausible stress factors has not been detected, their contribution (and possible complex interactions) to wheat growth disorders can not be excluded; so, P deficiency remains the only “visible” component of a black box. Both macronutrient (N, P) problems are not likely to be solved by fertilization, mainly because of a) potential rhizotoxic effects of other multiple factors, such as low pH, Al or Cu, which inhibit root growth and thus nutrient uptake, and b) continuous P sorption by different soil minerals (from Ca to Fe) along the pollution gradient. High soil acidity will be continuously generated by weathering of the substantial residues of sulphides in the soil (and exacerbated by ploughing), leading finally to mobilization and leaching of Cu and probably other micronutrients as well. In early stages of weathering elevated As in leaves and grains and related toxicity risks could also be expected. Although liming might temporarily alleviate the generated acidity, we believe that the only sustainable management option for this waste marginalized area in the long run would be to leave the soil unploughed and also consider restoring of the natural flooding regime.

Acknowledgement

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Chapter 2

3 Pioneers on post-mining land: new insights for spontaneous revegetation from an extreme locality

Nina Nikolic and Reinhard Böcker

Compiled draft of three manuscripts

"Grass is the forgiveness of nature – her constant benediction. Fields trampled with battle, saturated with blood, torn with the ruts of cannon, grow green again with grass, and carnage is forgotten".

(From: "Blue grass" pp. 100-116 in: A Collection of the Writings of John James Ingalls: Essays, Addresses, and Orations. Kansas City: Hudson-Kimberly, 1902)

Abstract

Post-mining wastelands provide an attractive environment to study the fundamental processes of succession, yet data from regions other than Central Europe, which include toxic substrata, are extremely scarce. We have studied the revegetation process on an exclusive model locality created by long-term and large-scale fluvial deposition of sulphidic Cu tailings over arable fields, where no reclamation had been undertaken. Results of soil (21 parameter) and vegetation (species abundance, biomass, foliar analyses) surveys were jointly analysed. In the surrounding of pronouncedly carstic geology and calcicole xerothermic vegetation, plant growth in the polluted floodplain is constrained by a mosaic of extreme acidity, nutrient deficiency and Cu toxicity which occur during weathering of the tailings sediments. We describe a highly patterned, non-random primary colonization, with strong vegetation gradients and explicit spatial segregation of primary vegetation types as a consequence of severe environmental filtering and fluvial mode of pollution deposition. Pioneer species are the well-known colonizers of post-mining sites, with different phytoceonological affiliations, geographic origin, and ecophysilogical adaptations, but they all maintain

homeostasis of leaf P concentrations across the pollution-induced soil gradient. The proposed conceptual model shows that succession relies on novel types of early vegetation which comprise not only novel combinations of species, but also the key role of species which are novel to the affected region, and do not survive outside of the polluted area. We demonstrate for the first time that pollution-induced severe nutrient deficiency can override the well-established importance of both surrounding vegetation and water level gradient for primary succession.

Keywords: primary succession; gradient analysis; post-mining restoration; pyritic Cu tailings; novel ecosystems.

3.1 Introduction

Mining activities have despoiled about 1% of the Earth's surface (Walker, 1999). Barren land where plant growth is constrained by different soil factors is a common post-mining legacy, often posing a challenge for efficient and cheap restoration of the productive use of these soils (Bradshaw, 1997; Bradshaw and Hüttl, 2001). On the other hand, barren post-mining landscapes provide a unique and exciting opportunity to study primary succession, with the high probability of discovering some general ecological principles (e.g. Wiegleb and Felinks, 2001a; Kirmer et al., 2008; Walker and del Moral, 2009). Ecological restoration, an approach which relies on spontaneous vegetation succession, is increasingly seen as a desirable, sustainable alternative to classical technical reclamation measures of anthropogenically degraded land (Pyšek et al., 2001; Prach et al., 2001a; Prach et al., 2007a; Prach and Hobbs, 2008). Understanding of successions is essential to the practice of ecological restoration; knowledge of spontaneous successions can determine the success of restoration measures, the likely time frame, and the key intervention points to achieve the desired vegetation cover (Walker et al., 2007; Prach and Walker, 2011). Despite a large body of theory on vegetation dynamics and succession to rely upon (Wiegleb and Felinks, 2001b; Rebele and Lehmann, 2002; Walker and del Moral, 2003, and the references therein), primary succession on man-made barren land is still insufficiently understood (Johnson et al., 1994; Wiegleb and Felinks, 2001a).

Post-mining primary succession has been most intensively studied on nutrient-poor, acidic substrates created by open cast mining of coal with high amounts of sulphides in Central Europe (German and Czeck mining areas: Prach, 1987; Pyšek and Pyšek, 1988; Pietsch, 1996; Schmiedeknecht, 1996; Pietsch and Schötz, 1999; Schulz and Wiegleb, 2000; Kirmer and Mahn, 2001; Hüttl and Weber, 2001; Wiegleb and Felinks, 2001a,b; Tischew and Kirmer, 2007; Felinks and Wiegand, 2008; Kirmer et al., 2008; etc.). The overriding importance of the surrounding vegetation for spontaneous revegetation of non-toxic barren land has been demonstrated (e.g. Borgegård, 1990; Prach et al., 2001b; Walker and del Moral, 2003; Rehounkova and Prach, 2006; Prach and Rehounkova, 2006; Kirmer et al., 2008). Of the regional species pool, only some were shown to take an important part in primary succession on these post-mining substrates (Prach and Pyšek, 1999); this drove attention to plant functional traits (life form, life strategy, pollination mode, and ability of lateral spread) as tools to predict colonization (Prach and Pyšek, 1999; Pywell et al., 2003; Rehounkova and Prach, 2010). Finally, general conceptual models of primary succession on barren post-mining land have been proposed (e.g. Pietsch, 1996; Schulz and Wiegleb, 2000; Tischew and Kirmer, 2007). However, the correlation of early vegetation development with soil properties could not have been established (Schulz and Wiegleb, 2000; Wiegleb and Felinks, 2001b; Baasch et al., 2009; Alday et al., 2011).

On the other hand, the most intractable issue in post-mining restoration are soil toxicity problems, namely excessive concentrations of plant-available metals, and severely low pH (Bradshaw, 1997). A combination of stress factors involving low pH and high metals is commonly found only on relatively small, distinctive areas around tailing ponds after processing of sulphidic metal ores. Studies of spontaneous revegetation of metalliferous spoils and tailings worldwide are very scarce (Gibson, 1982; Thompson and Proctor, 1983; Bagatto and Shorthouse, 1999; Borden and Black, 2005; Shu et al., 2005; Banásová et al., 2006; Martínez-Riuz and Marrs, 2007). Moreover, even more pronouncedly than with post-coal mining land, majority of studies published so far on spontaneous revegetation of barren land degraded by sulphidic metal tailings were carried out in applied frameworks (focused on management issues for reclamation, or, more commonly, phytoremediation; e.g. Wong, 2003); often they presented only a floristic inventory, and did not aim to interpret the data in the context of succession theory.

This work addresses two major knowledge gaps regarding primary succession on post-mining land, as indicated by Prach and Pyšek (2001) and Prach (2003): 1) extreme conditions which include metal toxicity, and 2) environmental conditions (including the regional species pool) which considerably differ from the Central European. The study was undertaken on an exceptional model locality which combines different soil constraints (Cu toxicity, nutrient deficiency, and low pH), affects a large area, and has been free of any restoration/rehabilitation measures. These unique conditions of a true "field laboratory" made possible the major research question: how would primary succession proceed under extreme environmental filtering, if nutrient-poor, strongly acidic barren soils are created amidst pronouncedly calcareous surrounding dominated by xerothermic calcicole vegetation. This study should enhance the understanding of ecological principles which underlay spontaneous revegetation processes.

3.2 Material and methods

3.2.1 Research locality

Massive sulphide (predominantly pyrite) deposits of copper ore in the Bor metallogenic zone (Timok magmatic complex) in Eastern Serbia, as well as out-of-date processing technology, have made the Bor Copper Mines and the city of Bor one of the four environmental hotspots in the country, affecting also the neighbouring countries Bulgaria and Romania (Wolkersdorfer and Bowel, 2005). The unique research locality has been created during over 50 years of uncontrolled discharge of highly sulphidic Cu tailings into the local river system (the two tributaries of the river Timok in Eastern Serbia). About 40 km (air distance) of the Timok flow (up to its confluence with the Danube) became severely polluted with tailings slurry. With regular floods twice a year, fostered by the bimodal rainfall patter (see Fig. 3.1), the tailings waste had been deposited (via lateral accretionary sedimentation processes) over the cropped alluvial fields in the Timok watershed (for more details see Nikolic et al., 2011; Nikolic and Nikolic, 2012). The pollution ceased allegedly in the early 1980's, while it took another decade approximately for the river bed to clean up, so that flooding waters could start to bring nutrients again instead of mine waste (informal interviews with local land users; official data still not available). Due to complex historic socio-political circumstances, no systematic reclamation of the polluted area has been undertaken. The true extent of the degraded land has never been published; so far, about 10,000 ha of the formerly fertile alluvial soils are still barren, and far larger area is severely degraded.

This study investigated 50 km of the Timok flow affected by the slurry mine discharge. This section is characterized by a) meandering flow; b) pronouncedly braided river pattern (a network of channel running more or less parallel to the main flow); and c) pronounced fluvial dynamics (and related erosion and deposition) and general lack of embankments and flood protection. These factors have contributed to the severity of degradation of the alluvial soils (see Miller, 1997), but also made possible an *ex-post* identification of important gradients (Nikolic et al., 2011; Nikolic and Nikolic, 2012).

The major sampling locations (satellite view available at: http://goo.gl/maps/U4C51) were at the meandering position, where large tracts of barren polluted soils are still easy to find (land belonging to the villages Čokonjar, Trnavac, Braćevac, Tamnič and Rajac); the control samples were from the alluvial fields of the Vražogrnac village, immediately before the polluted tributaries join the Timok. Nowadays, most of spontaneously revegetated land extends in perpendicular direction to the river channel along transects shorter than 1 km. The major land use surrounding the created wasteland is a small scale, subsistence oriented agriculture, where post-harvest burning of fields (though officially banned) is still a common practice.

3.2.2 Soils

The long-term release of sulphidic mine waste has drastically altered the alluvial soils in the Timok watershed: formerly pronouncedly calcareous soils (pH > 7.5; Antonović, 1974) were substituted by nutrient-poor, acidic soils additionally constrained by the excessive concentrations of available Cu or Al (Nikolic et al., 2011; Nikolic and Nikolic, 2012). Depth of the fluvially deposited mine waste ranges from few centimetres to over a metre. As unpolluted acidic soils do not occur outside of the floodplain research area at least within the distance of 10 km (Antonović, 1974), fluvial deposition of mining waste has, first of all, created a remarkable difference of about 4 pH units between polluted and unpolluted soils, what is a very severe filter. Furthermore, previous work, conducted mainly at the outer edge of the polluted area (Nikolic et al., 2011; Nikolic and Nikolic, 2012), demonstrated a gradual, very regular, and spatially explicit increase of the pollution deposition along the transects perpendicular to the river channel, which could very conveniently be indicated by soil S_{tot} concentrations. In general, nutrients (P, Ca, Mg, K), CEC, pH and percentage of clay fraction regularly decrease towards the river channel (Nikolic and Nikolic, 2012).

Finally, the two processes: a) oxidative weathering of pyrites in the tailings deposits (a major issue on sulphide-polluted land, Hüttl and Weber, 2001; Álvarez-

Ayuso et al., 2008); and b) pronounced fluvial geomorphic processes involving dynamic burying and re-exposing of the sediments, have made possible to encounter the two extremes of the tailings weathering at the same time. Namely, no plant growth was found to be possible both on relatively fresh tailings deposits (excessive concentrations of available Cu), and on highly weathered deposits (very low pH, nutrient deficiency, excessive concentrations of available Al; Nikolic and Nikolic, 2012). Normally, these two extremes are expected to be separated in time, but at this exceptional locality it has been possible to study the effect of both of these constraints on revegetation process at the same time, what is an invaluable input for space-for-time inferences. The weathering gradient of the pyritic Cu tailings was not found to be spatially explicit (Nikolic and Nikolic, 2012).

3.2.3 Climate and vegetation

The research area is under both continental and submediterranean climatic influences, characterized by dry hot summers, cold winters, and bimodal rainfall pattern with annual precipitation below 600 mm (Fig. 3.1). The former natural vegetation, prior to conversion to cropland and subsequent pollution by mining effluents, had been azonal alluvial forests of the Populetalia albae Br.-Bl. 1931 order. Nowadays the polluted alluvial land is abandoned, and some occasional cropping takes place only at the outer edge of the polluted area (see Nikolic et al., 2011).

Outside of the fluvial influence, the former agricultural land uses have been increasingly abandoned in the past decades (socio-economic reasons), and the landscape is now dominated by a matrix of different successional stages of fallow vegetation (so far, unfortunatelly uptodate vegetation maps of Serbia are not available). Spontaneous vegetation is represented by different light, xerothermic forests and scrubs of the Quercetalia pubescentis Br.-Bl. 1932 order. Forest association Quercetum frainetto-cerridis Rudski (1940)1949 (*sensu lato*) is a climazonal vegetation of most of Serbian and Eastern Balkan area. In the surrounding of the research area (Timok watershed, up to 30 km from the major research sites), two varieties of this association have been described (Jovanović 1997): termophytic var. geog. Carpino orientalis-Quercetum frainetto-cerridis Jov. 1953 on the south; and monodominant Quercetum frainetto Jov. 1982 on the north (Negotin valley; *Quercus cerris* apparently avoids calcareous soils).

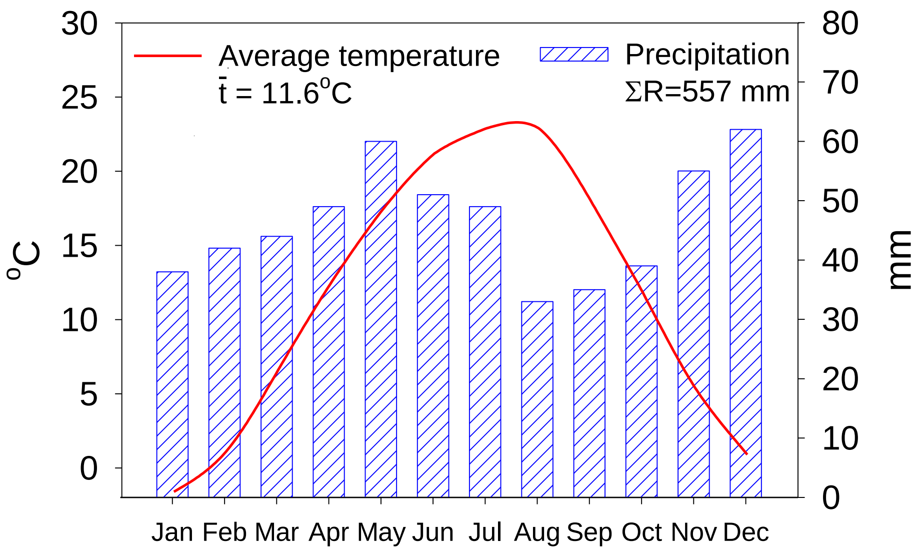

Fig. 3.1: Climatic conditions of the research area. Climate diagram according to Walter: t – average annual temperature; ΣR – total annual precipitation; data for the Meteorological Station Negotin, 1961-1999.

Because of the pronouncedly carstic geology, dissected terrain and shallow calcareous soils on steep slopes which surround the polluted alluvium (Petrović, 1974), the climazonal forest type is however often substituted by more xerothermic, extrazonal, polydominant vegetation of a relict character (Mišić, 1981, 1982). The most developed formation of this type is described as calcicole forest Carpino orientalis-Quercetum mixtum syringetosum Mišić 1967(1981) (Mišić, 1981). An edificatory species of this type of vegetation is *Syringa vulgaris* (relict endemic of the Balkan peninsula). Summer-green, submediterranean scrubs of different stages of degradation/regeneration are the most widespread vegetation type adjacent to the research locality, described in two alliances: Pruno tenellae-Syringion Jov. 1979 and Syringo-carpinion orientalis Jak. 1959 (review in Diklić and Vukićević, 1997; Mišić, 1997). Besides the xerothermic oaks, lilac and oriental hornbeam, the very common species on fallow land within 5 km from the polluted alluvium were observed to be *Fraxinus ornus*, *Paliurus spina-christi*, *Andropogon ischaemum*, *Clematis vitalba*, *Crataegus monogyna*, *Acer monospessulanum*, *Tilia tomentosa*, *Lycium vulgare*, *Echium vulgare*, etc. Moreover, subspontaneous groves of *Robinia pseudoacacia* are also fairly common. Overall, the mosaic vegetation surrounding the research locality is dominated by xerothermic calcicoles, many of which belong to the submediterranean chorotype (Diklić and Vukićević, 1997; Jovanović 1997; Mišić, 1997). It is important to note that acidophytic species of cooler and moister climate and (pseudo)atlantic floral element do not grow in the surrounding of the polluted floodplain; during this study they were not observed at least within the distance of 5 km, and the available literature indicates the absence of such vegetation in far broader surrounding.

3.2.4 Vegetation survey

3.2.4.1 Herbaceous pioneers

Herbaceous pioneers were considered to be only the species which were observed to colonize barren deposits of Cu tailings without any (visible) anthropogenic influence. Most common anthropogenic interference with the revegetation process in the polluted area is the disposal of different kinds of garbage and waste; heaps of these allochthonous materials are observed to sustain the growth of different and unstable species assemblages for the period of up to 3 years. Therefore, to avoid any "unspontaneous" colonization, sampling of herbaceous pioneers included only the localities where the consistent dominance of one species had been previously observed during the period of at least 3 years prior to the survey. Moreover, pioneer stands with the total vegetation cover of less than 30%, estimated in the standardized quadrates of 4 m x 4 m, were not included. Finally, species with less than 3 occurrences were deleted from further analyses.

Sampling strategy thus comprised firstly stratification according to the dominant pioneer species, and subsequent flexible systematic sampling according to the visual appearance of the vegetation (Smartt, 1978). The sampling was thus essentially preferential (*sensu* Botta-Dukát et al., 2007). Each sample consisted of a one 1 m × 1 m quadrate laid for destructive sampling; aboveground biomass was clipped, sorted by species, air dried and weighted (hence expressed as shoot dry weight, DW). Sampling was undertaken in the period end of May – beginning of July. The internet associated W3 TROPICOS nomenclatural database of the Missouri Botanical Garden, and the associated authority files (http://www.tropicos.org/Home.aspx), were used as reference.

3.2.4.2 Pioneer forests

Two distinct types of pioneer forests occurring on the alluvial land degraded by sulphidic Cu mine waste deposits were surveyed, totalling 25 forests dominated by silver birch (*Betula pendula*) and aspen (*Populus tremula*), and 23 forests dominated by two poplar species (*Populus alba* and *Populus nigra*). The selection of forest localities was opportunistic, with the main criteria being the absence of the visible anthropogenic influence (logging, burning, waste/garbage deposition, and bank fortification for flooding control). The selection of sampling units for vegetation survey in each locality was random. It should be noticed that some other types of forest vegetation occur throughout the area affected by the tailings deposition (most notably the stands dominated by *Robinia pseudoacacia*), but these are severely modified by anthropogenic influence, and as such are not included in this study. Moreover, while the pioneer character (colonization of the barren land) of the *Betula pendula-Populus tremula* stands was beyond any doubt and could be observed throughout the research area, the proofs for the pioneer character of poplar forests were more circumstantial. Because of the lack of the historic land cover data, we had to rely on the participatory interviews with the local land users about the historic extent of the barren polluted soil which is now under *Populus alba-Populus nigra* stands.

3.2.4.3 Forest vegetation survey

Forests were surveyed in the standardized 25 m × 25 m quadrates in each sample unit in the period May - July when most if the herbaceous species was in flowering. Cover-abundance value of each species was estimated on the extended nine-grade Braun-Blanquet scale (van der Maarel, 2007). Cover-abundance of each species was estimated as its summary cover-abundance in all the three forest vegetation layers. For the subsequent multivariate analyses, species not achieving the frequency of at least 15% in one forest type were excluded. Tiny species with very low abundance and/or only few small individuals ("r" class on the Braun-Blanquet scale) were not excluded. Plotless sampling, the point-centered quarter method (Cottam and Curtis, 1956), was used to assess the forest structure parameters. In brief, the distance to the closest tree (and its DBH – diameter at breast height) in each of the four quadrants centered around each of the 10 random sampling points was measured in every surveyed forest. As "trees" were considered all the encountered tree species with the DBH > 4 cm. Additionally, line intercept method along the transects running from the outer forest edge (towards the unpolluted zone) to the inner forest edge (towards the pollution source, i.e. the river channel) was used to assess the DBH of the pioneer species only (*B. pendula*, *P. tremula*, *P. alba* and *P. nigra*) in both forest types.

3.2.5 Leaf analyses

A composite sample of ten young, fully developed leaves was collected from each herbaceous pioneer species in every sample (i.e. in each 1 m × 1 m quadrate were aboveground biomass was destructively sampled) for mineral analyses. All the investigated pioneer species were sampled in flowering time, except *Chenopodium botrys*, which flowers later; therefore, *Ch. botrys* was resampled again in September, but no significant differences in leaf mineral composition were found. Plant tissue samples were thoroughly washed with deionised water, dried at 70 °C and digested with a mixture of HNO_3 and H_2O_2 (3:2) in a microwave oven. Upon digestion, the concentrations of mineral elements were determined by inductively coupled plasma optical emission spectrometry (ICP-OES; SpectroGenesis EOP II, Spectro Analytical Instruments GmbH, Kleve, Germany).

3.2.6 Soil sampling and analyses

In herbaceous pioneer stands, rhizosphere soil was collected together with biomass and plant tissue sampling. Pioneer species encountered in each sample (1 m x 1 m quadrates) were excavated, coarse soil was shaken off, and only soil in the zone of 2 mm along the root was collected for each species. Bulk soil samples in herbaceous stands were collected from the depth of 0-20 cm in each quadrate (rooting depth). Likewise, bulk soil in forest stands was sampled from the depth 0-40 cm as a composite sample consisting of 5 subsamples in each surveyed forest (i.e. in each 25 m × 25 m stands). Barren tailing sediments (relatively fresh and highly altered) were sampled as described by Nikolic and Nikolic (2012).

Soil samples were analysed in fine earth fraction (< 2 mm), after drying and sieving through a 2 mm mesh screen. pH was measured in water (soil : water = 1:2.5), soluble $CaCO_3$ was determined by calcimetry, and the concentrations of total C, N and S by the CHNOS elemental analyzer. Organic carbon (C_{org}) was calculated from total C and $CaCO_3$. Potential CEC was determined by ammonium acetate extraction buffered at pH 7 (with ethanol treatment adjusted for salty and carbonate samples). Different extraction procedures were applied to determine plant available concentrations of elements: ammonium lactate – ammonium acetate (AL) for P and K, ammonium acetate (NH_4-Acc) for Mg and Ca, KCl extraction for available Al, and hot water (70 °C) for B. Plant available fractions of metals (Fe, Cu, Zn and Mn) were extracted by the DTPA-TEA solution (buffered at pH 7.3), with the soil: solution ratio of 1:5 (Norvell, 1984).

The concentrations of chemical elements in soil samples subjected to different extraction procedures were determined by ICP-OES, with the exception of the P concentrations which were determined colorimetrically at 882 nm.

3.2.7 Statistical analyses

3.2.7.1 Ordination

All the multivariate analyses were done by the Nonmetric Multidimensional Scaling (NMS) with the PC-ORD 6 software (MjM Software Design, Gleneden Beach, USA). NMS is a robust, non-parametric ordination method based on rank distances, well suited for community data which are frequently non-normal, or are on discontinuous or arbitrary scales. The major advantages of the NMS over other commonly used ordination methods (Clarke, 1993) are: a) it avoids the assumption of linear relationship among variables; b) it relieves the “zero truncation problem” (see Beals, 1984), which is an issue in all ordinations of heterogeneous community data sets; and c) it is distance-preserving and allows any distance measure or relativization. NMS is a form of indirect (i.e. unconstrained) gradient analyses, where the sampling units are ordered solely by the structure in the response matrix. For the vegetation data, thus, sampling units are ordered solely according to the covariation and association among the species. Environmental variables (soil data) were passively projected (overlaid) on the ordination scores to produce joint plots, which visualize the linear relationship between soil variables and ordination axes. Overlaid soil variables radiate thus from the centroid of ordination scores; the angles and lengths of the radiating lines indicate the direction and strength of relationships of the soil variables with the ordination scores. The values in parenthesis denote the proportion of variance represented by each axis.

For the normal ordination (samples in species space) the Sørensen distance (city block, proportion-based) was used. This semi-metric distance measure is relatively robust to outliers, and ignores joint absences (asymmetrical approach). For the search for patterns in soil properties, samples were ordinated based on Euclidean distance. To bring the soil parameters, measured at different scales, at the equal footing for ordination, relativization by adjusting to standard deviate was applied. Biomass data were log-transformed by using the generalized log transform formula [$b = \log(x+x_{min}) - \log(x_{min})$] to deal with zeroes. This transformation tends to preserve the original order of magnitude in the data, and results in zero when the initial value was zero. The abundance-dominance data recorded on nine-grade Braun-Blanquet scale were transformed to a quasi-metric 1-9 ordinal transform scale of ordinal transform values (OTV; van der Maarel, 2007) which roughly correspond to the logarithm of percentage cover.

The number of significant NMS ordination axes to be interpreted was determined by testing the stress in relation to dimensionality by Monte Carlo randomization test with 250 runs. “Stress” is the measure of departure from monotonicity in the relationship between the distances in the original n-dimensional data matrix, and the distances in the reduced k-dimensional ordination space (McCune et al., 2002). This non-parametric test essentially compares the degree of stress in real data to the degree of stress in randomized data, and selects the highest dimensionality of ordination solution for which the stress is lower than for the 95% of the randomized runs (i.e. the probability of Type I error (p) < 0.05).

3.2.7.2 Habitat modelling

Variation in performance of the five herbaceous pioneer species was related to the measured soil parameters using habitat models. Multiplicative habitat modelling was done by Non-Parametric Multiplicative Regression (NPMR) with the HyperNiche software (MjM Software Design, Gleneden Beach, USA). NPMR (see review in McCune, 2006) is a new, robust approach which accommodates any type of species response (need not be defined in advance) to multiple habitat factors (i.e. to the situation when effect of one factor is influenced by the level of another, what is a common “real world” situation). NPMR was performed using Local Mean with a Gaussian weighting function (i.e. kernel density estimation); species response matrix contained biomass data relativized by total plot DW. Conservative overfitting control, better suited for the clumped data, was applied.

3.3 Results

3.3.1 Overview: soil gradients and land cover

The spontaneous revegetation on this exceptional locality created by the long term fluvial deposition of sulphidic Cu mine tailings proceeds on a mosaic of different soil constraints between the two extremes (relatively fresh tailings sediments, and highly weathered tailings sediments) where no plant growth is possible.

More than 50 years after the onset of pollution, and more than 18 years after cessation of the pollution input, the four major land cover types have developed on the barren ground (Fig. 3.2). The soils affected by the pollution still drastically differ from the "control" alluvial soils sampled before the tributaries carrying tailings waste join the Timok. In general, the majority of these degraded soils are poor in available nutrients and soil organic matter, have low CEC, and are additionally constrained either by low pH or high available Cu.

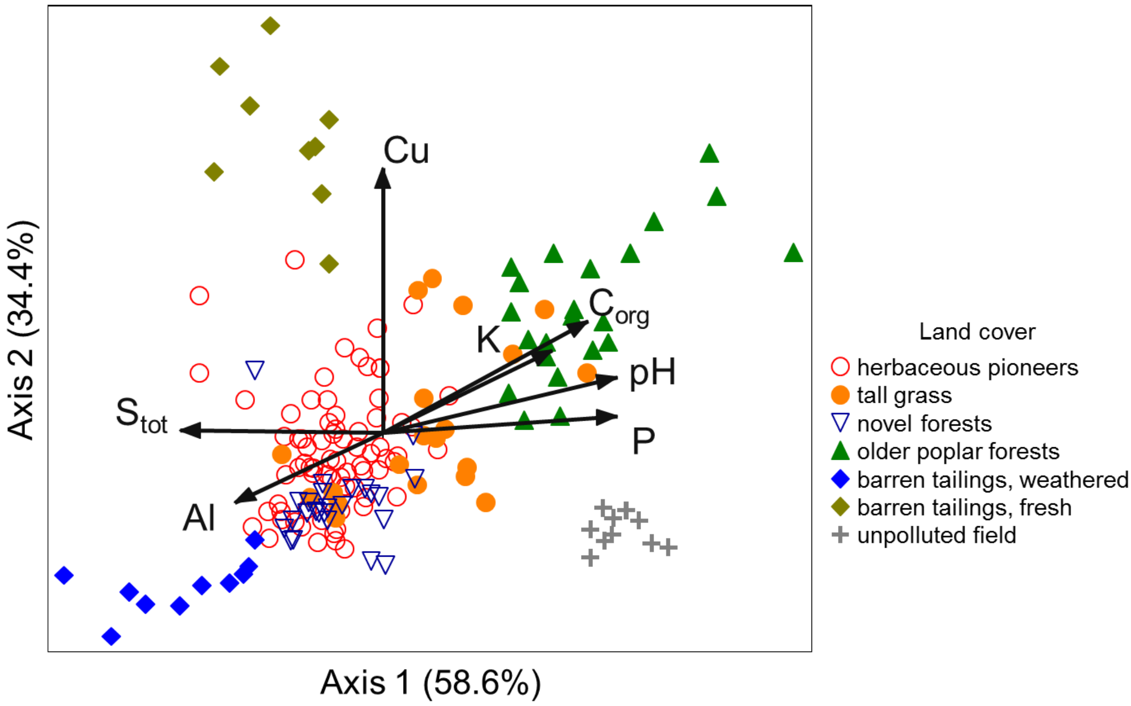

Fig. 3.2: Major gradients (NMS ordination) in soil properties of the land cover types occurring during the spontaneous revegetation of the alluvial land degraded by fluvial deposits of pyritic Cu-tailings. Data matrix: 171 soil samples, 21 soil chemical parameters (relativized by adjusting to standard deviate), Euclidean distance, varimax rotation; final stress for 2-d solution 12.94; ordination rotated by -30°. Parameters presented are correlated with the ordination scores by more than 40%; plant available concentrations of Cu, Al, P and K are shown. Herbaceous pioneers: stands dominated by *Rumex acetosella* and *Agrostis capillaris*; tall grass: strands dominated by *Calamagrostis epigeios*; novel forests: young formations of *Betula pendula* and *Populus tremula*; older poplar forests: *Populus alba-Populus nigra* forests at the outer edge of the polluted area.

Vegetation types are clearly ordered along the two main soil gradients: available Cu concentrations, and S_{tot} – pH (nutrients) along the spatial gradient. Ordination (Fig. 3.2) shows a large overlap of chemical properties of polluted soils colonized by different vegetation; the soils under the densest regrowth (poplar forests at the outer edge of the polluted area) are prominently set apart by the highest pH and available nutrients. On the other hand, soils colonized by the pioneer forests of birch (*B. pendula*) and aspen (*P. tremula*) show least variability in chemical constraints. The herbaceous stands formed by the two other pioneer species (*Persicaria lapathifoila* and *Chenopodium botrys*) are highly restricted to the specific microhabitats (less than 40 m^2 and 20 m^2) with patchy distribution, and their overall impact on the revegetation process is considerably smaller.

3.3.2 Herbaceous pioneers

3.3.2.1 Habitat preferences: non-random colonization

Only five herbaceous species were observed to be able to spontaneously colonize the barren deposits of pyritic Cu-tailings and to maintain dominance in the pioneer stands during at least 3 years: *Rumex acetosella*, *Agrostis capillaris*, *Calamagrostis epigeios*, *Persicaria lapathifolia* and *Chenopodium botrys* (Table 3.1). Although the five pioneer species might start the colonization of the barren ground together (see Fig. 3.3A), they achieve rather discrete dominance in early successional vegetation types (Table 3.2; Fig. 3.4). The matrix of the early herbaceous communities in the polluted region is however undoubtedly dominated by only two species, *Rumex acetosella* and *Agrostis capillaris*. Flowering of *R. acetosella* at the end of May and of *A. capillaris* in July are the only two distinct aspects of spontaneous vegetation in the area affected by mine tailings deposition (see Fig. 3.3B).

Despite a considerably overlapping chemical properties of the polluted soils undergoing primary succession of vegetation (Fig. 3.2), the pioneers tend to achieve dominance along different multiple soil gradients (Fig. 3.5). In particular, the clear “preferences” of particular pioneer species for certain combinations of soil properties could be demonstrated (Fig. 3.5). For instance, *P. lapathifolia* and *Ch. botrys* stands harbour the smallest number of species, and effectively exclude other pioneers (Tables 3.1 and 3.2). The former becomes dominant at higher concentrations of available Al and Fe, and lower sand fraction then other pioneers (Fig. 3.5). The latter is consistently demonstrated to dominate well-drained (higher sand proportion, lower available Fe concentrations), non-acidic (higher pH and in particular the absence of available Al) microhabitats close to the river channel (higher S_{tot}), with the highest concentrations of available metals (Cu and Zn are shown; Fig. 3.5).

Table 3.1: Overview of the major 5 types of pioneer vegetation on the barren land polluted by the fluvially deposited Cu tailings.

Parameters	Pioneer vegetation dominated by				
	Rumex acetosella	*Agrostis capillaris*	*Persicaria lapathifolia*	*Chenopodium botrys*	*Calamagrostis epigeios*
No. of samples	27	31	17	20	20
Total species no.	64	62	38	40	65
Average species no. per m^2	9.4	8.0	7.8	6.7	14.0
Average aboveground biomass (g DW m^{-2})	183.6	199.4	173.2	82.9	546.6
Average within-group distance (Sørensen)[a]	0.53	0.46	0.47	0.50	0.37

[a] group is defined by the dominant pioneer species

Table 3.2: Average share of pioneer species in the aboveground biomass in the major types of herbaceous pioneer vegetation occurring on the polluted soils.

Pioneer species	Type of pioneer vegetation stands				
	Rumex	Agrostis	Persicaria	Chenopodium	Calamagrostis
	Average rel. contribution to aboveground biomass per m^2 (%)				
R. acetosella	49.8	19.9	23.3	9.8	3.9
A. capillaris	18.5	56.3	14.3	21.9	10.1
P. lapathifolia	8.0	4.1	49.5	1.8	0.24
C. botrys	1.0	2.6	0.7	49.1	0.6
C. epigeios	1.8	5.8	0.2	2.0	67.4

This is well in accordance with the observed habitats of these two pioneers on the polluted soils: *P. persicaria* is found to dominate in microdepressions where water might stagnate for some time, while *Ch. botrys* preferentially colonizes sandy banks where tailings sediments have apparently been recently exposed due to a pronounced fluvial dynamics, and regular floods with calcareous water help maintain relatively high soil pH. Increased P-availability in soils of *Chenopodium botrys* appears to be the consequence of lower P sorption to Al and Fe (hydr)oxides at increased pH and not of increased P pool *per se* (based on data on total P concentrations, not shown).

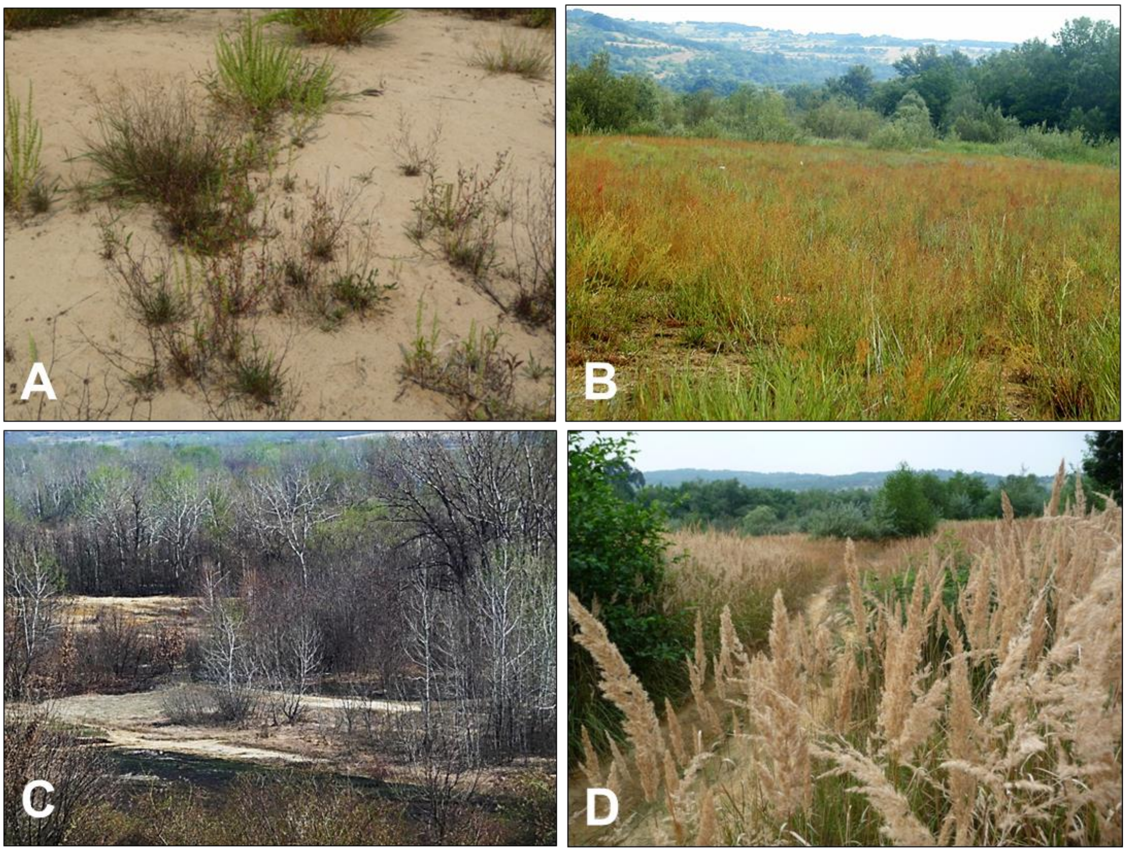

Fig. 3.3: Herbaceous pioneers colonizing the barren land polluted by fluvial deposition of sulphidc Cu tailings. A – *Rumex acetosella*, *Agrostis capillaris*, *Persicaria lapathifolia* and *Chenopodium botrys* starting together; their niches broadly overlap, but they achieve dominance under distinctive soil constraints; B – the two locally novel species, *R. acetosella* and *A. capillaris* commonly grow together and dominate the revegetation matrix; C – burning of the nearby fields unwittingly affects the vegetation succession on the polluted land; D – fires promote the spread of the clonal *Calamagrostis epigeios*.

On the other hand, stands dominated by *C. epigeios* (highest biomass production, overwhelming dominance of one species, Table 3.1 and Table 3.2; Fig. 3.4) are distinguished by significantly higher N_{tot} and C_{org} (Fig. 3.5); these soil improvements might well be a consequence of *C. epigeios* growth over years. Likewise, the increased available K in *C. epigeios* soils coincides with frequent fires this vegetation type is prone to (see also Fig. 3.3C and D). Uncontrolled fires were observed to spread from crop fields at the outer edge of the polluted area mostly in late autumn; dry standing biomass of *Calamagrostis* catches it easily, contrary to the stands of *Rumex* and *Agrostis* (personal observation, not shown). The early vegetation types on "wetter" habitats (stands of *R. acetosella*, *A. capillaris* and *P. lapathifolia*) are clearly distinguished from the "drier" habitats of *C. epigeios* (encountered further from the river channel) and *Ch. botrys* (encountered exclusively on well-drained locations) by the consistently higher concentrations of DTPA-extractable Fe in the rhizosphere soils (Fig. 3.5).

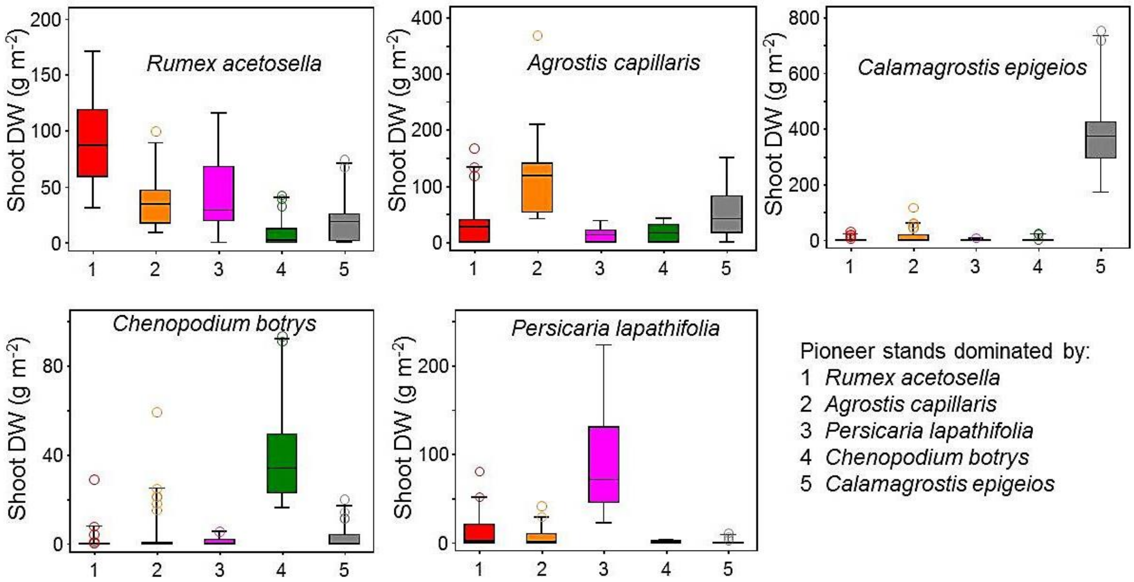

Fig. 3.4: Shoot biomass of five herbaceous pioneer species across the early vegetation types they dominate. Box-plots (scaled in percentiles) outline the frequency distribution of shoot dry weight in different pioneer stands; outliers deviate by more than 1.5 interquartile range.

The modelled responses of the pioneer colonizers to the key soil gradients are shown in Fig. 3.6. In the early vegetation stands, the abundance of pioneers is not only determined by the multiple soil constraints, but also by competition with other species (other pioneers, but also species encroaching the colonized microsites through facilitative interactions, see Fig. 3.13; Fig. 3.14; and Fig. 3.15).

The relative abundance of *A. capillaris* (Fig. 3.6) does not decrease with the growing concentrations of the major soil constraints (available Cu and Al), but is severely supressed by the competition at better sites characterized by increased N_{tot} (but also other nutrients which are not shown). The other clonal grass, *C. epigeios*, on the contrary increases in dominance with the increasing availability of nutrients, primarily K (which is a common indicator of recent fires). *Calamagrostis* shows less tolerance to the increased Cu concentrations, but appears to tolerate higher available Al levels.

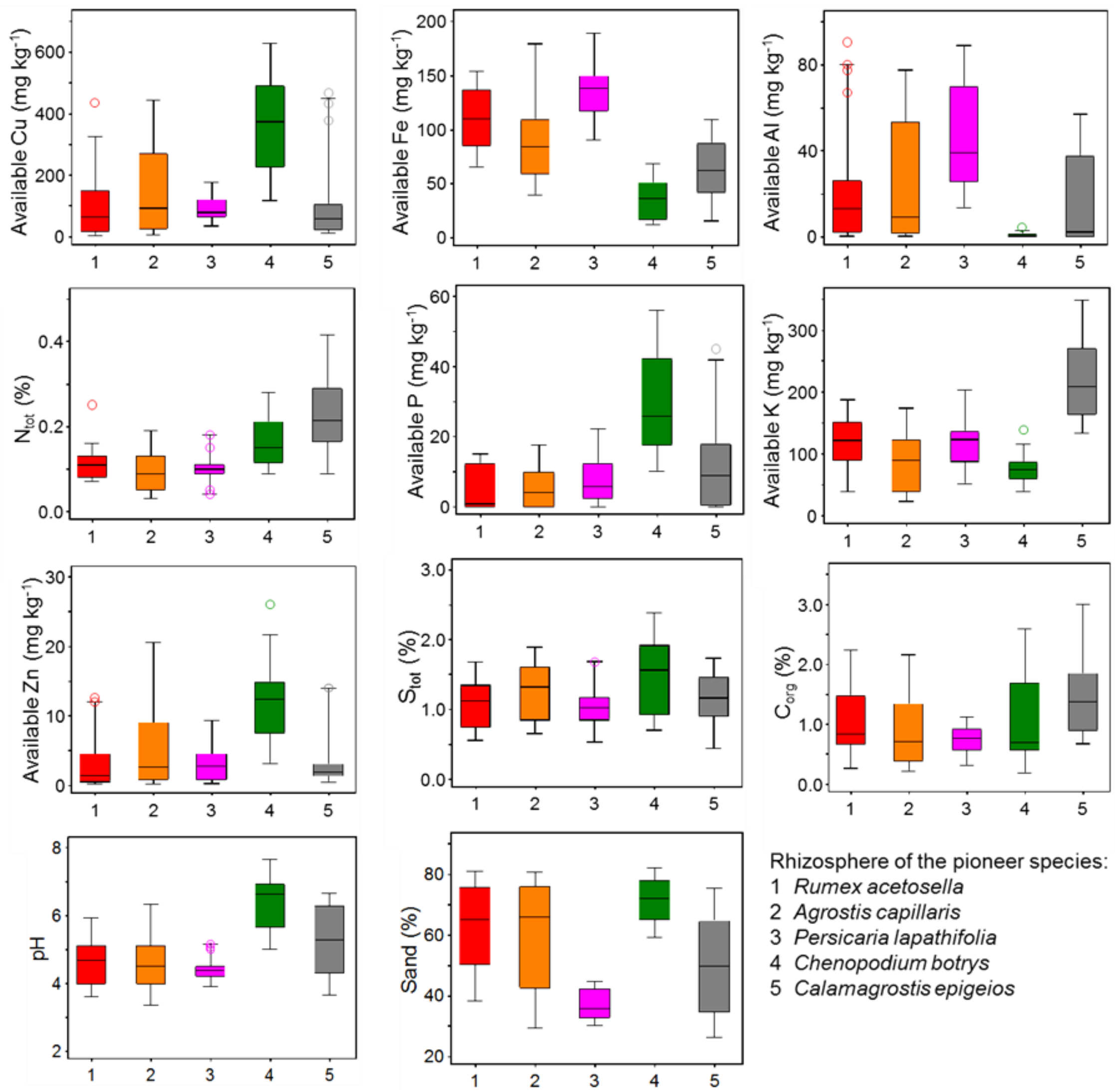

Fig. 3.5: Selected parameters in the rhizosphere soils of the five herbaceous pioneer species which colonize barren deposits of mine waste. Soil was collected from the 2 mm zone along the root. Box-plots (scaled in percentiles) outline the frequency distribution of each soil variable in the samples. Values deviating by more than 1.5 times interquartile range are marked as outliers. Plant available concentrations of mineral elements were determined by different extractions: DTPA for metals, ammonium acetate-ammonium lactate (AL) for P and K, and KCl for Al. Soil variables presented are correlated by > 10% with the ordination of pioneer vegetation stands.

R. acetosella, also a clonal species, dominates at low and medium levels of available Cu, and is (like *A. capillaris*) strongly supressed by competition with the growing availability of nutrients, primarily P. *Rumex* also increases dominance with the increasing available Fe (i.e. with the increasing proximity to the river channel), until at microdepression localities it gets substituted by *Persicaria lapathifolia*. *P. lapathifolia* is consistently present along the gradients of the both major soil constraints (available

Cu, but also available Al which is not shown) with very low abundances; it dominates only at very specific micro-locations where other species cannot survive. Finally, *Ch. botrys* clearly dominates non-acidic sites with the extreme concentrations of available Cu. Irrespectively of the Cu levels, this species avoids low pH (and high Al, not shown) spots. At pH about 5-6 *Chenopodium* slightly increases in dominance (nutrient-poor sites, *Ch. botrys* admixed with other pioneers), while at higher pH and not extreme available Cu concentrations it is severely supressed by competition with other immigrating species.

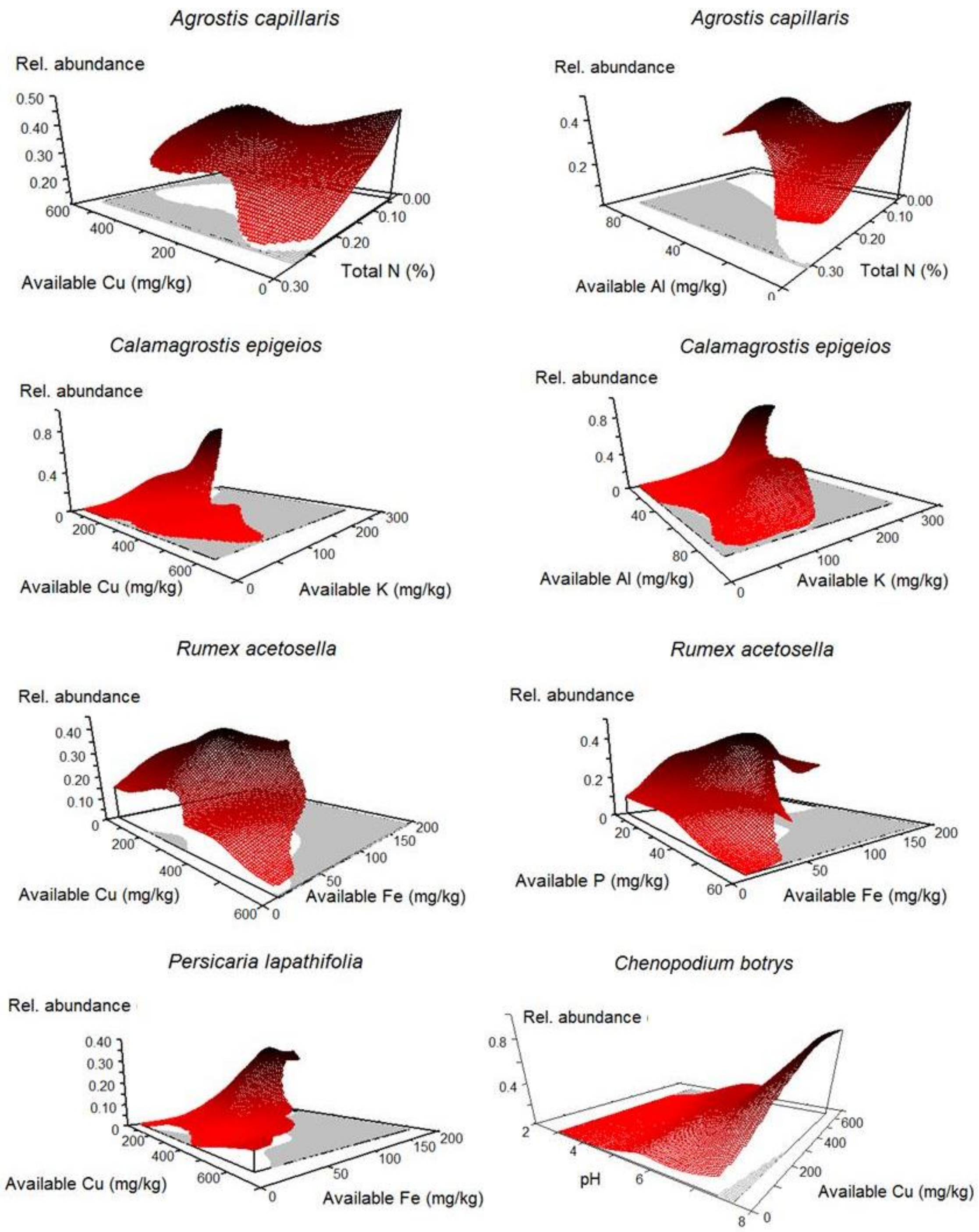

Fig. 3.6: Responses of the pioneers to the key soil parameters, modelled by Non-Parametric Multiplicative Regression (NPMR). Relative abundance – share of the species in the total aboveground DW per m^2, analysed across 115 relevées.

3.3.2.2 Adaptations to multiple abiotic stresses

Herbaceous pioneers colonizing the barren deposits of mine tailings are a heterogeneous group of plants with very different origin in the research area. Interestingly, *R. acetosella* and *A. capillaris*, the two key species of spontaneous revegetation, are observed to be locally novel species; they have been encountered neither on the unpolluted alluvial soils, nor in the non-flooded surrounding vegetation in the radius of 5 km from the sampled polluted area. Yet, these two species, so far, apparently dominate the successional matrix of spontaneously regenerating vegetation on this abandoned, severely degraded land. The other three herbaceous pioneers are not found to be limited to the polluted soils: *C. epigeios* is frequent in the fallow vegetation on the non-flooded surrounding, while *P. lapathifolia* and *Ch. botrys* are sporadically found on the non-polluted alluvial soils in the Timok watershed. The life form of the pioneers is different: *R. acetosella*, *A. capillaris* and *C. epigeios* are hemicryptophytes with dominant vegetative reproduction, while *P. lapathifolia* and *Ch. botrys* are annual therophytes.

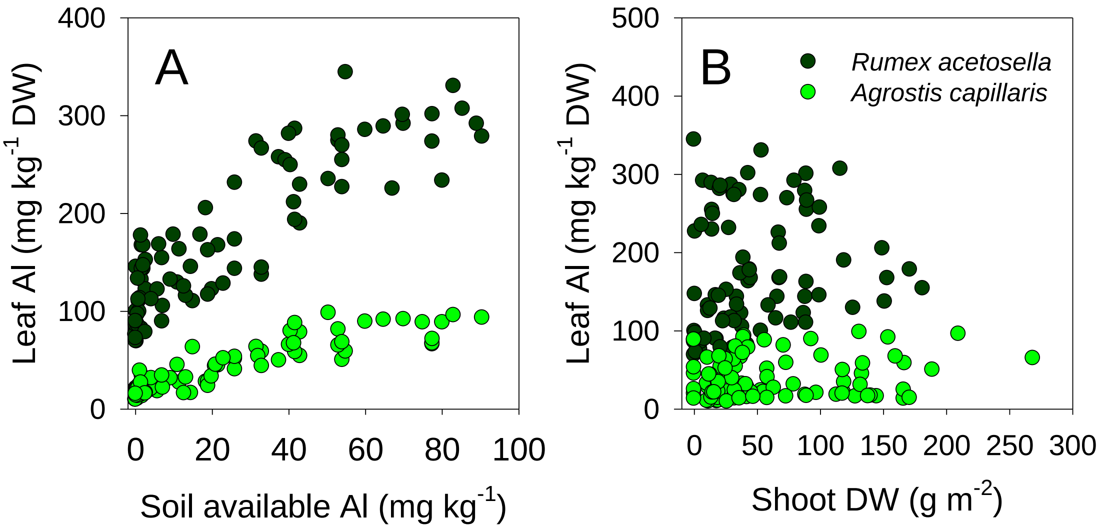

Fig. 3.7: The two main pioneer colonizers have contrasting adaptations to excessive concentrations of available Al in the polluted soils.

Likewise, the pioneer colonizers appear to have different adaptation strategies to the major soil stresses (excessive concentrations of available Cu and Al, severe P deficiency). The two major species in the spontaneous revegetation process, *R. acetosella* and *A. capillaris*, share similar habitats and tolerate excessive concentrations of available Al (see Fig. 3.4, Fig. 3.5; and Fig. 3.6). *R. acetosella* accumulates Al in shoot, and *A. capillaris* effectively excludes it (Fig. 3.7A); at the same time, the growth of both species is not affected by the excessive Al (Fig. 3.7B). *C. epigeios* has similar adaptation to high available Al as *A. capillaris* (not shown).

Although all the five pioneers tolerate high concentrations of available Cu in the polluted soils (Fig. 3.5 and Fig. 3.6), their adaptation strategies widely differ (Fig. 3.8 and Fig. 3.9). *R. acetosella* and *A. capillaris* (Fig. 3.8A and C), as well as *P. lapathifolia* (Fig. 3.9C) show a distinct biphasic pattern of Cu uptake and translocation to shoots, albeit with very different saturation levels. *C. epigeios*, on the other hand, shows the most efficient Cu exclusion strategy (Fig. 3.8E). In contrast, *Ch. botrys* has an almost linear Cu uptake pattern, achieving extremely high leaf Cu concentrations and no apparent saturation so far (Fig. 3.9). Furthermore, *Ch. botrys* is the only pioneer species whose growth is not negatively affected by elevated Cu uptake (Fig. 3.9B).

In the conditions of multiple abiotic stresses on these polluted soils, leaf P status is not solely a consequence of low soil P status. Leaf P concentrations reflect a "black box" result of a of all the putative rhizotoxic effects (e.g. low pH, high Al and Cu, see Fig. 3.5) which hamper root functioning and can lead to a restricted nutrient uptake. Leaf P concentrations along the gradients (Fig. 3.10 and Fig. 3.11) show a remarkable adaptation of all the pioneer species to P deficiency. Primary succession on these multiply constrained soils with low available P (see Fig. 3.5) starts with species capable to effectively maintain leaf P homeostasis (Figs. Fig. **3.10** and Fig**. 3.11**). Only *Ch. botrys*, normally dominant at higher soil P levels than other pioneers (Fig. 3.5) is supressed by severe P deficiency (Fig. 3.11). While the relation between leaf P status and relative abundance is not so clear for clonal species (Fig. 3.10), and *Ch. botrys* avoids extremely low P sites, *P. lapathifolia* shows low relative abundance at both ends of the leaf P gradient (Fig. 3.11): at the very low leaf status the vitality decreases, while at high leaf P it gets competitively supressed by other species.

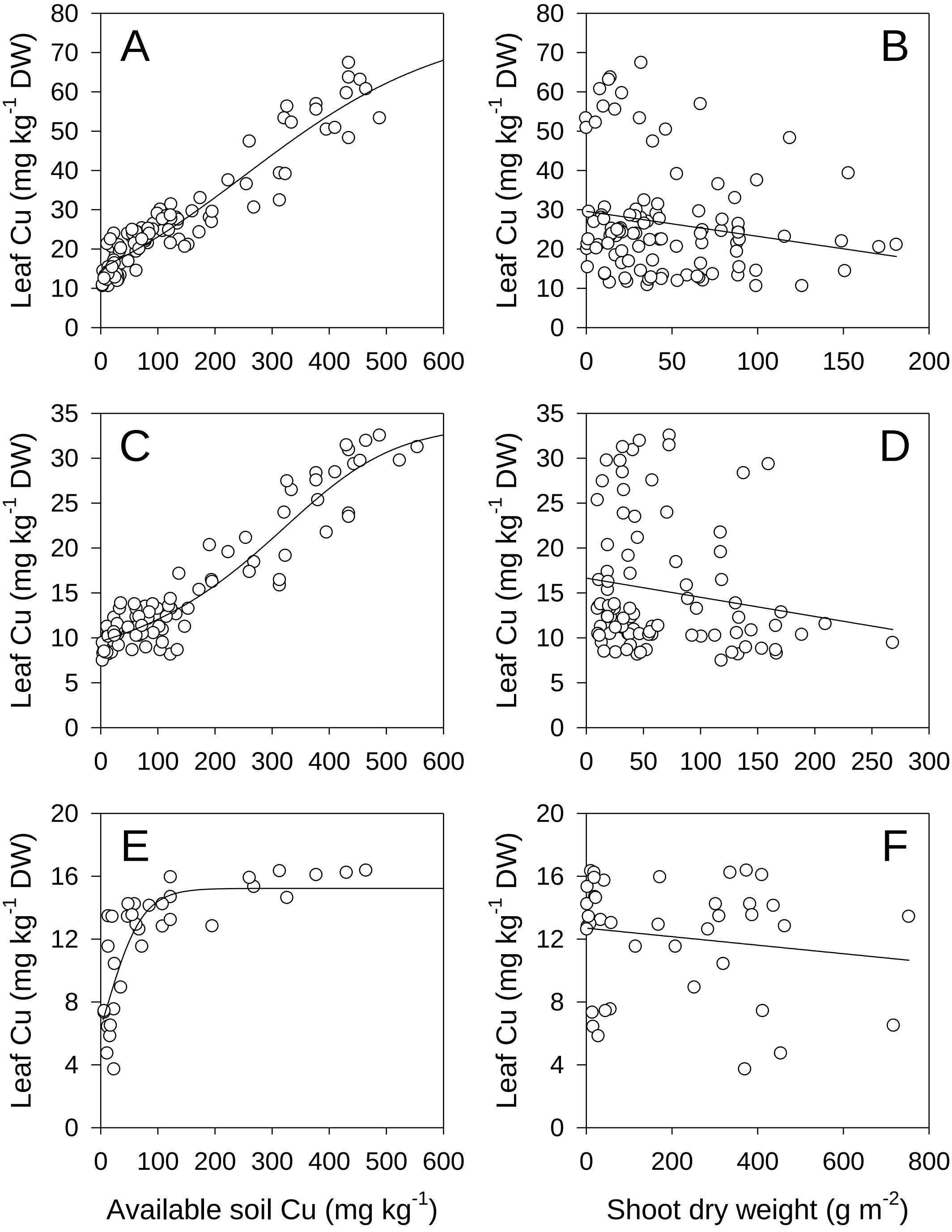

Fig. 3.8: Response of the clonal pioneer species to the gradient of available Cu in the alluvial soils polluted by mine tailings. A, B – *Rumex acetosella*; C, D – *Agrostis capillaris*; E, F – *Calamagrostis epigeios.*

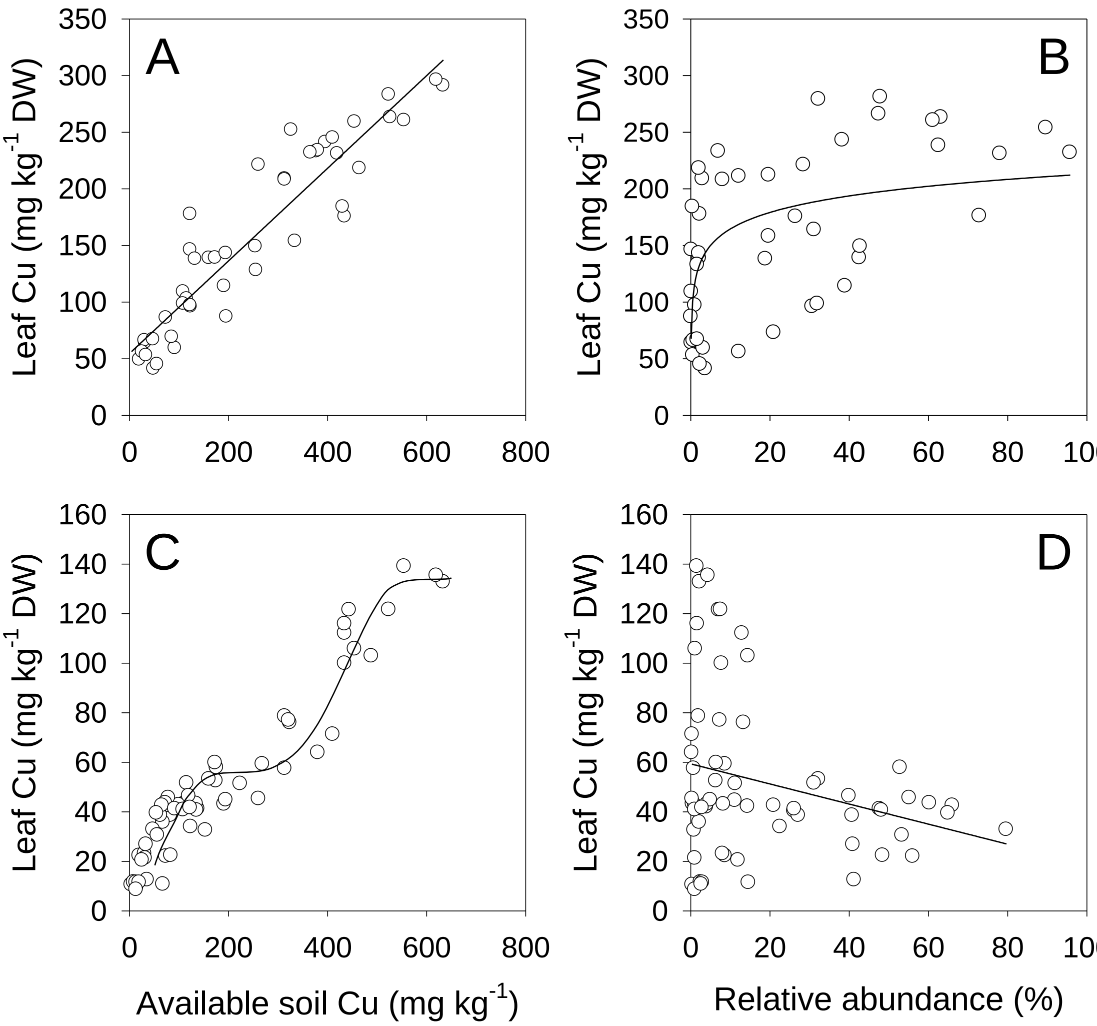

Fig. 3.9: Response of the therophytic pioneer species to the gradient of available Cu in the alluvial soils polluted by mine tailings. A, B – *Chenopodium botrys*; C, D – *Persicaria lapathifolia*. Relative abundance – species' share in the total aboveground DW m^{-2}.

Leaf Cu concentrations in *R. acetosella*, *P. lapathifolia* and less pronouncedly in *A. capillaris* might increase 3 times with increasing soil levels of the available Cu fraction (Fig. 3.8A and C; Fig. 3.9C), but this increase is accompanied by a severe growth inhibition (expressed as shoot biomass or relative abundance, Fig. 3.8B and D; Fig. 3.9D), while *Ch. botrys* achieves and maintains dominance at extremely high soil and leaf Cu levels (Fig. 3.9B).

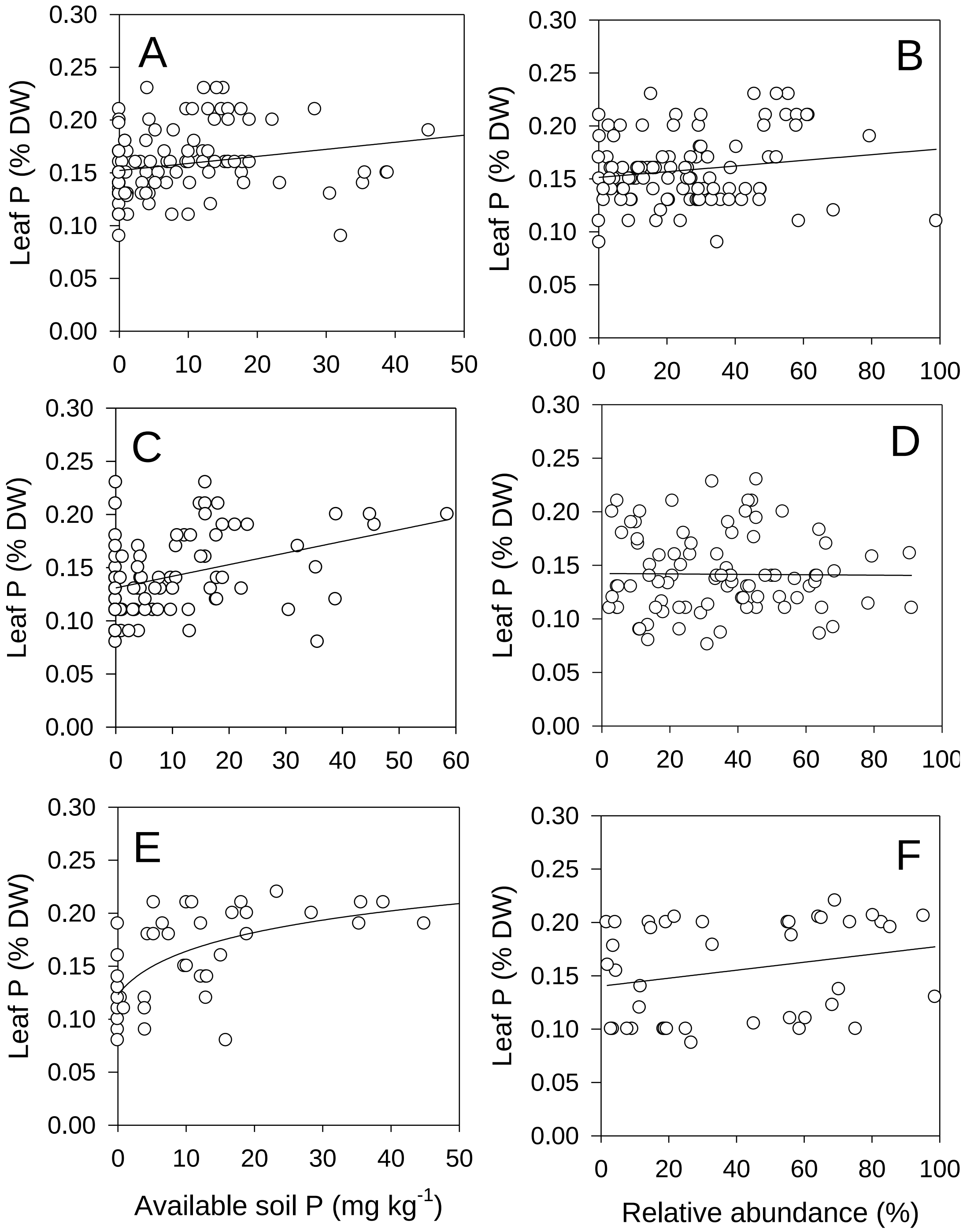

Fig. 3.10: Leaf P homeostasis along the gradients of soil available P and species dominance in the clonal pioneers colonizing the barren polluted alluvial soils. A, B – *Rumex acetosella*; C, D – *Agrostis capillaris*; E, F – *Calamagrostis epigeios*. Relative abundance – species' share in the total aboveground DW m^{-2}.

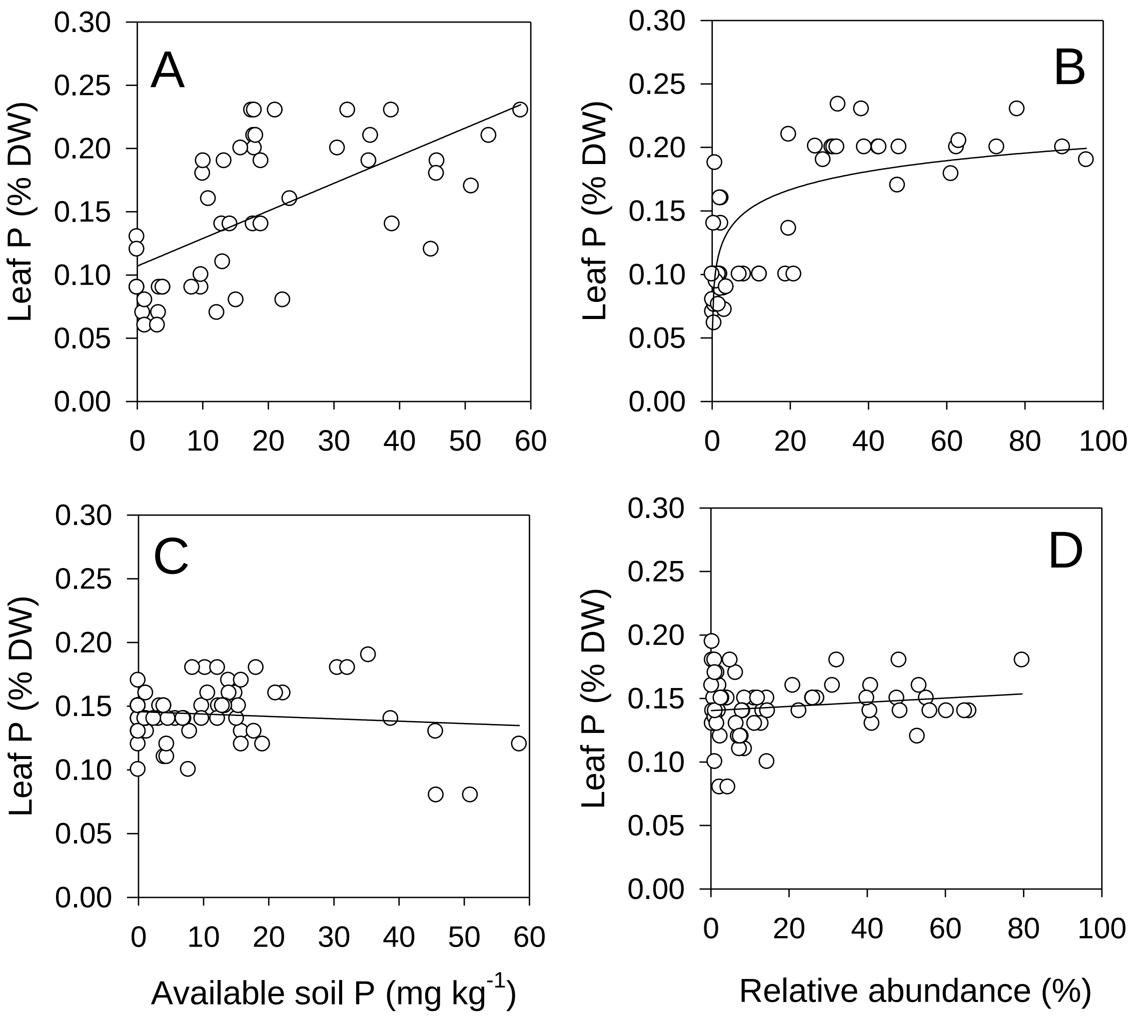

Fig. 3.11: Leaf P homeostasis along the gradients of species dominance and soil available P in the therophytic pioneers colonizing the alluvial soils polluted by mine tailings. A, B – *Chenopodium botrys*; C, D – *Persicaria lapathifolia*. Relative abundance – species' share in the total aboveground DW m^{-2}.

3.3.2.3 Early successional trends

The process of facilitated succession has led, over the period of at least 3 years, to the immigration of a total of 70 additional species to the initial stands of the five pioneers (*R. acetosella*, *A. capillaris*, *C. epigeios*, *P. lapathifolia* and *Ch. botrys*) on the barren deposits of mine tailings. About 27% of these accompanying species occur in all the five types of early herbaceous vegetation, while the three most similar types (dominated by *R. acetosella*, *A. capillaris*, and *P. lapathifolia*) share about 43% of the accompanying species. Unconstrained ordination (Fig. 3.12) shows three strong gradients which capture 83% of variation in these early vegetation stands. The gradients in species composition of the early vegetation are significantly correlated with the measured soil parameters (Fig. 3.12; Table 3.3). On the other hand, S_{tot} clearly indicates the relative position of a sample along a transect perpendicular to the river channel; higher S_{tot} indicates that a sample of early vegetation was taken at a position closer to the pollution source (i.e. to the river channel), while lower S_{tot} concentrations are encountered towards the outer edge of the polluted area, i.e. towards cropped fields on the unpolluted adjacent soils.

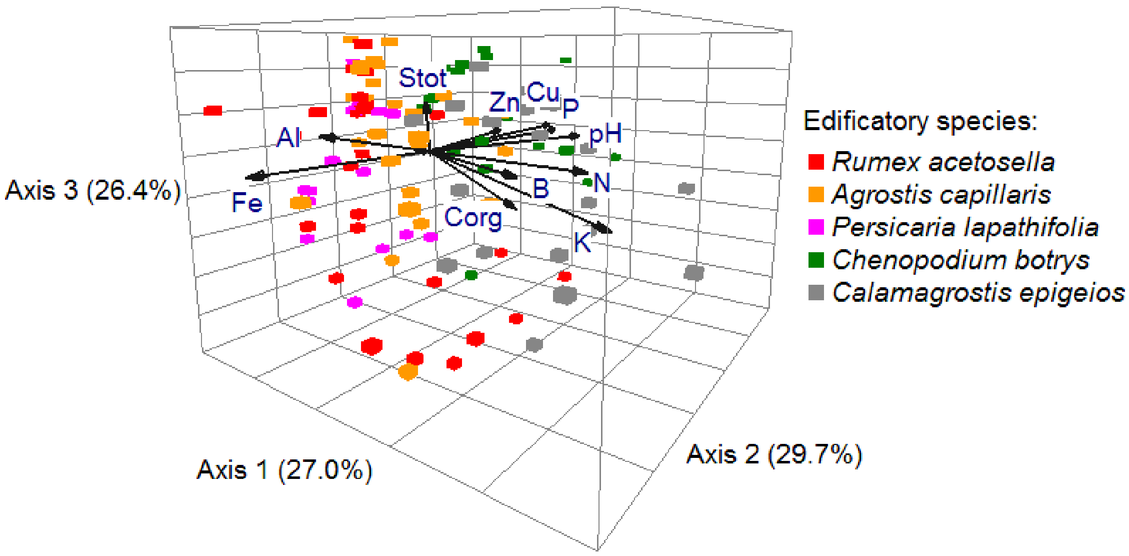

Fig. 3.12: Unconstrained ordination (Nonmetric Multidimensional Scaling, NMS) of early vegetation relevées dominated by one of the five pioneer species occurring on the polluted land. Selected soil parameters, correlated with the ordination scores by > 10%, are overlaid. Data matrix: 75 species, 115 stands, log-transformed biomass data, Sørensen distance, varimax rotation; final stress for 3-d solution 14.52.

The strongest separation of the early vegetation samples (stands dominated by R. acetosella, *A. capillaris* and *P. lapathifolia*, against the stands of *Ch. botrys* and *C. epigeios*) occurs along the first two ordination axes, which are correlated with the two main soil gradients: increasing pH, P, Cu – increasing Al and Fe along the Axis 2; and increasing K, N and C_{org} along the Axis 1 (Fig. 3.12; Table 3.3). The gradual transition of the abundance of the pioneers and the associated species along the major gradients is shown in Fig. 3.13; Fig. 3.14; and Fig. 3.15.

The Axis 1 of the NMS ordination (Fig. 3.13) effectively separates the relevées dominated by *C. epigeios*, which are characterized by the increased presence of ruderals and nutrient-demanding species like *Galium aparine*, *Tanacetum vulgare*, *Amorpha fruticosa*, *Rubus caesius*, *Linaria vulgaris*, etc. On the other hand, the NMS Axis 2 represents the gradient which sets apart the stands dominated by *Ch. botrys*; despite the overwhelming correlation with the extremely high concentrations of available Cu in soils (see also Fig. 3.5), no true "metallophytes" occur there. Instead, the vegetation gradient along the Axis 2 comprises increase of xerophytes like *Linaria genistifolia, Eryngium campestre, Echium vulgare, Acinos arvensis, Centaurea stoebe* subsp. *micranthos*, etc. The third gradient (Axis 3) in the early successional vegetation, though it also represents considerable amount of variation in species abundances (26.4%, Figures 3.12; 3.14 and 3.15 has the lowest correlation with the measured soil parameters (Table 3.3). Only S_{tot} can be correlated with the Axis 3 (Table 3.3), and, to a lesser extent (about only 7%), Mn concentrations (see Fig. 3.14). S_{tot} has, *per se*, no direct effect on plant growth, and the true status of Mn in alluvial soils very much depends on the fluctuating redox conditions in the rhizosphere, so the sole two soil variables correlated with the ordination cannot be considered for potential underlying causes of the observed floristic gradient.

Table 3.3: Correlation of the selected soil properties with the ordination of early vegetation relevées on the polluted soils. r^2 – Pearson's correlation coefficient; Tau – Kendall's rank correlation coefficient.

	Correlation with ordination scores					
	Axis 1		Axis 2		Axis 3	
Soil parameter	r^2	Tau	r^2	Tau	r^2	Tau
pH	0.07	-0.2	0.34	-0.37	0.01	-0.15
Available Cu	0.006	0.09	0.36	-0.39	0.009	-0.08
Available P	0.008	-0.056	0.36	-0.37	0.002	-0.17
Available K	0.31	-0.30	0.29	0.15	0.09	-0.26
N_{tot}	0.22	-0.30	0.08	-0.19	0.03	-0.21
S_{tot}	0.007	0.04	0.002	0.002	0.12	0.27

Moreover, S_{tot} concentrations imply not only a spatial gradient, but also indicate the landscape context in which the early succession proceeds: at higher S_{tot}, the novel birch-aspen forests with non ruderal undergrowth are the closest type of surrounding vegetation, while at the lower S_{tot} levels poplar forests with ruderal species, and cropped fields farther on unpolluted soils are the neighbouring vegetation (see the next section). Consistently, the major change in the associated species along the spatial gradient (Fig. 3.14 and Fig. 3.15) reflects the substitution of ruderals and weeds (e.g. *Daucus carota, Papaver rhoeas*, *Equisetum palustre*, *Chenopodium album*, *Polygonum aviculare, Vicia grandiflora*, *Amorpha fruticosa*, *Rubus caesius*, *Bidens frondosa*, *Silene alba*, etc.) with non-ruderals (*Andropogon ischaemum, Verbascum thapsus, Tragopogon dubius, Picris* *hieracioides*, *Campanula persicifolia*, etc.) by forest species (*Betula pendula*, *Populus tremula*, *Quercus cerris*).

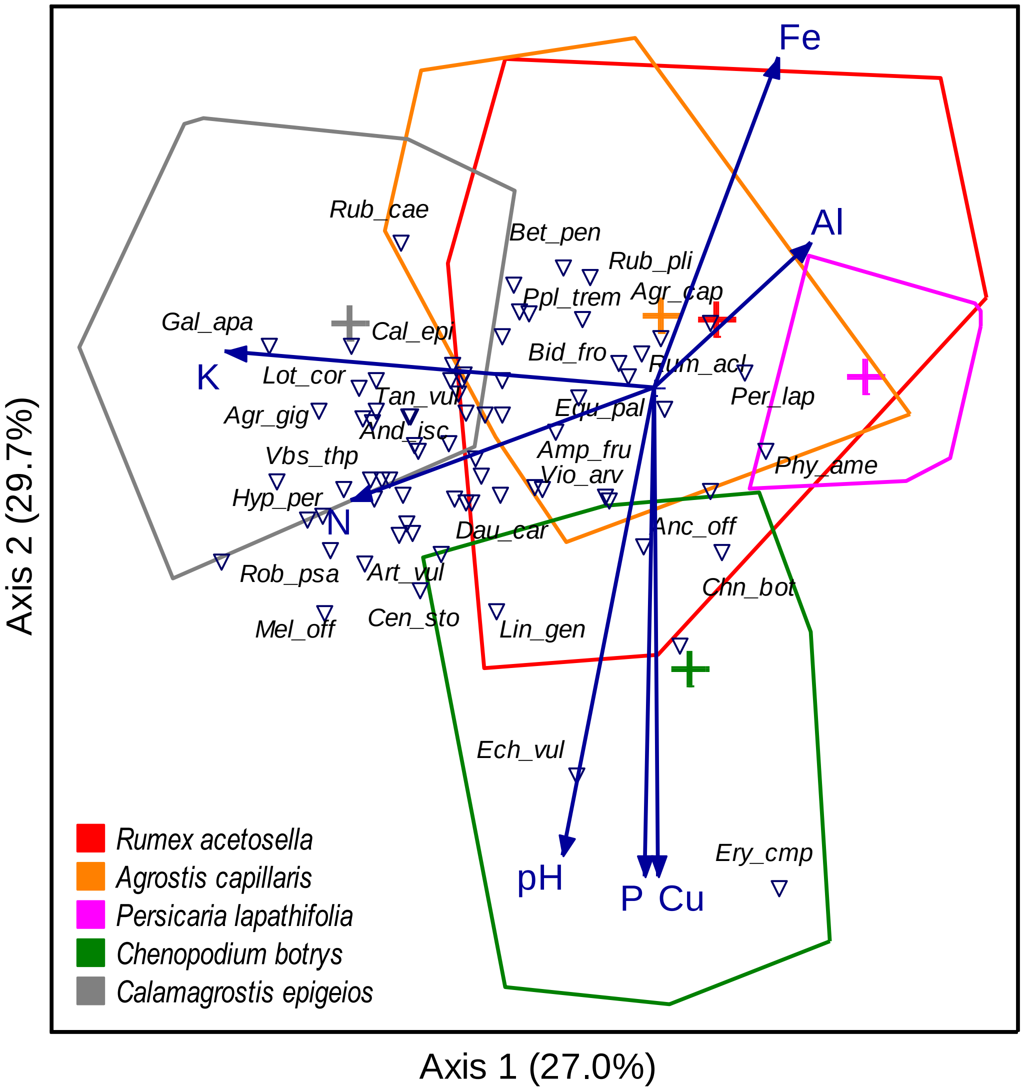

Fig. 3.13: Species tendencies along the Axis 1 and Axis 2 of the NMS ordination. Convex hulls for five early vegetation stands, dominated by different pioneer species are shown. Group centroids (crosses) show the average position of a sample within each early vegetation type. Points of species' central tendency (triangles) are weighted averages of species' abundance in sample units. Data matrix: 75 species, 115 stands, log-transformed biomass data, Sørensen distance, varimax rotation; final stress for 3-d solution 14.52. Species are abbreviated as in Supplement 3.1.

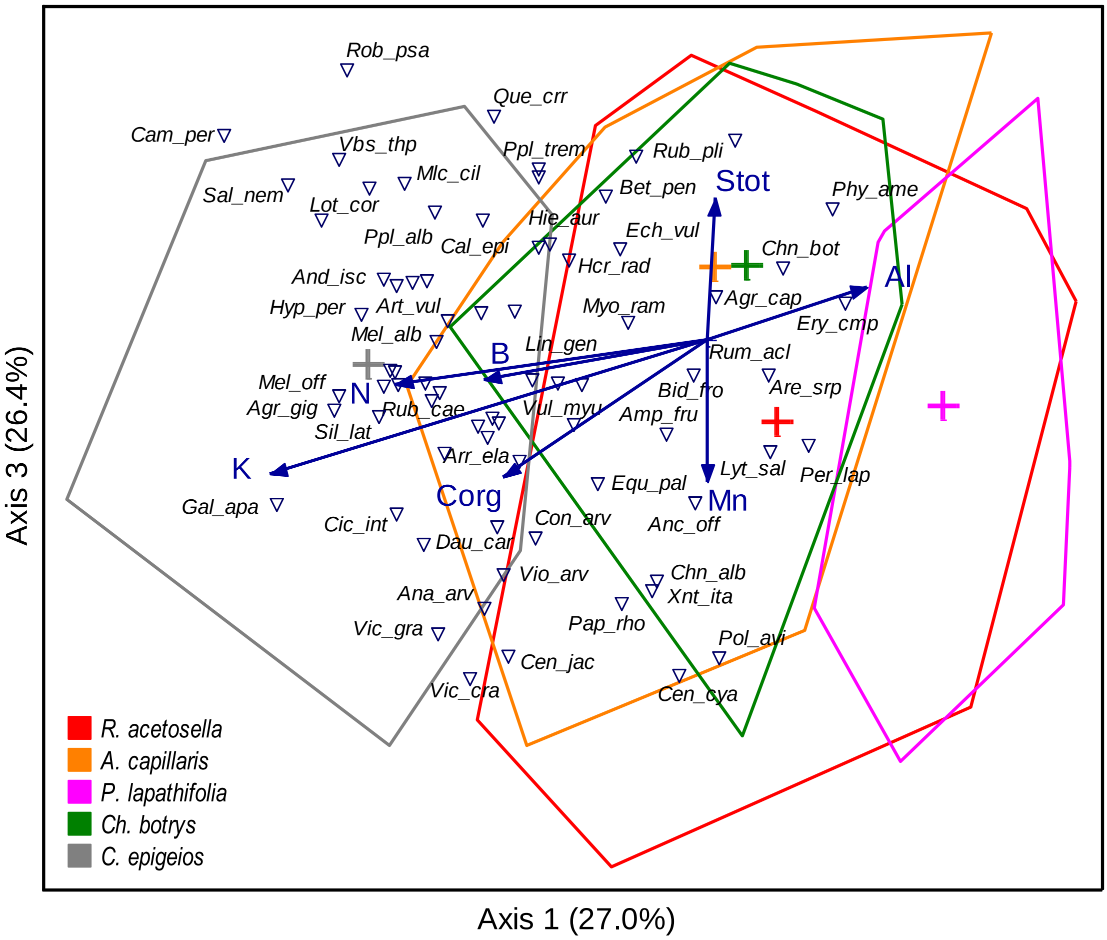

Fig. 3.14: Species tendencies along the Axis 1 and Axis 3 of the NMS ordination. Convex hulls for five early vegetation stands, dominated by different pioneer species are shown. Group centroids (crosses) show the average position of a sample within each early vegetation type. Points of species' central tendency (triangles) are weighted averages of species' abundance in sample units. Soil variables vectors are scaled by 280%. Data matrix: 75 species, 115 stands, log-transformed biomass data, Sørensen distance, varimax rotation; final stress for 3-d solution 14.52. Species are abbreviated as in Supplement 3.1.

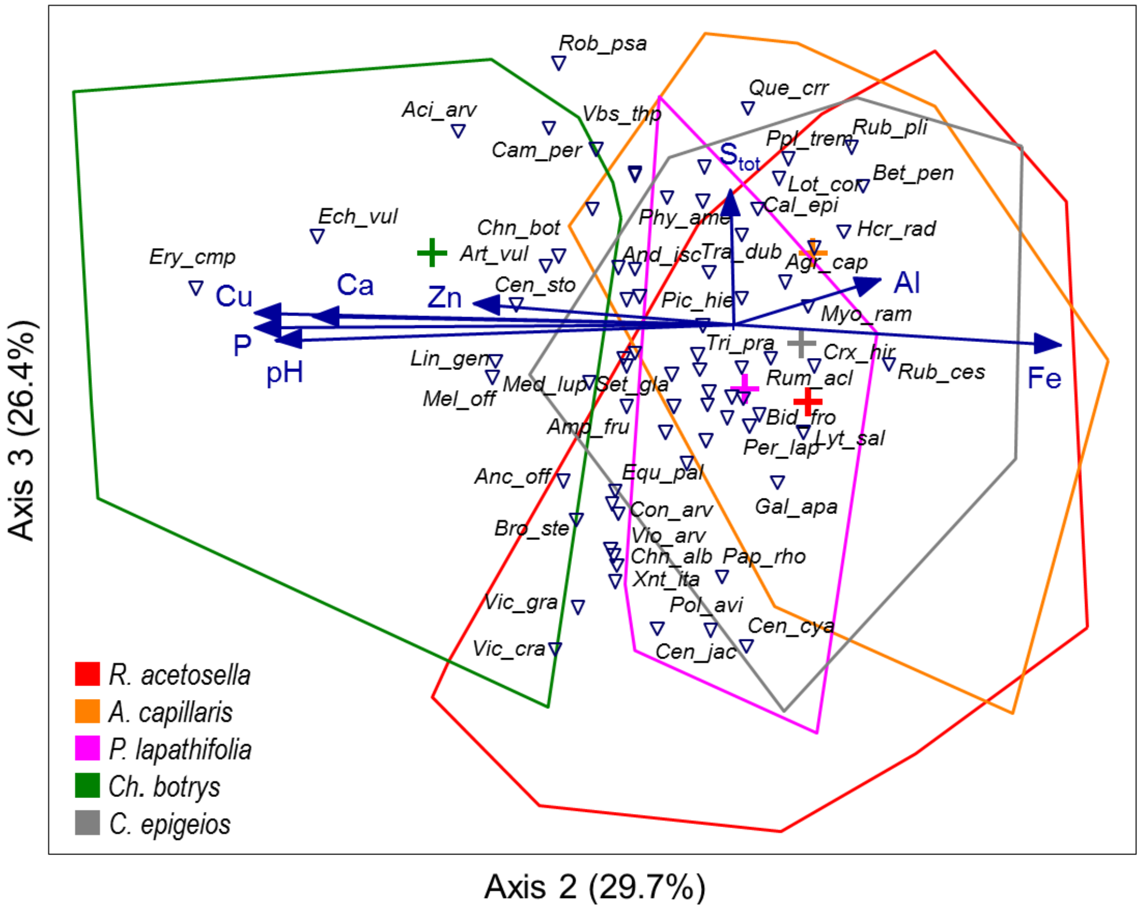

Fig. 3.15: Species tendencies along the Axis 2 and Axis 3 of the NMS ordination. Convex hulls for five early vegetation stands, dominated by different pioneer species are shown. Group centroids (crosses) show the average position of a sample within each early vegetation type. Points of species' central tendency (triangles) are weighted averages of species' abundance in sample units. Data matrix: 75 species, 115 stands, log-transformed biomass data, Sørensen distance, varimax rotation; final stress for 3-d solution 14.52. Species are abbreviated as in Supplement 3.1.

3.3.3 Pioneer forests colonizing the barren land

Two different types of forests colonize the barren fluvial deposits of the pyritic Cu-tailings. They occupy spatially distinct positions along the transects perpendicular to the water course (river channel), differ markedly in structure and species composition, and grow on soils with contrasting constraints (Table 3.4; Fig. 3.16; Fig. 3.17; Fig. 3.18; and Fig. 3.19). The relative age of these two forest types is indicated by trunk circumference and absolute dominance (Table 3.4, see also Fig. 3.20). Although no pre-specified sampling transects were envisaged by the study design, the soil properties of the two pioneer forest types are clearly ordered along the gradient of increasing pollution deposition (Fig. 3.17), which is most apparently indicated by the increasing S_{tot} concentrations as shown before (see Section 3.2.2).

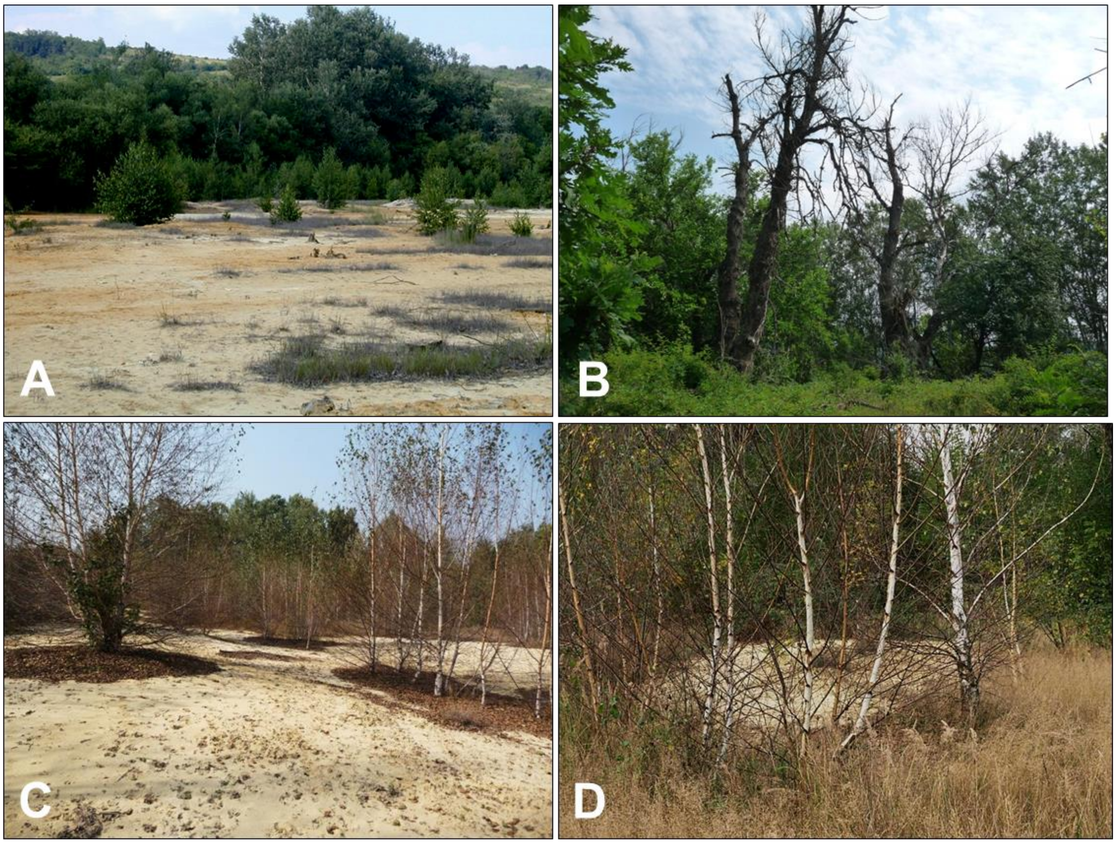

Fig. 3.16: Pioneer forests colonizing the barren land polluted by fluvial deposition of sulphidc Cu tailings. A – background: older poplar forests, spread from the unpolluted soils, with the clear boundary towards barren land; foreground: young stand of Betula pendula encroaching the barren land; B – sporadic dieback of *P. alba* and *P. nigra* at the boundary of the poplar forests area; C – pioneer birch trees facilitate the subsequent colonization by other species; older poplar forests in the background; D – birch-aspen forests closing up; barren patches remain only at microelevated localities.

Very strong soil gradient underlying the occurrence of the two distinct pioneer forest types comprises a directional increase of plant macronutrients (Ca, Mg, N, P, K), pH, soil organic matter, but also all the measured metals except Al and Fe along the distance gradient from the pollution source (Fig. 3.17). Selected soil parameters for the two forest types are shown in Fig. 3.18. Although the plant available concentration of all the measured metals except Al and Fe increase from younger stands occurring at the (relatively) middle transect position, towards older stands occurring towards the edge of the polluted area, only Cu is found in elevated concentrations which might constraint plant growth (Fig. 3.18). For instance, the available (DTPA-extractable) concentrations of Cd, Pb, Ni and Cr were below 0.5, 5, 1.5 and 0.5 mg kg^{-1}, respectively (not shown).

Table 3.4: Overview of the pioneer forests which colonize the barren alluvial land polluted by sulphidic Cu mining waste.

	Pioneer forest formations on barren tailings deposits	
Parameter	Younger birch-aspen stands	Older poplar stands
Dominant species	*Betula pendula* & *Populus tremula*	*Populus alba* & *Populus nigra*
No. of samples	25	23
Average species number	18.9	33.7
Average within-group distance (Sørensen)[a]	0.46	0.54
Median DBH (cm)	14.5	50.7
Total density (trees ha^{-1})	1402 ± 281	424 ± 38
Absolute domninance (m^2 trunks ha^{-1})	22.8 ± 5.2	81.9 ± 8.8
Ground layer vegetation	Non-ruderal	Ruderal
Major soil constraint	Low pH, nutrient deficiency	High Cu
Rel. position on the transect[b]	Middle portions	Outer edge

[a] group is defined by the forest type;
[b] transects run perpendicularly from the pollution source (river channel) towards unpolluted area.

Furthermore, though the concentrations of total soluble salts in the two groups of forests soils do not differ, and mostly remain within the non-saline limits (< 0.15%, Fig. 3.18), the composition of salts is very different (not shown). In birch-aspen forests soils, sulphates of Fe and Al are dominant, while in the poplar forests soils Ca-sulphate, but also soluble carbonates, prevail. Namely, low pH, high available Al, and an increased share of sulphates in S_{tot} (Fig. 3.18) indicate a very intensive historic oxidative weathering of the deposited tailings in the middle transect portions, which has led to the intensive Cu (and other metal) leaching in the soils currently colonized by *Betula pendula-Populus tremula* forests. On the other hand, at the outer edge of the polluted area, high levels of Cu in the soils of poplar forests are maintained; this is consistent with other indicators of slower weathering (pH, Al, and SO_4/S_{tot} ratio).

Higher concentrations of Stot and available Fe in the soils colonized by the birch- aspen forests indicate the higher deposition of the mine tailings and the closer relative position to the pollution source i.e. to the river channel. However, despite the higher deposition of the sulphidic Cu tailings, the concentrations of Cu are surprisingly low in these forest soils (Fig. 3.18). This apparent "Cu paradox" along the transect (higher soil Cu concentrations are found farther from the pollution source, where the pollution deposition was considerably lower) is consistent with the evidence for very different intensity of oxidative weathering in the soils colonized by the two types of pioneer forests.

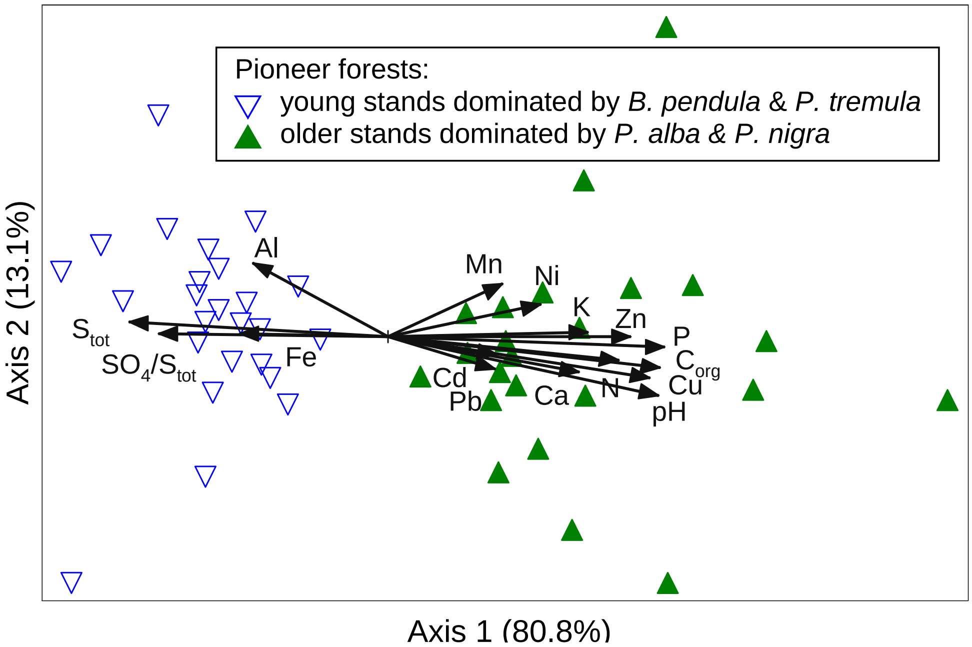

Fig. 3.17: Gradients in soil properties of the two major forest types which colonize barren fluvial deposits of the pyritic Cu-tailings. NMS ordination: data matrix of 48 soil samples, 21 soil chemical parameters (relativized by adjusting to standard deviate), Euclidean distance, final stress for 2-d solution 10.31; ordination rotated by -5°. Parameters presented are correlated with the ordination scores by more than 35%. Plant available concentrations of P and metals are shown. Pollution load is indicated by S_{tot} concentrations which regularly decrease with the distance from the river channel.

Floristically, these two forests are very different, with only one, overwhelming, gradient in vegetation properties (Fig. 3.19). This gradient in forest vegetation is tightly matching the previously discussed gradient in soil properties (Table 3.5; see also Fig. 3.17). The pioneer character of these two forest type is also reflected in unusual species composition; most apparently, species with different niches have similar scores along the major vegetation gradient (e.g. *Bromus sterilis* and *Salix fragilis*, *Bidens frondosa* and *Hypericum perforatum*, etc; Fig. 3.19).

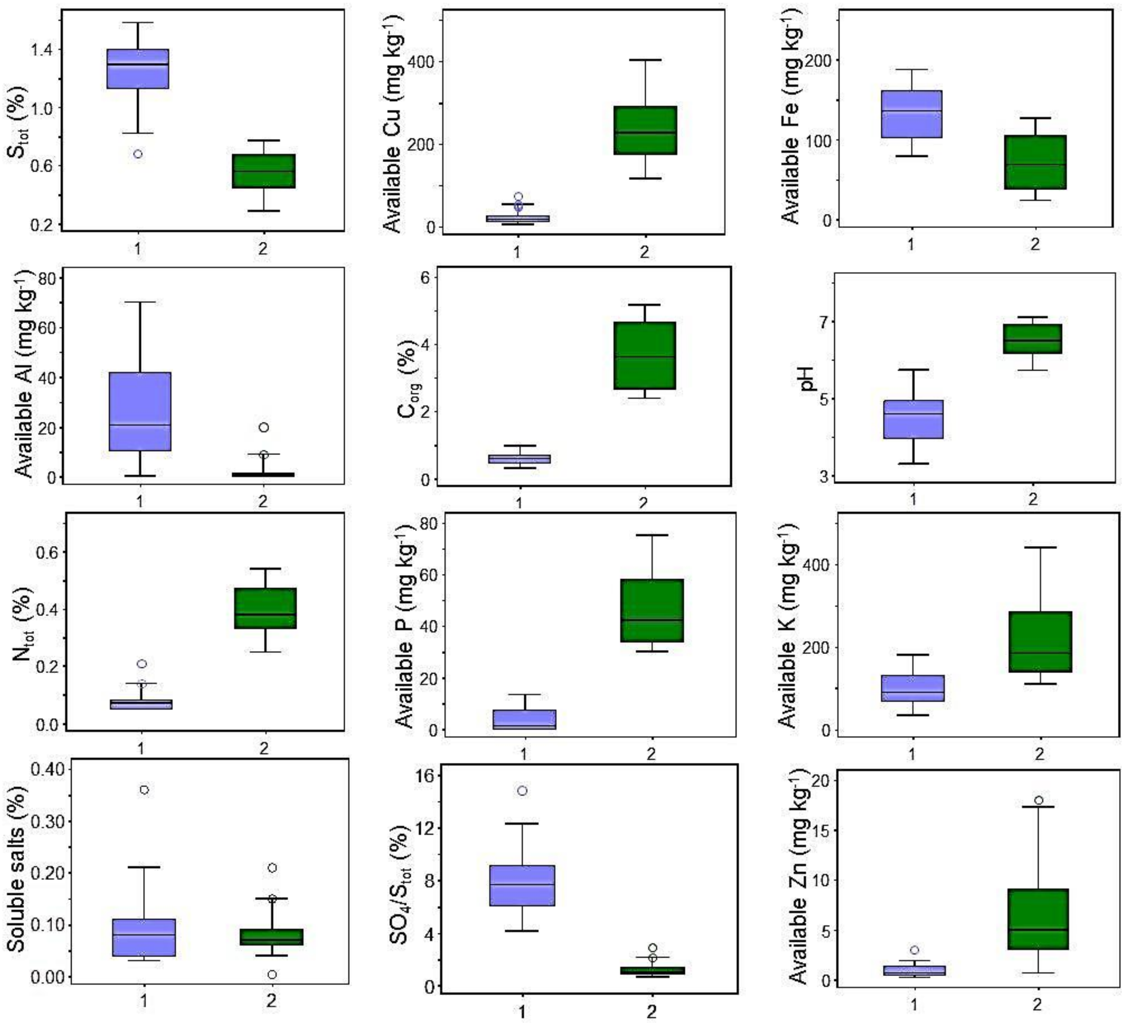

Fig. 3.18: Selected soil parameters of the two distinct forest formations colonizing the barren fluvial deposits of the mine waste. 1 – Young, novel stands dominated by *Betula pendula* and *Populus tremula*, occurring towards the inner zone of the polluted area. 2 – Older forests dominated by *Populus alba* and *Populus nigra*, encroaching from the outer edge of the polluted area (i.e. from the adjacent unpolluted soils). Box-plots are scaled in percentiles; cutoff for outliers is 1.5 times interquartile range. Plant available concentrations of mineral elements were determined by different extractions: DTPA for metals, ammonium acetate-ammonium lactate (AL) for P and K, and KCl for Al.

Table 3.5: Correlation of the selected soil properties with the ordination of vegetation samples in the two pioneer forest types on the polluted soils.

	Correlation with ordination scores for vegetation samples	
Soil parameter	Pearson's r^2	Kendall's tau
S_{tot}	0.76	-0.51
pH	0.73	0.66
Available Al	0.34	-0.35
C_{org}	0.84	0.61
Available Cu	0.76	0.54
Available P	0.78	0.59

Out of the 92 species included in the NMS ordination, 48 were found to occur in both forest types, albeit with very different abundances. The dominant species however are clearly confined to either forest type. Out of 59 species with frequency > 15%, recorded in the young birch-aspen forests, only 9 occur exclusively and consistently in these forest. *Echium vulgare* and *Vulpia myuros* are not considered to be truly confined to the *Betula pendula-Populus tremula* forests, but rather to appear opportunistically due to the lower overall vegetation cover there, since these two species have also been recorded in the surrounding vegetation on non-polluted soils (not shown). However, *Betula pendula*, *Populus tremula*, *Rumex acetosella*, *Agrostis capillaris*, and *Hypochaeris radicata* have been the exclusive components of all the surveyed birch-aspen stands with the frequency of 100%, while *Pteridium aquilinum, Rubus plicatus, Hieracium auriculoides, Hieracium pillosella* and occasionally *Quercus petraea* occur randomly with estimated cover < 5% , i.e. < 15% for *R. plicatus*. It is important to notice that these 9 species, confined to the younger *Betula pendula-Populus tremula* forests, appear to be the true novelties in the alluvial vegetation of the Timok region, since they have been encountered neither on the non-polluted alluvial land, nor within the distance of at least 10 km from the study area, in non-alluvial surrounding vegetation (own unpublished results, not shown). Another very interesting feature of these novel forests is that although the pioneer, edificatory species (*Betula pendula* and *Populus tremula*) are not pronouncedly drought tolerant, the accompanying species whose existence is facilitated by the establishment of birch and aspen (*Quercus cerris, Q. frainetto, Hypericum perforatum, Myosotis ramosissima, Hieracium auriculoides*) are strongly xerophytic.

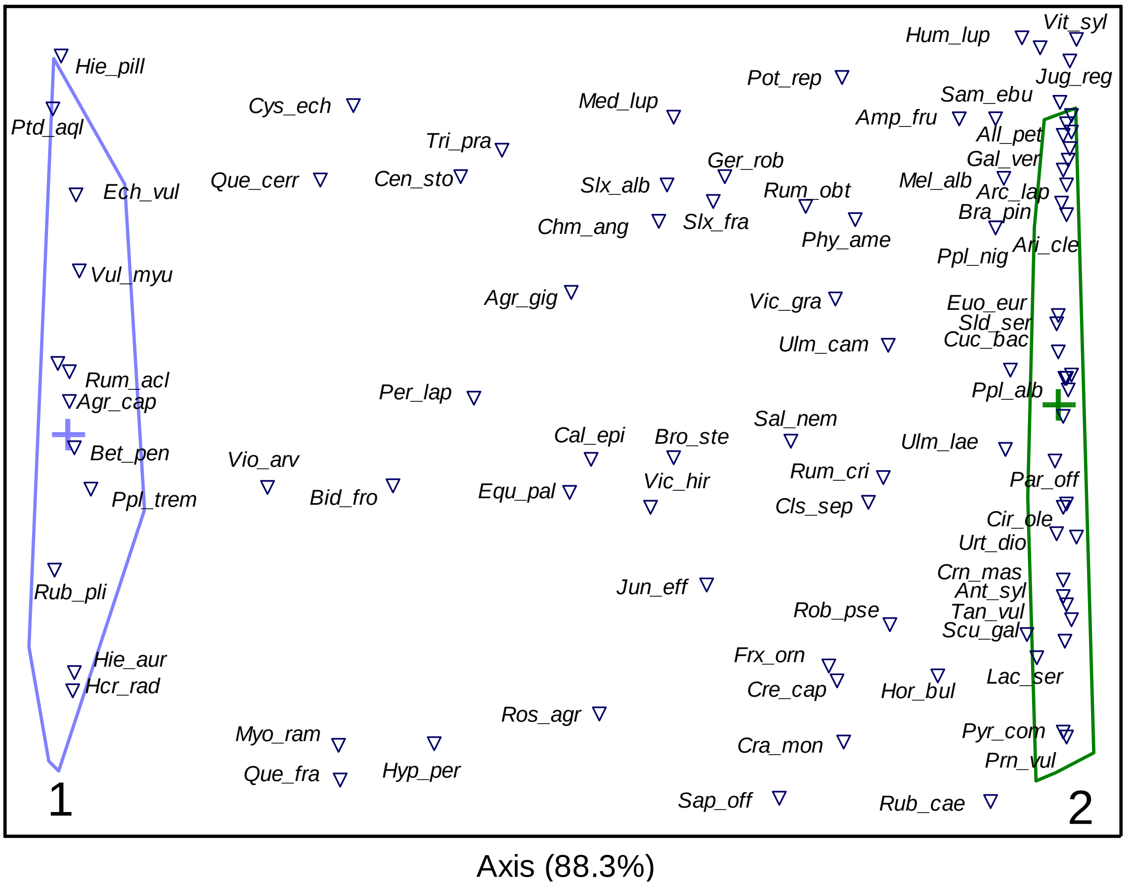

Fig. 3.19: Gradients in pioneer forest vegetation (NMS ordination) on the barren, fluvially deposited mine tailings. Data matrix: 92 species, 48 samples, Sørensen distance; final stress 6.21 after 72 iterations. 1 – Young, novel stands dominated by *Betula pendula* and *Populus tremula*, occurring towards the inner zone of the polluted area. 2 – Older forests dominated by *Populus alba* and *Populus nigra*, encroaching from the outer edge of the polluted area (i.e. from the adjacent unpolluted soils). Species central tendencies are indicated by triangles, group centroids by crosses, and convex hulls encompass all the samples. Species are abbreviated as in Supplement 3.1.

Contrary to the young birch-aspen forests, dominated by non-ruderal species adapted to oligotrophic, nutrient poor habitats, older poplar forests advancing from the outer edge of the polluted area are dominated by many common ruderal species which are encountered along lowland rivers in this region. For instance, nitrophytes like *Rubus caesius, Galium aparine, Anthriscus sylvestris, Lamium album* and *Urtica dioica* occur throughout this forest type with frequencies > 60%. Peculiar appearance of fruit trees (*Juglans regia*, *Pyrus communis*, *Malus domestica*, and *Cydonia oblonga*) in this forest is a historic relict from the pre-pollution time. Namely, participatory surveys with local farmers revealed that these very trees had existed as shade trees in the former agricultural fields (common local practice), before the fluvial deposition of mine tailings prevented further use of this land for cropping. Besides the common species of alluvial poplar forests (e.g. *Cucubalus baccifer, Scutellaria gelericulata, Ulmus leavis, Solanum dulcamara, Humulus lupulus*), the species typical of xerothermic, calcareous surrounding are also an interesting feature of this forest (e.g. *Clematis vitalba, Rosa agrestis, Fraxiuns ornus, Hordeum bulbosum*). Another surprising finding is an apparent paradox: the pronouncedly drought tolerant species (e.g. *Quercus cerris, Q. frainetto, Hypericum perforatum, Myosotis ramosissima, Hieracium auriculoides, Rosa agrestis, Centaurea stoebe*) tend to prevail in the birch-aspen forests (see their scores along the Axis 1, Fig. 3.19), which are closer to the river channel (indicated by soil S_{tot} concentrations, Fig. 3.17 and Fig. 3.18) and allegedly more exposed to regular floods. Likewise, the two submediterranean grasses with rather similar environmental requirements (*Hordeum bulbosum* and *Cynosurus echinatus*) have different scores along the major gradient (Fig. 3.19). These results indicate that exceptionally strong filtering by main soil constraints (nutrient deficiency, low pH, and possibly Cu and Al toxicity) has an overwhelming effect on revegetation process, overrunning other potential modifiers like soil moisture.

Finally, the quantitative assessment of the pioneer forests, besides differences in structure (Table 3.4, Fig. 3.16) reveals an interesting trend in regeneration layers of the four dominant tree species (Fig. 3.20). Most strikingly, the regeneration layer (young individuals with smaller DBH) of the *Populus alba* and *Populus nigra* in the older poplar forests does not extend beyond a certain point on the transect. Instead, the prominent decrease of young individuals is visible already after soil S_{tot} concentration of about 0.6%. Towards the river channel, at S_{tot} about 0.8%, *P. alba* and *P. nigra* forests make an unusually sharp boundary (see also Fig. 3.16A), and do not spread towards the more polluted area. This is consistent with the observed sharp decline of poplars in the borderline zone of these forests (see Fig. 3.16B). The younger, birch-aspen forests show however an opposite trend (Fig. 3.20): the largest (and the oldest) individuals are concentrated in a relatively narrow range along the transect (from about 1.2-1.3% S_{tot}), and the regeneration layer (trees with smaller DBH) is spreading both towards the river channel (i.e. pollution source) and towards the unpolluted area. This is likely to have important implications for the future dynamics of these pioneer forests.

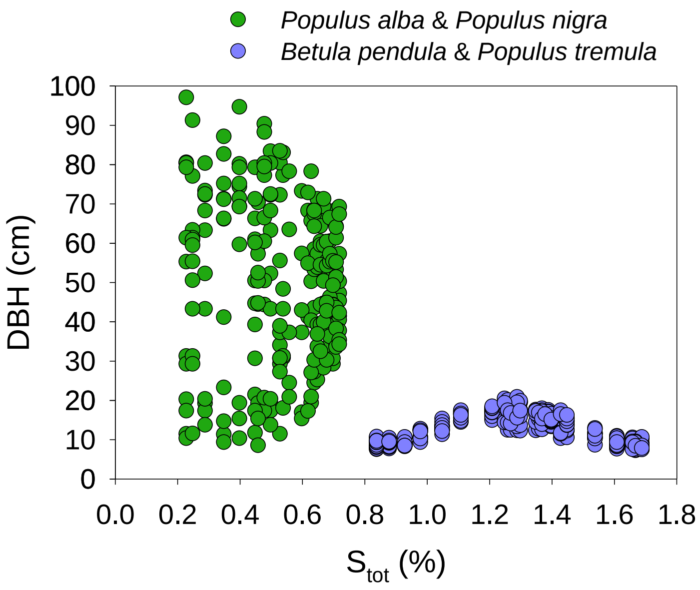

Fig. 3.20: Individual tree size (DBH – diameter at breast height) along the soil pollution gradient in the two pioneer forest types.

3.4 Discussion

3.4.1 Highly patterned primary succession

The first striking feature of the primary succession on these very complexly degraded soils is an extremely high proportion of the "variance explained" (Fig. 3.2; Fig. 3.12; Fig. 3.13; Fig. 3.14; Fig. 3.15; Fig. 3.17; and Fig. 3.19). In particular, very strong gradients in primary vegetation structure (Fig. 3.12 and Fig. 3.19), and, moreover very high correlation with soil properties (Table 3.3 and Table 3.4) show the effect of a strong pattern which shapes up both soil and vegetation, allowing for surprisingly little randomness. In this exploratory study, major gradients were shown to represent up to 80% of variation in herbaceous and forest early stands; 30% to over 70% of this variation is correlated with the measured soil parameters, what is several times higher than in an average ecological experiment (Møller and Jennions, 2002). In particular, this is primarily in strong contrast with the reported low correlation of soil parameters and primary succession (Schulz and Wiegleb, 2000; Wiegleb and Felinks, 2001b). Secondly, high degree of structure in pioneer vegetation groups is surprising for primary succession, which is usually a highly stochastic process, dependent on random events (Walker and del Moral, 2003).

Soil degraded by mining wastes have been shown to have an immanently irregular patchy distribution of chemical constraints over very short distances (less than 10 m, e.g. Prach, 1987; Hüttl and Weber, 2001; Néel et al., 2003), what is an intrinsic difficulty for gradient analysis approach. Moreover, on the most thoroughly researched tailings dam failure case (the Azñalcóllar accident in 1998), natural river flooding dynamics on damaged alluvial soils were found to augment this small-scale spatial heterogeneity (Gallart et al., 1999; Burgos et al., 2006). However, in the Azñalcóllar case the layer of mine slurry was mechanically removed, while in this study the unconstrained pollution had been going on for about 50 years. The fluvially polluted soils of this research locality thus combine two important features: Firstly, increased spatial variability at the micro level (few meters and less) is important for the creation of microhabitats suitable for pioneer colonization (see e.g. Shu et al., 2005). Secondly, the well-known phenomenon that fluvial sediments deposition regularly decreases in the lateral direction (see e.g. Asselman and Middelkoop, 1995) brought about spatially explicit gradients of regularly changing soil properties (at a scale of few hundreds of meters). The former feature increased the chance to observe the clear patterns of the herbaceous pioneer vegetation (small-scale variability, all stands relatively close to the pollution source), while the latter one was crucial for the unusually clear distinction of the two pioneer forest types (medium-scale variability, along the transects to unpolluted soils).

The second factor (besides the clear fluvially-induced soil gradients), which we consider responsible for this highly patterned colonization process, is a strong effect of abiotic constraints which operate as environmental filters. Environmental filters reduce the number of potential species which might establish under new abiotic conditions (Keddy, 1992); this is particularly apparent when the conditions in the immediate surrounding are pronouncedly different, like in our case. Moreover, in polluted areas environmental filters tend to favour facilitation over competition (see Gallagher et al., 2011). Once established, pioneer species facilitate the establishment of other accompanying species in early vegetation stands, fostering thus non-random plant-plant interactions (Felinks and Wiegand, 2008).

3.4.2 The effect of surrounding vegetation *vs*. the effect of environmental filters

3.4.2.1 The pioneers are common species of post-mining sites

All the five pioneer species which successfully colonize these complexly degraded soils (*Agrostis capillaris, Rumex acetosella, Calamagrostis epigeios, Chenopodium botrys, Persicaria lapathifolia*, *Populus alba*, *Populus nigra*, *Betula* *pendula* and *Populus tremula*) have been already described from different post-mining sites worldwide.

For instance, Cu tolerance of a pseudometallophyte *A. capillaris* is well known; even some Cu-tolerant populations were commercially bred for phytoremediation (Thompson and Proctor, 1983; Cole and Smith, 1984; Lepp et al., 1997; Balabane et al., 1999; Dahmani-Muller et al., 2000). Likewise, the Cu tolerance of *R. acetosella* is well established; the species was even used for metal prospecting i.e. for indicating sulphidic Cu ore veins (Kelepertsis and Andrulakis, 1983; Kelepertsis et al., 1985; Reeves et al., 1986; Babalonas et al., 1997). Banásová et al. (2006) found a consistently increased dominance of *R. acetosella* on former Cu mine soils, compared to the non-polluted surrounding. On severely Cu-polluted sites, *A. capillaris* and *R. acetosella* are described to grow together (Becker and Brändel, 2007; Bes et al., 2010). The ability of *C. epigeios* to grow on metal (particularly Cu) polluted soils has been documented (e.g. Lehmann, 1997; Lehmann and Rebele, 2004). *P. lapathifolia* (syn. *Polygonum lapathifolium*) was also found to tolerate heavy metals (Cui et al., 2007; Guleryuz et al., 2008). Eltrop et al. (1991) and Marguí et al. (2007) found that *Betula pendula* tolerates high concentrations of heavy metals in post-mining soils. Finally, metal tolerance of different *Populus* species has been reported (Domínguez et al., 2008; Guerra et al., 2011).

Likewise, the pioneers from our study are also reported from nutrient-poor, acidic post mining sites, most commonly created by coal mining. *P. lapathifolia* was for instance reported as a pioneer from the Czeck Republic (e.g. Prach, 1987; Pyšek and Pyšek, 1988), while Croxton (1928) identified dominance of a similar *Persicaria* species as a key stage in regeneration of coal stripland with lot of pyritic waste in the USA. *Polygonum* (*Persicaria*) and *R. acetosella* were encountered even in spontaneous vegetation developing after lignite mining in Texas, in the surrounding of a subtropical oak savannah in Texas (Skousen et al., 1990). Dominance *C. epigeios*, capable to temporary arrest succession on post mining land, was observed throughout Europe (Prach, 1987; Pyšek and Pyšek, 1988; Pietsch, 1996; Pietsch and Schötz, 1999; Shulz and Wiegleb, 2000; Kirmer and Mahn, 2001; Wiegleb and Felinks, 2001a; Hodacová and Prach, 2003). *Betula pendula* has been the most commonly reported woody species in these studies, while *P. tremula* is mentioned occasionally (see also Prach and Pyšek, 2001; Prach et al., 2001b). *A. capillaris* and *R. acetosella* were reported in different early vegetation stands in Lusatia (Germany, Wiegleb and Felinks, 2001a). So far, *Ch. botrys* is still seldomly reported, but it has also been recorded on nutrient-poor coal mining spoils (e.g. Drexler, 2005 in Germany), as well as on Cu- rich mine soils (e.g. Nouri et al., 2009 in Iran).

Nevertheless, none of these studies mentioned above has reported the sole dominance of any of our four pioneers (i.e. excluding *C. epigeios*), neither on metal-polluted nor on acidic and nutrient poor post-mining sites. Moreover, “our” five species have not even been reported so far as pioneers, but as constituents of the early vegetation types.

3.4.2.2 The role of the surrounding vegetation

The most surprising finding of our study is a limited effect of surrounding vegetation on primary succession. So far, the literature suggests that the effect of surrounding vegetation (as a propagule source) on primary succession in post-mining areas, particularly in very early stages, could not be overemphasized (e.g. Borgegård, 1990; Prach et al., 2001b; Walker and del Moral, 2003; Rehounkova and Prach, 2006; (Prach and Rehounkova, 2006; Tischew and Kirmer, 2007; Kirmer et al., 2008; Baasch et al., 2009; Alday et al., 2011). Our study has clearly demonstrated the effect of the surrounding vegetation on the very early vegetation types, but has also showed that the major pioneer species, necessary to initiate the succession on the most severely polluted land, are not related to the surrounding vegetation.

3.4.2.2.1 Adjacent vegetation affects early successional trends

Once the colonization process starts, the landscape context and the surrounding vegetation have a strong effect on the accompanying species in the pioneer vegetation stands (Fig. 3.14 and Fig. 3.15). In fact, a large portion of the variation in vegetation data (about 26%, along the Axis 3, Fig. 3.14 and Fig. 3.15) represents the variation in abundances of the accompanying species and can be related to the spatial position of the relevées. Hence, the landscape context and the surrounding vegetation actually induce a shift in the accompanying species characteristics, from non-ruderals (closer to birch – aspen forests) to ruderals/weeds (closer to cropped fields/poplar forests). While this pattern occurs along the transects shorter than 1 km in our study, Prach et al. (2001b) have described a similar pattern in seres old 10-67 years, occurring at the country-wise scale: non-ruderal seres develop in forested landscapes on acidic, moist, nutrient-poor sites, and ruderal on more fertile sites in agricultural/urban surrounding.

3.4.2.2.2 Local species have limited effect as pioneers

On the other hand, the major pioneer species (*Rumex acetosella*, *Agrostis capillaris*, *Betula pendula* and *Populus tremula*) do not occur in the surrounding vegetation, at least not up to the distance of 5 km, and the literature suggests even several times farther (see Section 3.2.3). Though this study did not attempt to estimate the relative area covered by each of the pioneer vegetation types, the differences in the extent of early vegetation types are very apparent (as mentioned in Section 3.3.1; see also Fig. 3.3 and Fig. 3.16). Pioneer poplar forests (*Populus alba*-*Populus nigra* type) are of course established by the species from the immediate surroundings, i.e. by vegetative lateral spread from the remnants of the riparian forests/groves out of reach of the major floods. These forests are however strictly limited to the outer edge of the polluted zone (Fig. 3.20). Moreover, recent observations on unclear dieback of *P. alba* and *P. nigra* trees at the border of this forest type area towards more intensively polluted land Fig. 3.16B) cast doubts on a potential of these two pioneer trees to extend its existence any further. Likewise, the two therophytic pioneers (*Ch. botrys* and *P. lapathifolia*) might have originated from the pre-pollution riparian vegetation; they are described in Euro-Siberian annual communities on muddy alluvial substrates in the Bidention tripartitae Tüxen et al. ex von Rochow 1951 alliance (Lakušić, 2005). However, these two pioneers achieve dominance only at very specific microsites of very limited extent (see Section 3.3.1), while they get easily supressed outside of these microsites, firstly by other pioneers (Table 3.2). Furthermore, *C. epigeios* has been encountered in the surrounding non-flooded vegetation; however, its dominance in fallows and wastelands throughout Serbia cannot be separated from severe anthropogenic disturbances (Lakušić, 2005). Though it was observed to colonize barren deposits of mine tailings in this study, we can still not be sure if its appearance and dominance are independent of anthropogenic interventions via uncontrolled burning (see Fig. 3.3C and D), more so considering its strong co-occurrence with elevated soil K concentrations (Fig. 3.5; Fig. 3.13).

3.4.2.2.3 Where do the major pioneers come from?

Therefore, the spontaneous revegetation of this multiply constrained post-mining land in the largest extent relies on four species (*R. acetosella, A. capillaris, B. pendula* and *P. tremula*), which are common on disturbed acidic soils in Central Europe (Ellenberg, 1988b). In Serbia, these species are common in the western part of the country, in the Illyric phytogeographic province, on middle altitudes, podsolised soils, and significantly more humid climate, in the disturbed habitats of acidophytic oak forests of the Quercion robori–petraeae Br.-Bl. 1932 alliance. Moreover, some acidophytic accompanying species in the pioneer vegetation at our research locality (*Hieracium pillosella*, *Rubus plicatus*, *Pteridium aquilinum*, *Quercus petraea*, see Supplement 3.1) which do not appear in the calcareous surrounding of the research locality, are common in those habitats (Lakušić, 2005). In Eastern Serbia, however, pioneer forest associations described as Betuletum pendulae Glišić 1950 and Populo tremuli-Betuletum pendulae Glišić (1950)1975 are found on higher altitudes in the zone of *Fagus moesiaca* and *Quercus petraea* forests, as secondary formations after mass logging and forest fires (Dinić, 2006). Interestingly, older reports (Jovanović, 1948, 1950) mention birch–aspen forests in the surrounding of another mine from the same metallogenic zone (Majdanpek, about 60 km from our research locality, in the submountainous zone of beech and sessile oak) to grow selectively on acidic mine waste spoils. The long-term research from the German coal-mining districts has already shown that nutrient-poor post-mining sites act as large seed traps at the landscape level, what might eliminate the problem of long-distance dispersal (Tichew and Kirmer, 2007; Kirmer et al., 2008).

3.4.2.3 Environmental filters and functional adaptations

3.4.2.3.1 Severe filtering brings about truly “novel” formations

Environmental filters are processes and conditions which constrain the establishment of species with unviable physiological limitations. Plant assemblage or redevelopment can be viewed as a process in which a series of filters allow for the selection of certain species from a regional pool (e.g. Keddy, 1992). Fig. 3.21 shows the major environmental filters (climatic conditions and severe soil constraints) which select for the successful survivors on our research locality.

Regional species pool mostly consists of xerothermic plants adapted to calcareous soils (see Section 3.2.3). Oxidative weathering of sulphides from the deposited tailings has caused a huge difference of 4 pH units compared to the unpolluted surrounding (see also Chapter 4). Besides, plants might need separate adaptations to cope with high acidity and high available Al concentrations (Kidd and Proctor, 2001). Moreover, plants adapted to calcareous soils possess physiologically different mechanisms to cope with P deficiency as compared to acidophytic species

(Neumann and Römheld, 2002). While mining-induced acidic soils amidst dry, non-acidic surrounding can severely constrain vegetation development due to a lack of suitable propagules (Moreno-de las Heras et al., 2008), our study shows that when the spontaneous revegetation eventually starts, it relies on a small group of the widely known post-mining pioneers. Moreover, our work shows that under the conditions of severe environmental filtering, the same well-known species will occur, irrespectively whether these species are present, or can even survive, in the surrounding vegetation.

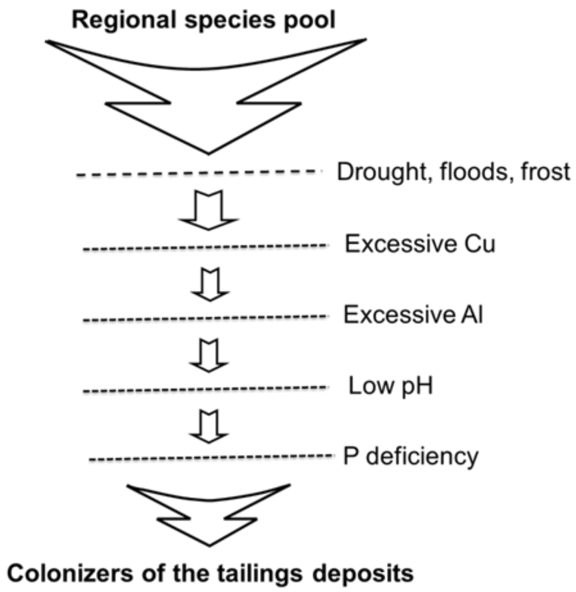

Fig. 3.21: Series of environmental filters which act as "sieves" selects for the successful colonizers of alluvial soils polluted by pyritic Cu tailings sediments

So far, the vast majority of ecological studies which came up with the conclusion of the surmounting influence of the local/regional species pool on post-mining primary successions where likewise conducted on soils altered by mining operations, but the overall differences of soils and early vegetation between the degraded and the surrounding areas were not so drastic. For instance, in the Central-European coal mining areas, *Betula pendula* and *Populus tremula*, the key pioneers, were very frequent species in the surrounding vegetation which consisted of mixed acidophytic oak forests, *Pinus sylvestris* stands, with overall importance of the Atlantic floral elements typical for heathland vegetation (Pietsch, 1996; Kirmer and Mahn, 2001; Tichew and Kirmer, 2007; Kirmer et al., 2008; Kopec et al., 2011). Besides, the difference of soil acidity induced by the sulphidic mine waste on the polluted soils, and the adjacent unaffected forest soils, was never bigger than 2 pH units (e.g. Hüttl and Weber, 2001; Schaaf, 2001).

In our study, on the contrary, the pronouncedly acidophytic pioneers are not even able to survive in the xerothermic surrounding. *Betula pendula* is obviously encountered outside of its range (Fig. 3.22; Dinić, 2006). Besides, not only that *B. pendula* does not occur in the surrounding vegetation, but it has been observed not even to be able to survive when planted by local farmers just some tens of meters outside of the polluted alluvium (own observation). Due to the distinct physiological adaptations, acidophytic species (also called calcifuges) commonly suffer from micronutrient deficiency (Fe, Zn and Mn), and P deficiency when grown in calcareous soils; their distribution is further modified by a complex climate gradient (e.g. Holzner, 1978; Tyler, 1992; Neumann and Römheld, 2002; Tyler, 2003).

Fig. 3.22: Distribution range of *Betula pendula* in Europe (after Vakkari, 2009). The approximate position of the major sampling sites is indicated by the arrow tip.

The unusual species combinations, atypical for the surrounding, relatively undisturbed vegetation, are a common feature of primary successions on post-mining land (Walker and del Moral, 2003). However, to our best knowledge, it has not been reported so far that the succession heavily relied on allochthonous species, which were not encountered, and could not survive in the surrounding. For instance, in the very thoroughly studied primary succession upon the St. Helens volcano explosion, a novel mixture of 6 pioneers, which, as such, had not existed in the region, was described (del Moral and Jones, 2002). Yet, only one of them (*Agrostis pallens*) was not present in the surrounding vegetation, and that was due to its avoidance of shade in the surrounding forests (del Moral, pers. comm., September 2012). Moreover, Kirmer et al. (2008) have shown that the distances of the surrounding vegetation of more than 17 km might be relevant for the colonization process, particularly in the areas surrounded by non-forest, agricultural landscapes. Yet, more than 60% of the species from the immediate surrounding were able to colonize these sites (Tichew and Kirmer, 2007). The emerging concept of "novel ecosystems" in restoration ecology acknowledges the new combinations of species which are brought about through spontaneous succession on man-made wastelands (Hobbs et al., 2006; Hobbs et al., 2009). The exceptional research locality addressed by our study demonstrates not only novel combinations of the already existing species, but also unusual combinations of species which are regionally new and whose presence is limited to the degraded soils. It has a potential for further research towards elucidating the drivers of novelty, what is deemed a research priority (Harris et al., 2013).

3.4.2.3.2 Functional adaptations of pioneers to mineral stress

Pioneer assemblages on post mining land are often just a "Frankenstein combination of species"; the predictability of species composition of early vegetation is usually very low (e.g. Tichew and Kirmer, 2007). It has been shown nevertheless that, as a consequence of the filtering effect of environmental conditions, consistent associations of plant attributes (traits) and environmental conditions are maintained irrespectively of the species involved (Wright et al., 2001; Cornwell and Ackerly, 2009; Venn et al., 2011).

In restoration ecology research however, the traits considered have been usually only those easy to measure (Ellenberg's indicator indices, life strategy, life form, dispersal mode, etc.), and the success was limited (e.g. Walker et al., 2006; Kirmer et al., 2008; Alday et al., 2011). On the other hand, the "physiotype concept" (a set of genetically determined physiological traits, and in particular related to the plant metabolism of mineral elements), was shown to be able to give new insights into major ecological questions of interest with respect to plants' distribution patterns and adaptations to abiotic environment stresses (Choo and Albert, 1997 and the references therein). Herbaceous pioneers colonizing our multiply constrained soils are phytosociologically distant (see Section 3.4.2.2); one is obligatory non-mycorrhizal (*R. acetosella*), they differ in life form and dispersal mode. They have likewise very different functional adaptations to major imposed soil stresses (Al tolerance strategy: Fig. 3.7; excessive Cu concentrations: Fig. 3.8 and Fig. 3.9). Yet, they all have one indicator in common: homeostasis of leaf P concentrations (Fig. 3.10 and Fig. 3.11) across a variety of soil constraints.

Heavy metals affect metabolism of essential nutrients in plants, and the phytotoxic effects of heavy metals are manifested in disturbed mineral composition of plant tissues (Monni et al., 2000). For instance, it has been demonstrated that nutrient uptake can be strongly restricted due to the Cu–induced root growth inhibition (Adalsteinsson, 1994). However, very little is known about the relationship between the Cu-tolerance and the tolerance to general nutrient deficiency in species capable to colonize Cu-rich mining wastes (Ke et al., 2007). So far, Ke et al. (2007) demonstrated in another wetland *Rumex* species that a Cu tolerant population was also capable to maintain the homeostasis of leaf mineral composition under general nutrient deficiency. Moreover, these authors suggest that in particular the plants' ability to maintain leaf P homeostasis under nutrient deficiency has an important role in Cu tolerance and uptake. Likewise, Eltrop et al. (1991) found that Pb tolerance in *Betula pendula* was dependent the P nutrition status. The competitive *Calamagrostis epigeios* tends to avoid waterlogging when it invades wetlands; yet, it has been demonstrated that the effect of nutrient limitations (available P in soils comparable to about 15 mg kg^{-1} AL-P) overrides the effect of habitat humidity preferences (Bakker et al., 2007). In our study, nutrient limitations (and, perhaps, the previously described increased sand content; Nikolic and Nikolic, 2012), has brought about an apparent "paradox": the pronouncedly xerophytic species are found closer to the water channel (see Fig. 3.19), what further underlines the importance of abiotic (primarily nutritional) limitations for the revegetation process.

Overriding effect of metals on vegetation patterns in complexly polluted soils is usually assumed (Becker and Brändel, 2007). The major effects of nutrient deficiency are seldom reported, for instance from non-toxic substrata (Ash et al., 1994; Kirmer et al., 2008), and metal mine spoils (Alvarez et al., 1974; Clark and Clark, 1981). In our study, nutrient limitations showed significant correlations with vegetation structure in early herbaceous stands (Fig. 3.13; Fig. 3.14; Fig. 3.15; Table 3.3), while the effect of Cu essentially only separated pioneer stands dominated by *Ch. botrys* (Fig. 3.13). In the pioneer forests developing along the spatially longer transects and longer gradients of soil properties, it was not possible to separate the effect of higher Cu from the co-occurring higher C_{org} and nutrient levels (Fig. 3.19; see Table 3.5). Similar phenomenon (high Cu concentrations co-occur with high nutrient levels) was reported by other studies (e.g. Strandberg et al., 2006), but the effect of nutrients on spontaneous vegetation of Cu-contaminated soils is seldom discussed. An interesting finding of this study is also a very high leaf Cu concentration in *Chenopodium botrys*, which does not adversely affect plant growth (Fig. 3.9). This is a rather rare phenomenon, because Cu tolerance in most plant species is based on effective exclusion from shoots (Broadely et al., 2012). Though other species might also show extreme concentration of Cu in leaves (Fig. 3.8 and Fig. 3.9), their abundance is consequently severely depressed, what then decreases the overall importance of this trait in revegetation process.

3.4.3 The conceptual model of primary succession

Vegetation dynamics in post-mining ecosystems are generally considered a type of primary succession with low probability of vegetation convergence (Wiegleb and Felinks, 2001a; Walker and del Moral, 2003; Tichew and Kirmer, 2007). Our study shows that in a simplified system where anthropogenic interferences are minimized, severe environmental filtering (comprising really drastic change as compared to the unaffected surrounding) can bring about discrete early vegetation types, with regular and predictable occurrence in space, and, we infer, possibly in time (Fig. 3.23). The study was undertaken more than 65 years after the onset of the fluvial pollution; unfortunately no historic land cover data were available, so the conceptual model offers a "reconstruction" of one possible scenario which fits the observations. The model relies on two major space-for-time substitutions: a) trends of Cu concentrations during aging of deposited pyritic Cu tailings (also discussed in Nikolic and Nikolic, 2012); and b) inferred trends of flooding intensity.

In brief, the model shows that, after sufficient amount of the tailings has been deposited, all former vegetation destroyed and previous land uses abandoned, the process of primary succession on this exceptional locality starts with the vegetative spread of the two poplar species from the edge of the polluted area (from the remnant trees/groves of the pre-pollution natural vegetation, Fig. 3.23A). Meanwhile, the regular floods which deposit new layers of the pyritic mine waste twice a year prevent any significant establishment of other vegetation types. Weathering of the barren tailings deposits (and Cu leaching) proceeds along the transect, while it is considerably prevented in soils under newly established poplars (Fig. 3.23A).

Eventually, after about 40 years from the first accident, the pollution finally ceases; the model assumes that after a while Timok starts to bring tailings-fee flood waters to the degraded alluvial land (Fig. 3.23 B, C and D). In the past, calcareous flooding waters of the Timok used to be an important nutrient input for local cropping practices (Antonović, 1974). Our unpublished results of the fresh river mud deposits collected after floods (pH 7-7.5; available nutrients (mg kg^{-1}): P, 20-45; K, 120-150; Ca, 280-350; Cu, 10-20) so far support the notion that the Timok floods currently act as pollution-free, relatively nutrient rich input.

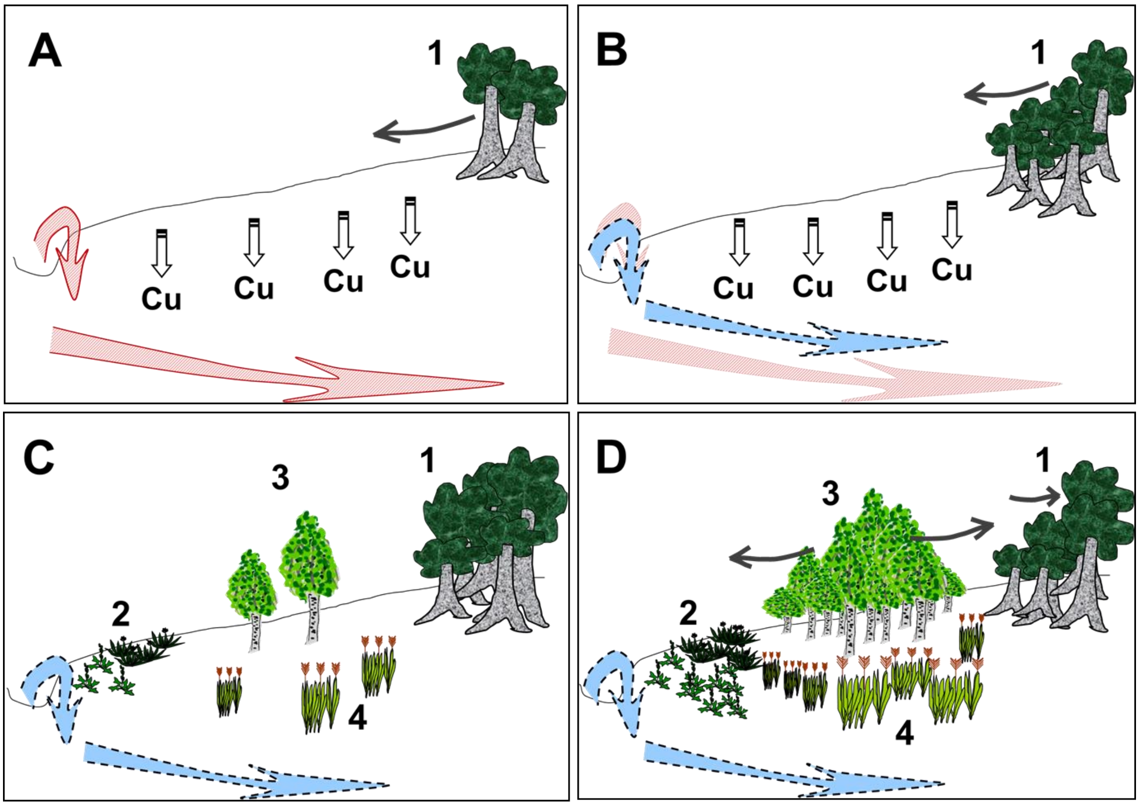

Fig. 3.23: Conceptual model of spontaneous revegetation of the research locality. 1 – forests dominated by *Populus alba* and *Populus nigra*; 2 – herbaceous pioneer stands dominated by *Rumex acetosella* and *Agrostis capillaris*; 3 – novel pioneer forests of *Betula pendula* and *Populus tremula*; 4 – tall grass stands of *Calamagrostis epigeios*. Line arrows denote the inferred trends in early vegetation dynamics; solid red arrows represent the fluvial deposition of the mining waste, while blue dashed arrows indicate the restored flooding by clear water after cessation of the pollution input.

We therefore hypothesize that the regime of flooding upon cessation of pollution (Fig. 3.23B) fosters the subsequent development of the pioneer herbaceous vegetation close to the river channel (Fig. 3.23C, see also Fig. 3.3). Gradually, pioneer birch-aspen forests develop at barren mid-portions of the transects, on nutrient-poor soils where Cu concentrations are already low (Fig. 3.23C); also *Calamagrostis* stands occasionally appear. Finally (Fig. 3.23D), our model predicts a spread of novel *Betula pendula*-

Populus tremula forests (see Fig. 3.20), and a retreat of older *Populus alba-Populus nigra* forests (see Fig. 3.16C, also Fig. 3.20). It is possible that *C. epigeios* stands might also extend with the increasing nutrient availability (see Fig. 3.3D). So far, the non-vegetated patches within the birch-aspen forests are found only at microlelevated dry sites (Fig. 3.16D); similar findings in a non-fluvial setup are reported by Prach (1987).

The spatially explicit Cu behaviour along the transects (higher soil Cu concentrations are found farther from the pollution source, an apparent “Cu paradox”, Fig. 3.18) coincides with the spatial succession of sparser and younger birch aspen-forest closer to the pollution source, by denser and older poplar forests at the outer zone of the polluted area (Table 3.4, Fig. 3.20). Earlier, Thompson and Proctor (1983) also mentioned that the highest soil Cu levels coincided with highest density of vegetation cover and highest soil organic matter. Higher metal concentrations in polluted soils under denser spontaneous vegetation was reported by some other studies as well (e.g. Sänger and Jetschke, 2004; Gallagher et al., 2008; Gallagher et al., 2011). These authors argue that the explanation of this phenomenon is some kind of a preference of forest species which dominate densest vegetation (*Betula* and *Populus*, incidentally very similar as in our study) for polluted soils with higher levels of metals.

In our study, however, it was possible to demonstrate that this phenomenon occurs as a consequence of spontaneous vegetation development on a substrate with highly dynamic chemical properties. Namely, older and denser riparian poplar forests, developed on the lowest levels of tailings deposition (as indicated by soil S_{tot} concentrations, Fig. 3.18) have actually phytostabilized the Cu from the tailings deposits, and thus prevented its leaching. Taken into consideration that the size of individuals from the *Populus alba-Populus nigra* stands is larger (Table 3.4, Fig. 3.20) and soil organic matter several times higher (indicated by C_{org} concentrations, Fig. 3.18), phytostabilization of Cu (and other metals) at the outer edge of the polluted zone can be inferred to had occurred earlier, before the other type of pioneer forests (birch-aspen) was initiated. Meanwhile, the weathering (via pyrite oxidation) of the tailings deposits on the non-vegetated soils has apparently been rather intensive (in accordance with the values of pH, Al, and SO_4/S_{tot} ratio, Fig. 3.18; schematically presented in Fig. 3.23A). Pyrite oxidation is shown to be the key drive of metal leaching in the alluvial soils polluted by pyrite-rich mine tailings (Álvarez-Ayuso et al., 2008). Our previous study showed that up to 8 t ha^{-1} of Cu can be leached below the depth of 30 cm during the weathering of barren tailings deposits (Nikolic and Nikolic, 2012). On the other hand, soil organic matter is known to prevent pyrite oxidation (Walker et al., 2004; Rigby et al., 2006) and thus contribute to a lower metal leaching. Over 50% of total soil Cu in acidic soils can be stabilized with soil organic matter; Cu retention was shown to be more dependent on Cu saturation and total amount of soil organic matter than on some of its' quality characteristics (Fernández-Calviño et al., 2008; Fernández-Calviño et al., 2010). Balabane et al. (1999) further advocate the existence of a positive feedback mechanism which maintains both high concentrations of stabilized heavy metals (impaired metal leaching), and persistence of high concentrations of soil organic matter (due to impaired biodegradation) in vegetated polluted soils.

Two issues, which warrant further investigation, are assumed by the proposed conceptual model: a) the decrease of flooding intensity during the period of several decades the research locality was formed; and b) sudden dieback of poplar trees at the edge of the current *Populus alba-Populus nigra* forests towards river channel (see Fig. 3.16B). These phenomena might explain an interesting observation in Fig. 3.20. Indeed, the comparison of the climatic characteristic for the periods 1961-1990 and 1991-2000 shows a decrease of annual precipitation by 19.6%, a decrease of the Lang's rain factor by 24.5% and an increase of de Marton's drought index by 22% in the latter period (Anđelković and Živković, 2007). It is quite probable that these climatic changes have decreased the reach of the flooding waters in recent times, and caused the existence of a strange unforested "band" between the two pioneer forest types (Fig. 3.20). The recent shrivelling of the young leaves and dieback of poplar trees was also mentioned by Marković et al. (2007). So far, this phenomenon has not been investigated. Our preliminary results which are not a part of this study, however, have shown that the only nutritional disorder indicated by the leaf mineral analysis was Mn deficiency; leaf Mn in drying poplars was below 20 mg kg^{-1} (for *Populus* species adequate leaf Mn levels are 35-100 mg kg^{-1}, Bergmann, 1992). Further research is needed to investigate the possible causes of low Mn availability on the highly weathered (oxidized), barren and not flooded mine waste deposits.

Water levels were shown to be the key factor structuring the floodplain vegetation, both at small scale (Gergely et al., 2001) and larger scales (Prach, 1996); zonation of vegetation along the water level gradients is one of the best studied phenomena in plant ecology. In this study, flooding water has had a key role both in bringing the pollution, and in fostering a spontaneous restoration by bringing the clear water again. However, the influence of water level gradients on pioneer vegetation on this locality was overridden by the effect of severe pollution-induced soil constraints, primarily by nutrient deficiencies.

The proposed conceptual model of spontaneous revegetation shows thus a spread of a novel formation dominated by *Betula pendula* and *Populus tremula*, with random and slow immigration of local floral elements. In this new, more xerophytic and oligotrophic habitat the reestablishment of the original alluvial vegetation and land use is unlikely.

3.5 Conclusions

This study describes a highly patterned, non-random primary colonization, with explicit spatial segregation of primary vegetation types as a consequence of severe environmental filtering and fluvial mode of pollution deposition. Pioneers species are the commonly reported colonizers of post-mining sites, with different phytoceonological affiliation, geographic origin, and widely different adaptations to major soil constraints, but they have in common an ability to maintain homeostasis of leaf P concentrations across a whole pollution-induced soil gradient. Succession relies on novel types of early vegetation which comprise not only novel combinations of species, but also the key role of species which are novel to the affected region. It is highly unlikely that spontaneous restoration under these conditions would allow the reestablishment of the original (or close to original) alluvial vegetation or agricultural land use. For the first time, this study has demonstrated that pollution-induced severe nutrient deficiency can override the well-established importance of both surrounding vegetation and water level gradient for primary succession.

Supplement 3.1: List of species occurring in the pioneer vegetation on the barren land degraded by fluvial deposition of sulphidic Cu tailings. H- herbaceous stands; F – forests. Nomenclature follows the W3 TROPICOS database of the Missouri Botanical Garden.

Species	Reference	Occurrence in pioneer associations		Abbrev.
		H	F	
Acinos arvensis	Dandy	x		Aci_arv
Agrostis capillaris	L.	x	x	Agr_cap
Agrostis gigantea	Roth	x	x	Agr_gig
Alliaria petiolata	(M. Bieb.) Cavara & Grande		x	All_pet
Amorpha fruticosa	L.	x	x	Amp_fru
Anagallis arvensis	L.	x		Ana_arv
Anchusa officinalis	L.	x		Anc_off
Andropogon ischaemum	L.	x		And_isc
Anthriscus sylvestris	(L.) Hoffm.		x	Ant_syl
Arctium lappa	L.		x	Arc_lap
Arenaria serpyllifolia	L.	x		Are_srp
Aristolochia clematitis	L.		x	Ari_cle
Arrhenatherum elatius	(L.) P. Beauv. ex J. Presl & C. Presl	x		Arr_ela
Artemisia vulgaris	L.	x		Art_vul
Betula pendula	Roth	x	x	Bet_pen
Brachypodium pinnatum	(L.) P. Beauv.		x	Bra_pin
Bromus sterilis	L.	x	x	Bro_ste
Calamagrostis epigeios	(L.) Roth	x	x	Cal_epi
Calystegia sepium	(L.) R. Br.		x	Cls_sep
Campanula persicifolia	L.	x		Cam_per
Carex hirta	L.	x		Crx_hir
Centaurea cyanus	L.	x		Cen_cya
Centaurea jacea	L.	x	x	Cen_jac
Centaurea stoebe ssp. *micranthos*	(S.G. Gmel. ex Gugler) Hayek	x	x	Cen_sto
Chamaenerion angustifolium	(L.) Scop.		x	Chm_ang
Chelidonium majus	L.		x	Chd_maj
Chenopodium album	L.	x		Chn_alb
Chenopodium botrys	L.	x		Chn_bot
Chondrilla juncea	L.	x		Cho_jun
Cichorium intybus	L.	x		Cic_int
Cirsium oleraceum	(L.)Scop.		x	Cir_ole
Clematis vitalba	L.		x	Cle_vit
Convolvulus arvensis	L.	x		Con_arv
Cornus mas	L.		x	Crn_mas
Crataegus monogyna	Jacq.		x	Cra_mon
Crepis capillaris	(L.) Wallr.		x	Cre_cap
Crepis conyzifolia	(Gouan) A. Kern.	x		Cre_con
Crepis tectorum	L.	x		Cre_tct
Cucubalus baccifer	L.		x	Cuc_bac
Cydonia oblonga	Mill.		x	Cyd_obl
Cynodon dactylon	(L.) Pers.	x		Cyn_dct
Cynosurus echinatus	L.		x	Cys_ech
Dactylis glomerata	L.	x	x	Dct_glo
Daucus carota	L.	x		Dau_car
Dipsacus fullonum	L.	x	x	Dps_syl
Echium vulgare	L.	x	x	Ech_vul
Equisetum palustre	L.	x	x	Equ_pal

Species	Reference	Occurrence in pioneer associations		Abbrev.
		H	F	
Erigeron canadensis	L.	x		Eri_can
Eryngium campestre	L.	x		Ery_cmp
Euonymus europaeus	L.		x	Euo_eur
Fraxinus ornus	L.		x	Frx_orn
Galinsoga parviflora	Cav.		x	Gls_par
Galium aparine	L.	x	x	Gal_apa
Galium verum	L.		x	Gal_ver
Geranium robertianum	L.		x	Ger_rob
Geum urbanum	L.		x	Geu_urb
Hieracium × auriculoides	Láng	x	x	Hie_aur
Hieracium pilosella	L.		x	Hie_pill
Hypochaeris radicata	L.	x	x	Hcr_rad
Hordeum bulbosum	L.		x	Hor_bul
Humulus lupulus	L.		x	Hum_lup
Hypericum perforatum	L.	x	x	Hyp_per
Juglans regia	L.		x	Jug_reg
Juncus effusus	L.		x	Jun_eff
Lactuca serriola	L.		x	Lac_ser
Lamium album	L.		x	Lam_alb
Linaria genistifolia	(L.) Mill.	x		Lin_gen
Linaria vulgaris	Mill.	x		Lin_vul
Lotus corniculatus	L.	x		Lot_cor
Lythrum salicaria	L.	x		Lyt_sal
Malus domestica	Borkh.		x	Mal_dom
Malva sylvestris	L.		x	Mlv_syl
Medicago lupulina	L.	x	x	Med_lup
Melica ciliata	L.	x		Mlc_cil
Melilotus albus	Medik.	x	x	Mel_alb
Melilotus officinalis	(L.) Lam.	x		Mel_off
Myosotis ramosissima	Rochel	x	x	Myo_ram
Papaver rhoeas	L.	x		Pap_rho
Parietaria officinalis	L.		x	Par_off
Persicaria lapathifolia	(L.) Gray	x	x	Per_lap
Petasites hybridus	(L.) G. Gaertn., B. Mey. & Scherb.		x	Pet_hyb
Phytolacca americana	L.	x	x	Phy_ame
Picris hieracioides	L.	x		Pic_hie
Plantago lanceolata	L.	x		Pla_lan
Poa pratensis	L.	x	x	Poa_pra
Polygonum aviculare	L.	x		Pol_avi
Populus alba	L.	x	x	Ppl_alb
Populus nigra	L.		x	Ppl_nig
Populus tremula	L.	x	x	Ppl_trem
Potentilla reptans	L.		x	Pot_rep
Prunella vulgaris	L.		x	Prn_vul
Pteridium aquilinum	(L.) Kuhn		x	Ptd_aql
Pyrus communis	L.		x	Pyr_com
Quercus cerris	L.	x	x	Que_cerr
Quercus frainetto	Ten.		x	Que_fra
Quercus petraea	(Matt.) Liebl.		x	Que_pet
Robinia pseudoacacia	L.	x	x	Rob_psa
Rosa agrestis	Savi		x	Ros_agr
Rubus ceasius	L.	x	x	Rub_cae
Rubus plicatus	Weihe & Nees	x	x	Rub_pli

Species	Reference	Occurrence in pioneer associations		Abbrev.
		H	F	
Rumex acetosella	L.	x	x	Rum_acl
Rumex crispus	L.		x	Rum_cri
Rumex obtusifolius	L.		x	Rum_obt
Salix alba	L.		x	Slx_alb
Salix fragilis	L.		x	Slx_fra
Salvia nemorosa	L.	x	x	Sal_nem
Sambucus ebulus	L.		x	Sam_ebu
Saponaria officinalis	L.	x	x	Sap_off
Scutellaria galericulata	L.		x	Scu_gal
Senecio rupestris	Waldst.	x		Sen_rup
Setaria glauca	(L.) P. Beauv.	x		Set_gla
Silene latifolia subsp. *alba*	(Mill.) Greuter & Burdet	x		Sil_lat
Solanum dulcamara	L.		x	Sol_dul
Solidago serotina	Retz		x	Sld_ser
Tanacetum vulgare	L.	x	x	Tan_vul
Tragopogon dubius	Scop.	x	x	Tra_dub
Trifolium pratense	L.	x	x	Tri_pra
Tussilago farfara	L.		x	Tus_far
Ulmus laevis	Pall.		x	Ulm_lae
Ulmus minor	Mill.		x	Ulm_cam
Urtica dioica	L.		x	Urt_dio
Verbascum thapsus	L.	x		Vbs_thp
Vicia cracca	L.	x		Vic_cra
Vicia grandiflora	Scop.		x	Vic_gra
Vicia hirsuta	(L.) Gray	x	x	Vic_hir
Viola arvensis	Murray	x	x	Vio_arv
Vitis sylvestris	Blume		x	Vit_syl
Vulpia myuros	(L.) C.C. Gmel.	x	x	Vul_myu
Xanthium italicum	Moretti	x		Xnt_ita

Chapter 3

4 Weed gradient caused by fluvial deposition of mining waste: environmental filters and functional adaptations at a field scale

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Summary

1. Low generality has, so far, been the major drawback in community ecology. A focus on explicit environmental gradients and plant functional traits is more likely to reveal general principles, particularly in a fairly simple model system as annual weed vegetation.
2. Exceptional research locality in Eastern Serbia: highly acidic, nutrient poor soils in a xerothermic calcareous surrounding are created by the long-term fluvial deposition of sulphidic Cu-tailings over the arable fields.
3. Research question: how would cereal weed assemblages respond to the sole and drastic change of soil if all the other factors are held constant.
4. We have analyzed soil parameters, leaf mineral composition and species abundance/biomass along the soil pollution gradient.
5. Highly systematic variation in weed assemblages along the clear soil gradient at the field scale comprises a patterned substitution of calcicoles by acidophytes, increase of circumpolar chorotype, decrease of Ellenberg indices for pH and continentality, decrease of therophytes, succession of Ca physiotypes, homeostasis of leaf Cu and of ratio of N:P in weed vegetation.
6. Species' ability to maintain leaf P homeostasis along the soil gradient appears to be the major filter underlying the soil-induced vegetation structure.
7. *Synthesis*. This study shows that plant functional adaptations to mineral stress can elucidate unusual species combinations which are typically observed as a consequence of strong environmental filtering, as for instance in post-mining sites.

Keywords: cereal weeds; calcicole – calcifuge behaviour; gradient analysis; N:P ratio; nutrient deficiency; post-mining soils;

4.1 Introduction

A need to identify more predictable ecosystem responses and general ecological principles is increasingly calling for a major shift in plant community ecology approach, from a “nomenclatural” one, centered on species identities, towards a focus on explicit environmental gradients and ecophysiological plant traits (McGill et al., 2006). Compared to other types of spontaneous vegetation, annual weed vegetation is a fairly simple and predictable system; hence, weeds are more likely to elicit the assembly rules and the relationship between plant attributes and current environment (Lososová et al., 2008; Petit and Fried, 2012).

Phytosociological approach, based on a premise of characteristic and predictable co-occurrence of plant species under similar environmental conditions, has dominated the extensive studies of European weed communities over the past 60 years. Recently, gradients in annual weed vegetation were shown to be caused by environmental (climate and soil) and management (crop, season, agricultural practices and landscape structure) factors (e.g. Hallgren et al., 1999; Chytrý et al., 2003; Lososová et al., 2004; Radics et al., 2004; Gabriel et al., 2005; Pyšek et al., 2005; Glemnitz et al., 2006; Fried et al., 2008; Cimalová and Lososová, 2009; Šilc et al., 2009; Pinke et al., 2010; Pinke et al., 2012). The lack of consensus about the relative importance of individual factors for the patterning of weed vegetation has arisen as a consequence of widely differing sampling designs of these studies (as discussed by e.g. Fried et al., 2008; and Šilc et al., 2009). Yet, broad-scale environmental gradients involving a set of co-varying soil and climatic variables (commonly soil pH, precipitation, or altitude), particularly within one crop type like cereals, consistently appear to be responsible for the major discontinuity between weed communities on the lowland drier basic soils and more acidic soils in moister areas. However, because of the known interdependence of soil pH and climate on larger scales, it has not been possible to assess the separate effect of soil on cereal weed vegetation (Fried et al., 2008; Cimalová and Lososová, 2009). Moreover, as all the studies so far were based on large sets of vegetation data but very modest soil parameters, at best pH and texture (and even these might not be directly comparable;

Chytrý et al., 2003), the influence of other important soil properties on cereal weeds is very little understood.

Landscapes degraded by mining activities, on the other hand, provide an exciting opportunity to study the effect of severe environmental filters on spontaneous vegetation, and thus to understand some important ecological principles which might not be apparent under “normal” conditions (e.g. Kirmer et al., 2008; Walker and del Moral, 2009). Agricultural soils, however, are commonly taken out of use and subsequent cropping is usually banned following pollution by mining wastes (as in the most thoroughly researched Azñalcóllar case, see Grimalt et al. (1999).

This survey was undertaken on an exceptional model locality in Eastern Serbia, where pyrite-rich tailings from a copper mine had been deposited over alluvial fields during almost 50 years of regular annual flooding, and some partially damaged fields at the outer zone of the polluted area are still regularly cropped (Nikolic et al., 2011; Nikolic and Nikolic, 2012). This large scale accident has caused a drastic change from unpolluted calcareous loamy soils towards acidic, nutrient poor, sandier soils over very short distances. The gradients in soil properties and cereal crops response were shown to be clear, consistent and spatially explicit (Nikolic et al., 2011; Nikolic and Nikolic, 2012). The objective of this study is to investigate the change of cereal weeds along the pollution-induced soil gradient. The major research question is: how would cereal weed assemblages respond to the sole change of soil if all the other factors (altitude, climate, season, year, agricultural practices, surrounding vegetation, and landscape context) are held constant. The elucidation of the effect of soil constraints on weed community patterning should also enhance the understanding of the effects of environmental filters on spontaneous vegetation.

4.2 Materials and methods

4.2.1 Research locality

4.2.2 Soil

Arable alluvial soils in the lower course of the Timok river in Eastern Serbia, traditionally used for grain production, were originally of loamy texture, calcareous (pH > 7.5), and relatively poor in available nitrogen, phosphorus and soil organic matter; regular floods used to provide an important nutrient input for local cropping practices (Antonović, 1974). Outside the floodplain research area, unpolluted acidic soils do not occur within the distance of 10 km, and acidic arable soils are found much farther

(Antonović, 1974). Since 1940's, however, these soils have been drastically altered by the long-term release of mining waste from the Bor copper mines directly into the local river system (for details see Nikolic and Nikolic, 2012). More than 10,000 ha of arable land is permanently lost for agriculture, and much more is severely degraded.

In brief, fluvial deposition (via regular floods) of highly sulphidic (pyritic) copper tailings over arable alluvial land has created multiple constraints for plant growth: nutrient deficiency (primarily P and microelements), excessive concentrations of available Cu, and, as a result of the oxidative weathering of deposited pyrite, very low pH (about 4) and excessive concentrations of available Al. Intensity of soil pollution (well indicated by the soil S_{tot} concentrations) regularly decreases along the spatial gradient (relative distance from the water channel, modified by the microelevation gradient), and is highly correlated with the visual symptoms in the crop field. In general, the gradient of soil pollution from the almost unaffected carbonate alluvial soils towards the acidic, highly altered soil comprises a directional change of major soil properties: pH, CEC, carbonates, concentrations of plant available P, Ca, Mg, K and B decrease; concentrations of total S, available Fe, Cu and Al increase along the gradient, while total N is consistently low (for details see Nikolic et al., 2011, and Nikolic and Nikolic, 2012).

4.2.3 Climate and vegetation

The research area is under both continental and submediterranean climatic influences characterized by dry hot summers, cold winters, and bimodal rainfall pattern with annual precipitation below 600 mm (Fig. 4.1). The former natural vegetation, prior to conversion to cropland and subsequent pollution by mining effluents, had been azonal alluvial forests of the Populetalia albae Br.-Bl. 1931 order. Alluvial forests had largely been converted to cropped fields; since 1950's, however, cropping had to be abandoned because of the large-scale pollution by mining effluents. Fields were converted to wastelands, and only recently, upon the cessation of the pollution influx, the spontaneous revegetation process of this barren land has begun, predominantly by very tolerant species such as *Rumex acetosella, Agrostis capillaris, Betula pendula* and *Populus tremula* (Chapter 2).

Outside of the fluvial influence, the thermpohilous varieties of the climazonal Quercion frainetto-cerridis Rudski 1940 (1949) forests are, because of carstic geology, dissected terrain and shallow calcareous soils on steep slopes, often substituted by more xerothermic extrazonal vegetation of the Quercion pubescentis-petraeae Br.-Bl. 1931 alliance (Mišić, 1997). The former agricultural land uses (annual food crops in the lowland, mostly viticulture on steeper terrains) have been increasingly abandoned in the past decades, and the landscape is now dominated by a matrix of different successional stages of fallow vegetation.

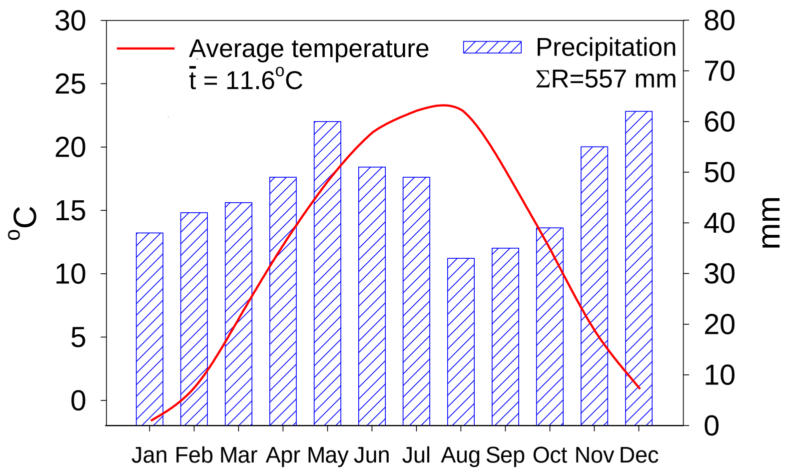

Fig. 4.1: Climatic conditions of the research area. Climate diagram according to Walter: t – average annual temperature; ΣR – total annual precipitation; data for the Meteorological Station Negotin, 1961-1999.

On the prevailing neutral to calcareous soils in the lowlands of the Timok valley, the two major associations of winter cereal weeds have been recorded: *Consolida regalis-Polygonum aviculare* ass. var. lathyrethosum aphacae Kojić 1973 (crop aspect) and *Stachys annua-Ajuga chamaepitys* ass. var. typicum Slav. 1952 (stubble aspect). These therophytic communities belong to the Caucalidion R.Tx.1950 alliance and are characterized by the dominance of Submediterranean and Eurasian floristic elements (Milijić, 1980).

4.2.4 Field trails

The survey included 26 fields of winter cereals (18 wheat and 8 fodder barley fields), located within the 5 km radius from the N 44º 04´34″, E 22º31′10″ (about 70 km downstream from the pollution source), at meander positions where the Timok river flow slows down and creates a braided channel pattern. Satellite image of the research area (fields belonging to the villages Rajac, Braćevac and Tamnič) is available at: http://maps.google.com/maps?ll=44.082508,22.519602&z=13&t=h&hl=en). All the fields were at the outer edge of the affected area, in a rather narrow band between the unpolluted soils on the upper part, and severely polluted soils where cropping had been abandoned on the lower part the microelevation gradient in the floodplain. Hence, all the surveyed fields were partially damaged by the fluvially deposited tailings. Despite the very visible delineation of the soil severely affected by tailings deposition, the whole area of a field is nevertheless uniformly cropped (local cultural issue).

In the predominant low-input system, rainfed winter cereals yield between 2 and 4 t ha^{-1} on non-polluted soils in the research area. Fields were selected on the basis of detailed participatory surveys with local farmers (loamy soil texture, extensive crop management and the absence of waterlogging in the year of the trial). The surveyed fields were fertilized with 50-75 kg ha^{-1} of NPK (15:15:15) at sowing, followed by about 50 kg N ha^{-1} early in spring; also, low doses of manure (up to 10 t ha^{-1} each 2-4 years) were usually applied. Herbicides are sparingly applied in this low-input system: systemics (commonly Glyphosate) are applied in the autumn before sowing, and occasionally some auxinic herbicides (IAA derivates) during the stem elongation phase.

4.2.5 Weed survey

While the selection of the survey fields was rather opportunistic, vegetation (and soil) sampling within each field was carried out according to the flexible systematic model (Smartt, 1978), a form of stratified sampling where samples were allocated on the basis of the visual symptoms in the cereal crop (Supplement 4.1). Relative yield reduction of a cereal crop was calculated *a posteriori* (relative to the "no symptom" plots), and found to closely correspond to the observed visual differentiation of the crop field. Intensity of soil pollution (well indicated by the soil S_{tot} concentrations) regularly decreases along the spatial gradient (distance from the water channel) and is highly correlated with the visual symptoms in the crop field. In each field, it was possible to clearly distinguish two to four (depending on the site-specific topography) physiognomically uniform areas (zones) along the transect perpendicular to the river channel (as previously described by Nikolic et al., 2011). Of a total of 100 samples (relevées), 26 samples each were allocated to Zones 1, 3 and 4, while Zone 2 contained 22 samples (plots). The internet associated W3 TROPICOS nomenclatural database of the Missouri Botanical Garden, and the associated authority files (http://www.tropicos.org/Home.aspx), were used as reference.

To reduce the noise and increase the chance to capture the response of weed vegetation to soil only, we have excluded vernal ephemeres and high-summer (i.e. post-harvest, stubble) species, which are assumed to be less dependent on soil, and more on the season (e.g. Lososová et al., 2006). The vegetation of the selected 26 cereal fields was continuously monitored (check each 10 days) during the period from 15th May to 15th July 2009 (i.e. till crop harvest). Cover-abundance of weed species was estimated on the extended nine-grade Braun-Blanquet scale (van der Maarel, 2007) at every check, in the standardised area of 30 m^2; the final scores used for each species were the highest recorded values during the 3-months monitoring.

4.2.6 Plant sampling and analyses

Weed shoot biomass and leaf sampling were carried out at the onset of milky development of the cereal crop, immediately after the anthesis (Zadoks growth stage Z71-75), when the typical weed association (Consolido-Polygonetum avicularis) is fully developed. In each sample, one 1 m × 1 m quadrate was laid for destructive sampling; biomass was clipped, sorted by species, air dried and weighted. For each weed species encountered, ten leaves were taken for a composite sample for tissue elemental analyses, as well as 20 flag (youngest fully emerged) leaves from a cereal crop. Crop individuals were sampled for chemical analysis only if they were alive and able to set at least 4 seeds in the spike.

Plant tissue samples were thoroughly washed with deionised water, dried at 70 °C and digested with a mixture of HNO_3 and H_2O_2 (3:2) in a microwave oven. Upon digestion, the concentrations of nutrients (P, K, S, Ca, Mg, Fe, Cu, Zn, Mn, B, Mo, and Ni) and other elements (Al, As, Pb, Cd, and Cr) were determined by inductively coupled plasma optical emission spectrometry (ICP-OES; SpectroGenesis EOP II, Spectro Analytical Instruments GmbH, Kleve, Germany). The concentrations of C and N in leaf samples were determined by the CHNOS elemental analyzer (Vario ELIII, Elementar Analysensysteme GmbH, Hanau, Germany). Elemental concentrations in weed vegetation (per 1 m^2) were calculated as an average of leaf mineral concentrations in each species, weighted by the relative contribution of species to the total plot biomass.

4.2.7 Soil sampling and analyses

A composite soil sample (taken at mid to late April) was obtained by mixing three subsamples from the depth of 0-30 cm, taken in each visually distinguished zone of cereal crop appearance, i.e. in each weed relevé. Soil samples were analysed in fine earth fraction (< 2 mm), after drying and sieving through a 2 mm mesh screen. pH was measured in water (soil : water = 1:2.5), soluble $CaCO_3$ was determined by calcimetry, and the concentrations of total C, N and S by the CHNOS elemental analyzer. Organic carbon (C_{org}) was calculated from total C and $CaCO_3$. Potential CEC was determined by ammonium acetate extraction buffered at pH 7 (with ethanol treatment adjusted for salty and carbonate samples). The precision and accuracy of the analysis was assessed with the reference sample NCS ZC 73005 Soil (CNAC for Iron and Steel, Beijing, China). Different extraction procedures were applied to determine plant available concentrations of elements: ammonium lactate – ammonium acetate (AL) for P and K, ammonium acetate (NH_4-Acc) for Mg and Ca, KCl extraction for available Al, and hot water (70 °C) for B. Plant available fractions of metals (Fe, Cu, Zn and Mn) were extracted by the DTPA-TEA solution (buffered at pH 7.3), with the soil: solution ratio of 1:5 (Norvell, 1984). The concentrations of chemical elements in soil samples subjected to different extraction procedures were determined by ICP-OES, with the exception of the P concentrations which were determined colorimetrically at 882 nm.

4.2.8 Statistical analyses

For all the subsequent analyses the records on nine-grade Braun-Blanquet scale were transformed to a quasi-metric 1-9 ordinal transform scale of ordinal transform values (OTV, van der Maarel, 2007) which roughly correspond to the logarithm of percentage cover. Species not achieving at least 20% frequency in at least one sampling zone, and species with overall frequency less than 10%, were excluded from further analyses; as well as outliers flagged by more than 2.3 standard deviations from the average distance. The final set comprised 84 weed species recorded in a total of 100 relevées (Supplement 4.2).

All multivariate analyses were done by PC-ORD6 software (MjM Software Design, Gleneden Beach, USA). In all analyses except CCA Sørensen distance was used. Only one data transformation was applied: relativization of OTV by sample unit totals. This transformation facilitates the focus on a shift in species composition along the gradient by emphasizing the relative contribution of a species to the total abundance in a sample unit. Hence this transformation accounts for the different competition pressure with cereal crop along the gradient, and the resulting different weed abundances which are not directly related to soil properties.

To test whether the visual zones of crop growth disorders (Supplement 4.1), used as a basis for sampling, really reflect the significant difference in weed vegetation along the gradient, the nonparametric Multi-Response Permutation Procedure (MRPP; Mielke and Berry, 2001) was used. MRPP compares if the distances among sample units within the zone are significantly smaller than it would be expected if they were randomly assigned to other zones. The reported test statistic T describes the separation between the samples in different zones; the more negative is T, the stronger is the separation. The chance-corrected within-group agreement (A) describes the homogeneity within the zones compared to the random expectation; A increases with the increasing similarity among the samples assigned to one zone.

Indicator Species Analysis (ISA) was done following the method of Dufrêne and Legendre (1997). This method gives indicator value (in % of perfect indication) of a species, based on combining relative abundance and relative frequency. Weed species with very high indication (Indicator Value > 30%, P value of the Monte Carlo test < 0.01) for a certain visual zone of cereal crop disorder (see Supplement 4.1) were further classified by agglomerative cluster analysis with the space-conserving flexible beta method (β = -0.25). The resulting dendrogram is scaled by the Wishart's objective function.

The unconstrained ordination of weed vegetation data was done by Nonmetric Multidimensional Scaling (NMS), using OTV data and Sørensen distance. A final 2-d solutions with instability < 10^{-5} and final stress (Monte Carlo test for stress in relation to dimensionality) of 17.55 for unrelativized and 17.76 for relativized OTV data were selected after 98 and 68 iterations for the relativized and unrelativized data respectively. The solutions were varimax rotated.

Envelope curves, i.e. fitted smoothed lines (by nonparametric regression model) which include 2σ of a running species abundance mean, plotted against the first NMS axis, were used to visualize the species response to the major soil gradient. Envelope curves show upper bounds of a solid species response curve. To measure the strength of association between the species, 2 x 2 contingency tables of presence-absence were used. Plexus values (φ coefficient) were calculated as a standardized χ^2 statistic for species association; Yates' correction was applied. φ coefficient ranges from +1 (perfect positive association) to -1 (perfect negative association).

To analyze the portion of weed community structure which can be linearly related to the major soil gradient, Canonical Correspondence Analysis (CCA, ter Braak,

1986) was used. Axes were scaled by Hill's method, LC scores were interpreted (sample scores were linear combinations of soil variables). Monte Carlo test for species-environment correlations, based on 999 runs with randomized data was used to test the hypothesis "no relationship between species and environmental matrices". Univariate analysis (ANOVA) was done by STATISTICA 6 (StatSoft Inc., Tulsa, USA). Variables with approximately lognormal distribution were log transformed prior to analyses. A posteriori comparison of means was done by Tukey's test, with $\alpha = 0.05$. Ellenberg indictor values for each plot were calculated as averages weighted by the species cover-abundance (OTV).

4.3 Results

4.3.1 Floristic changes along the transects

In the cereal fields partially degraded by deposited mine tailings, the total plant biomass (crop + weeds) decreases along the transects which correspond to the spatial gradient perpendicular to the water channel (Fig. 4.2). The increasing pollution load (indicated by increasing soil S_{tot} concentrations) causes a prompt and severe growth reduction of cereal crop, while the total weed biomass remains higher than on the relatively unaffected ($S_{tot} < 0.2\%$) soils along the gradient, and has a clearly hump-shaped response.

The floristic composition of weed assemblages (recorded over the 3-months survey period) significantly varies among all the *a priori* delineated zones of crop growth disorders (Table 4.1). MRPP test shows the strongest separation between the samples at the beginning and at the end of the pollution load gradient, i.e. between the Zones 1 and 4.

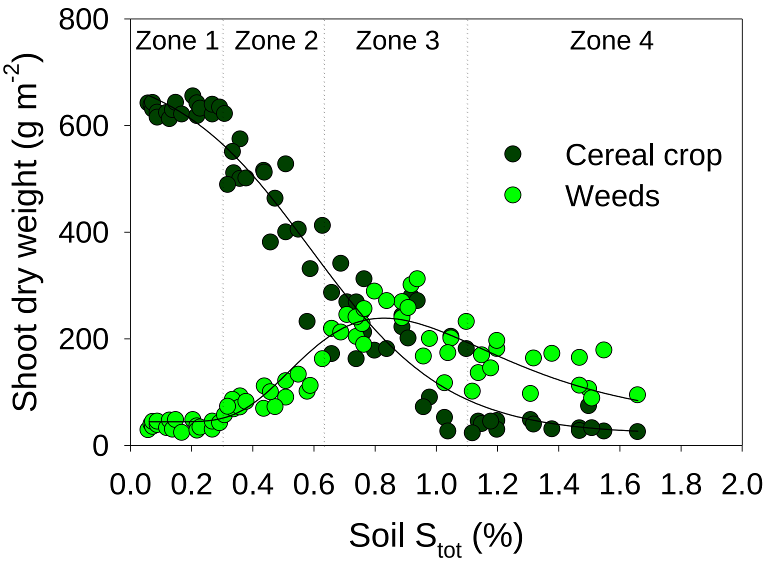

Fig. 4.2: Aboveground biomass of cereal crop (milky ripeness, Z71-75) and weeds along the soil pollution gradient

Table 4.1: MRPP test (Sørensen distance) shows that weed assemblages significantly differ among the visual zones of crop growth on polluted soils.

	Absolute abundance		Relative abundance	
Overall comparison	$A = 0.236, P < 10^{-8}$		$A = 0.214, P < 10^{-8}$	
Multiple comparisons among the zones	T	A	T	A
1 vs 2	-7.10	0.022	-4.66	0.014
1 vs 3	-29.26	0.122	-25.95	0.091
1 vs 4	-33.55	0.252	-33.50	0.239
2 vs 3	-21.26	0.079	-19.34	0.067
2 vs 4	-31.12	0.274	-30.84	0.253
3 vs 4	-33.14	0.245	-32.38	0.222

A: chance-corrected within-group agreement.

Sampling zone has the lowest effect on separating weed samples from Zones 1 and 2. During the 3-months survey, the lowest number of species and the lowest floristic distance among the fields was recorded on the most severely degraded soils (Zone 4, Table 4.2) The analysis of species association further showed that, along the whole transect, the φ coefficient higher than + 0.5 was only found in the Zone 4, namely among the following species: *Rumex acetosella*, *Agrostis capillaris*, and *Persicaria lapathifolia* (see also cluster C1, Fig. 4.3).

Table 4.2: Floristic homogeneity and species richness of weed samples in different field zones along the soil pollution gradient. Maximal abundance of all the species recorded during the survey is used.

	Visual zones in polluted cereal fields			
	Zone 1	Zone 2	Zone 3	Zone 4
Weighted mean distance (relative Sørensen)	0.70	0.64	0.60	0.54
Average species number per sample	27.1	36	35.2	10.9
Total species number recorded [a]	76	80	81	61

[a] during the 3-month survey

Table 4.3: The change in species composition (presence/absence data) of cereal weeds observed during the 3-months survey of 100 samples along the soil gradient.

	Shared species	
Visual zones in polluted cereal fields	Number	% of total in both zones
1 and 2	76	95.0
1 and 3	73	93.4
1 and 4	53	63.1
2 and 3	76	89.4
2 and 4	56	65.9
3 and 4	60	73.2

The mere presence/absence of weed species along the gradient however does not indicate the clear separation of weed assemblages among the zones as shown by the MRPP test. Of the total of 84 weed species recorded in the survey, 63% are present on both relatively undamaged calcareous soils of the Zone 1, and on the most severely altered acidic soils where drastic reduction of crop growth occurs in Zone 4 (Table 4.3). Furthermore, Table 4.2 and Table 4.4 show that floristic composition between the weed assemblages assigned to Zone 1 and the Zone 2 (i.e. up to about 30% of the soil pollution-induced crop yield reduction) differs very little (though this difference is statistically significant). This difference is decreased by relativization of the species abundances (Table 4.1). That is, the soil pollution-induced change of cereal weed samples between Zones 1 and 2 is mainly induced by the increased abundances. In the milky ripeness phase, this 2.5-fold increase in weed biomass occurs with decreased competition from cereal crop (Fig. 4.2).

Though not a single species was found to be confined to one visual zone of crop growth disorders, indicator species analysis (ISA) performed on the relativized maximal abundances recorded during the survey, pointed out 44 species which very clearly indicate the observed zones of crop growth disorders along the soil gradient (P value of the randomization test < 0.01) . These 44 species (Fig. 4.3) have indicator values (IV) larger than 30%. For the clarity of presentation, samples assigned to Zones 1 and 2 are grouped together, since they are found to be composed of almost the same species (Table 4.3), and differ mostly in absolute abundances. The clustering of species (Fig. 4.3) shows the three major groups of weeds with similar response to the change of soil properties along the transects (denoted A, B and C). These three groups account for about 80% of variation in distances between weed species (based on their relative abundances) along the soil gradient. The succession of these three groups along the soil gradient in damaged fields is rather visually apparent over short distances (Fig. 4.4).

4.3.2 Underlying soil gradient

The soil gradient induced by fluvial deposition of sulphidic Cu tailings is often visually apparent on non-vegetated fields (Fig. 4.4A). There is a very strong pattern in weed community along the induced soil gradient (Fig. 4.4 B, C, D). The clear-cut succession of the three major weed groups (as defined in Fig. 4.3) along the transect through the damaged fields is remarkable (Fig. 4.4). Highly altered, acidic soils are dominated by *Rumex acetosella* and *Agrostis capillaris* (the green “band”, Fig. 4.4) at the lowest portions of the microelevation gradient and closest to the water channel. Middle portions of the transect are dominated by some widespread species (see group B, Fig. 4.3), while weeds of the relatively unaffected calcareous soils (group A, Fig. 4.3) are rather inconspicuous due to the better cereal crop growth (see Fig. 4.2). Accordingly, free ordination of the weed relevées shows a very clear separation of vegetation along the NMS Axis 1 (Fig. 4.5). A strong first ordination axis shows that about 70% of variation in weed vegetation is actually accounted for by sampling zones based on the visual symptoms of cereal crop growth disorders (see Supplement 4.1).

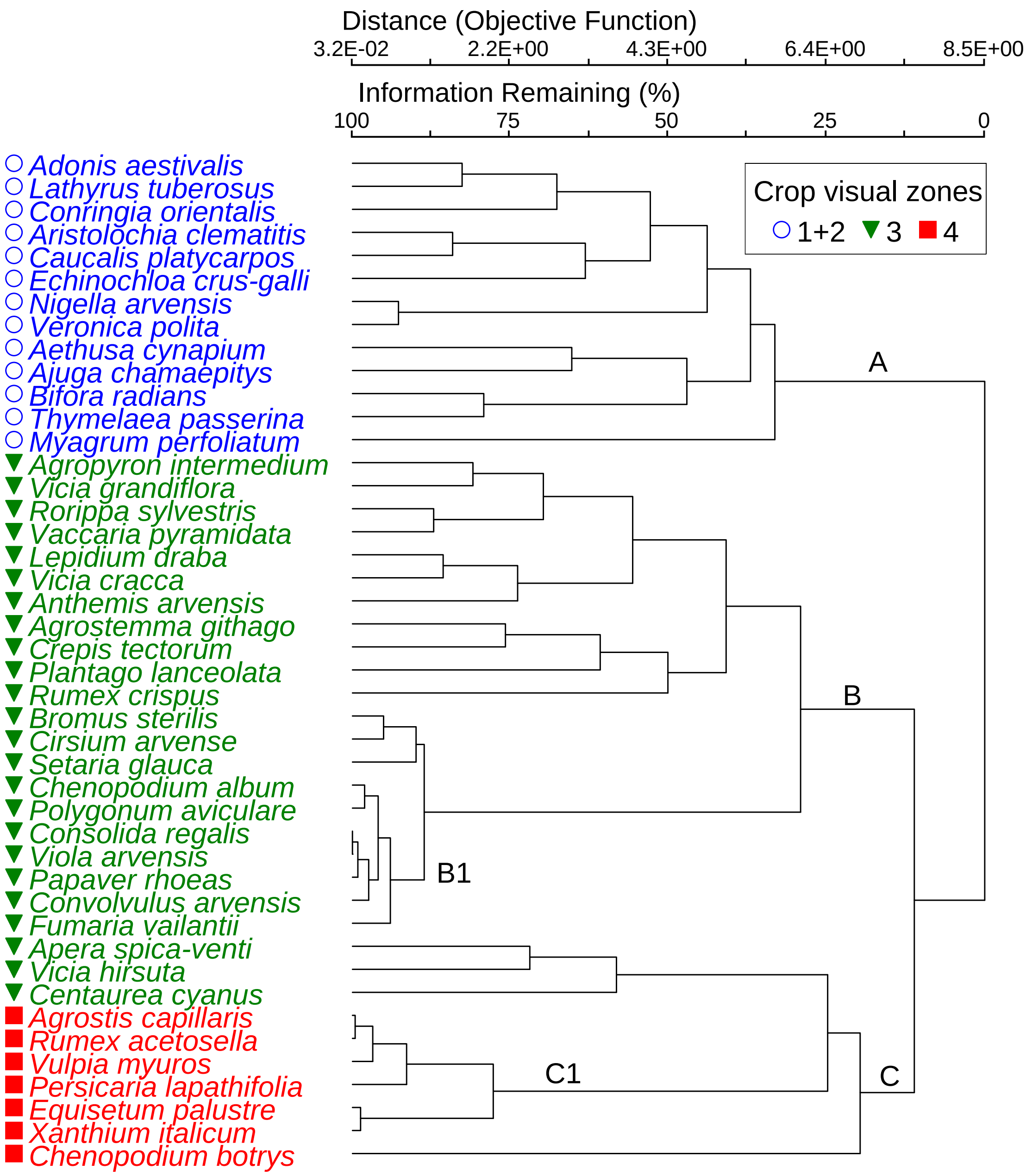

Fig. 4.3: Classification of weed species which highly indicate (IV> 30%, P < 0.01) visual zones of crop growth disorders along the soil gradient. A – species indicating relatively unaltered calcareous soil. B – species of broad valence dominant in the middle portions of the soil gradient. C – species indicating most severely altered, nutrient-poor acidic soils. B1 and C1 – weeds with IV > 50%, and with major contribution to the weed biomass (measured in crop milky ripeness) in the Zones 3 and 4, respectively. Clustering: relative abundance, Sørensen distance, flexible linkage (β=-0.25).

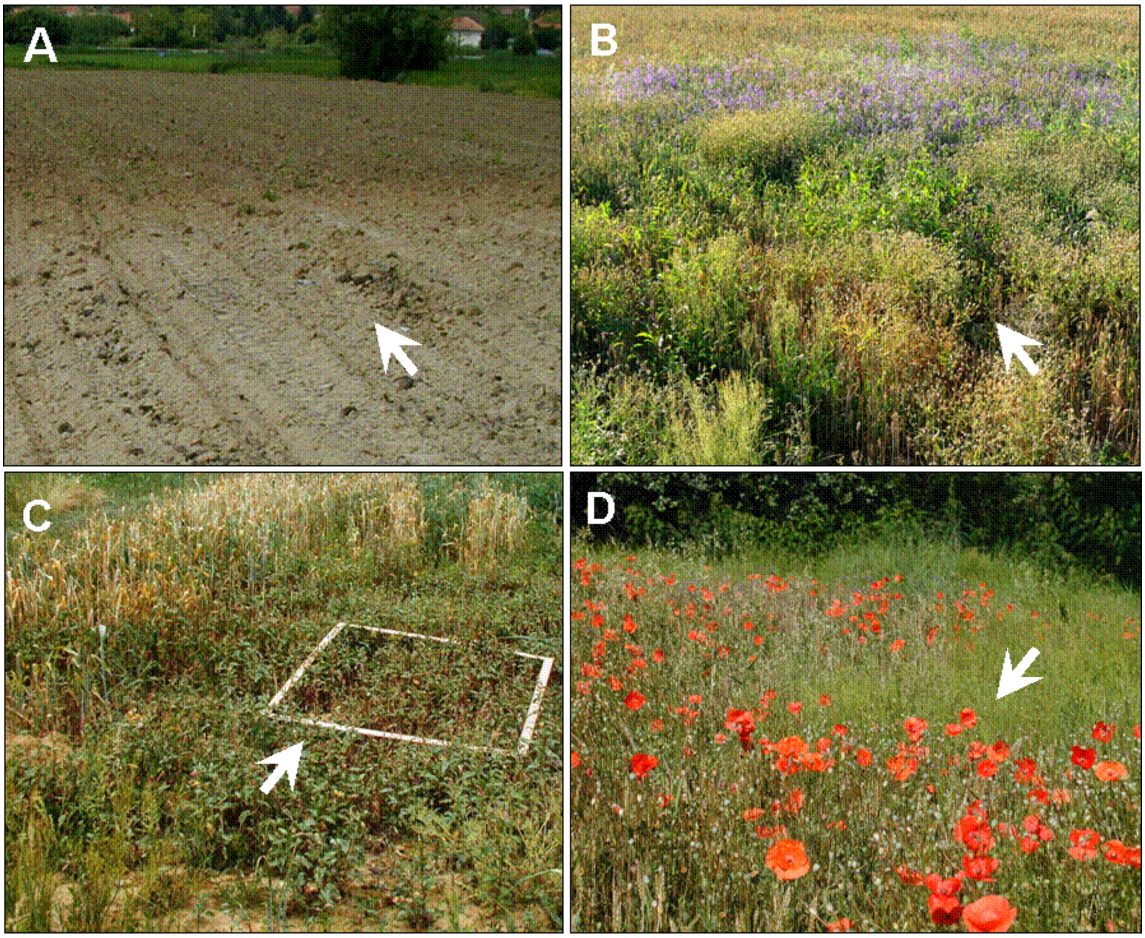

Fig. 4.4: Gradients in cereal fields partially damaged by the deposition of mining waste. Pollution load decreases from the arrow bottom (acidic soils, Zone IV, dominance of *Agrostis capillaris* and *Rumex acetosella*) towards the arrow tip (relatively unaltered calcareous soils). A – Soil gradient (before crop emergence); B – Zone III, facies with *Consolida regalis* (violet flowers); C – Zone IV weeds, facies with *Persicaria lapathifolia*; D – Zone III, facies with *Papaver rhoeas* (red flowers).

Sixteen soil chemical parameters which significantly change along the transects through partially damaged fields (plant available concentrations of Ca, Mg, K, P, B, Fe, Zn, Mn, Al, Cu; C_{org}, N_{tot}, S_{tot}, $CaCO_3$, pH, CEC) were overlaid on the weed samples ordination; nine were correlated by more than 10% with ordination scores (Fig. 4.5, Table 4.4). Soil parameters which might directly affect plant growth in the encountered concentration ranges (Table 4.4) are very strongly correlated with the NMS Axis 1: Kendall correlation (tau) of pH, Ca, P and Al are -0.72, -0.62, -0.61 and 0.63 respectively (for the unrelativized abundances, Fig. 4.5A; and even higher for the relativized data, Fig. 4.5B).

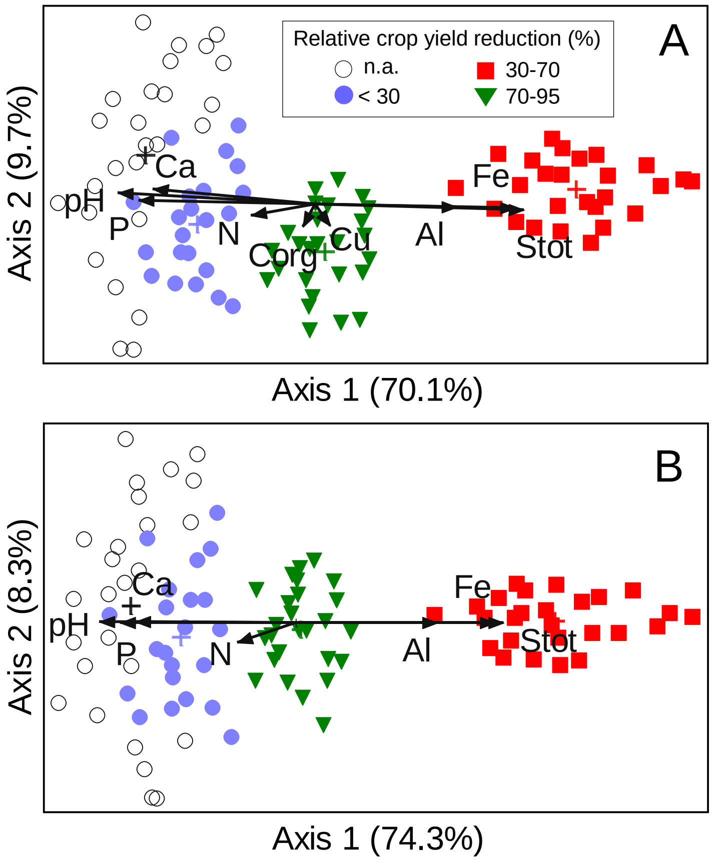

Fig. 4.5: Unconstrained ordination (NMS) of weed samples along the transects in cereal fields partially damaged by mine tailings. Data matrix: 84 species (Supplement 4.1) in 100 samples, maximal species abundances (OTV) recorded during 3 months-survey. A– untrelativized OTV; B – relativized OTV. The values in parenthesis denote the proportion of variance represented by each axis. Superimposed soil variables correlated by more than 10% with weed samples ordination are shown. The angles and lengths of the radiating lines indicate the direction and strength of relationships of the soil variables with the ordination scores. Crosses denote group centroids.

There is a very high degree of multicollinearity among the parameters which have the strongest correlation with the NMS Axis 1 (Fig. 4.5). Despite the rather high concentrations of plant available Cu, little systematic variations of weed vegetation is found to coincide with the gradient of soil Cu concentrations. Multiple linear regression (CCA, Table 4.5) shows that soil pH accounts for about 14% of the total variance (χ^2 distances) in weed vegetation data. Soil pH is strongly correlated with other major constraints, namely P, Al and Ca, whose effect is thus not accounted for by CCA.

Table 4.4: Soil chemical properties along the pollution gradient correlated by > 10% with the NMS ordination scores of weed relevées. Plant available concentrations of elements (obtained by different extractions) are shown. Mean values ± SD followed by the same letter in a row are not different ($P < 0.05$).

Parameter	Visual zones of crop growth disorders			
	1	2	3	4
	Relative yield reduction (%)			
	n.a.	< 30	30-70	70-95
S_{tot} (%)	0.17 ± 0.07 a	0.5 ± 0.2 b	0.9 ± 0.3 c	1.3 ± 0.2 d
pH (in H_2O)	7.9 ± 0.3 a	6.9 ± 0.3 b	5.6 ± 0.6 c	4.6 ± 0.5 d
C_{org} (%)	1.8 ± 0.8 a	1.9 ± 0.9 a	2.0 ± 0.9 a	1.6 ± 0.8 a
N_{tot} (%)	0.19 ± 0.07 a	0.18 ± 0.07 a	0.16 ± 0.04 a	0.13 ± 0.02 b
$Ca_{NH4\text{-}Ac\text{-}extr.}$ (mg kg^{-1})	331 ± 36 a	283 ± 42 b	166 ± 33 c	139 ± 38 c
$P_{AL\text{-}extr.}$ (mg kg^{-1})	86 ± 14 a	75 ± 12 a	52 ± 20 b	28 ±12 c
$Fe_{DTPA\text{-}extr.}$(mg kg^{-1})	9 ± 6 a	25 ± 10 b	61 ± 17 c	97 ± 18 d
$Cu_{DTPA\text{-}extr.}$ (mg kg^{-1})	23± 10 a	70 ± 42 b	112 ± 51 c	82 ± 56 bc
$Al_{KCl\text{-}extr}$ (mg kg^{-1})	0.3 ± 0.2 a	0.7 ± 0.6 a	27 ± 17 b	39 ± 25c

Table 4.5: Multiple linear regression of weed relevées on soil pH.

Canonical Corespondence Analysis summary	
% of variance explained by Axis 1	13.7
Intra-set correlation (pH and Axis 1)	1.0
Standardized canonical coefficient [a]	1.185
Species-environment correlation (Pearson) [b]	0.874, P=0.01

[a] unique contribution to regression solution, centered and standardized

[b] correlation between sample scores derived from the species data and sample scores derived from weighted linear regression on soil pH; P value of the Monte Carlo randomization test is shown.

Details of the soil-induced change in weed species dominance along the major soil gradient are shown in Fig. 4.6. Soil gradient is represented by the strongest ordination axis (NMS Axis 1) as demonstrated in Fig. 4.5. Species response curves of the three major weed groups (obtained by the indicator species analysis and classification, Fig. 4.3), have a distinct pattern along the gradient. Species typical for the unpolluted calcareous soils (Fig. 4.6A and D) gradually decrease with the pollution-induced soil acidification. Species known to have a rather broad tolerance achieve the maximal abundance in the middle portions of the gradient (Fig. 4.6B), but their relative abundance (Fig. 4.6E) tends to remain rather constant over a range of soil conditions. Finally, a group of species adapted to acidic, nutrient poor soils gradually increases along the gradient (Fig. 4.6C and F); at the most severely altered soils they face very little competition from either cereal crop (see Fig. 4.2) or other groups of weeds.

4.3.3 Ecophysiological adaptations of weed vegetation induced by the soil gradient

A patterned change in floristic composition and dominance relations of the weed assemblages (Fig. 4.3; Fig. 4.4; Fig. 4.5; and Fig. 4.6) along a clear, anthropogenically induced soil gradient (decrease of pH and available nutrients and increase of Al and Cu; Table 4.4, Fig. 4.4A and Fig. 4.5) underlies a gradient in major ecological adaptations at the weed vegetation level (Fig. 4.7). Over very short distances (up to few tens of meters, depending on the microlelevation gradient), the spontaneous vegetation indicates significantly different habitat conditions (Fig. 4.7A) which clearly reflect the induced change of a calcareous soil: species of different potential niches, which have their optima in more acidic, nitrogen-poor and moister habitats clearly dominate the most severely altered, acidified soils (Zone 4). Under the same climatic and management conditions, in the same field, drastic soil alteration favours perennials with vegetative propagation (hemicryptophytes), typical of cooler and moister climates, at the expense of therophytes (Fig. 4.7B) which normally dominate cereal weed communities. This is generally in the same line as the observed decrease of the continentality index (Fig. 4.7A). Likewise, the induced soil acidification and nutrient impoverishment underlies the marked substitution of the local floral elements (most markedly of the (Sub)pontic and Submediteranean) by the adapted species of very broad phytogeographical provenance (Circumpolar chorotype; Fig. 4.7C).

The concentration of mineral elements in the foliage of weed species might reveal differences in physiological adaptations of the dominant species along the soil gradient. In general, the pollution-induced enrichment of soil by S and available Fe and Al fractions is the most prominent soil change (more than 5-fold increase compared to the relatively unpolluted soils, Table 4.4); expectedly, leaf concentrations of Fe, and to a lesser degree of S and Al both in weeds and in crops systematically increase along the pollution gradient (not shown).

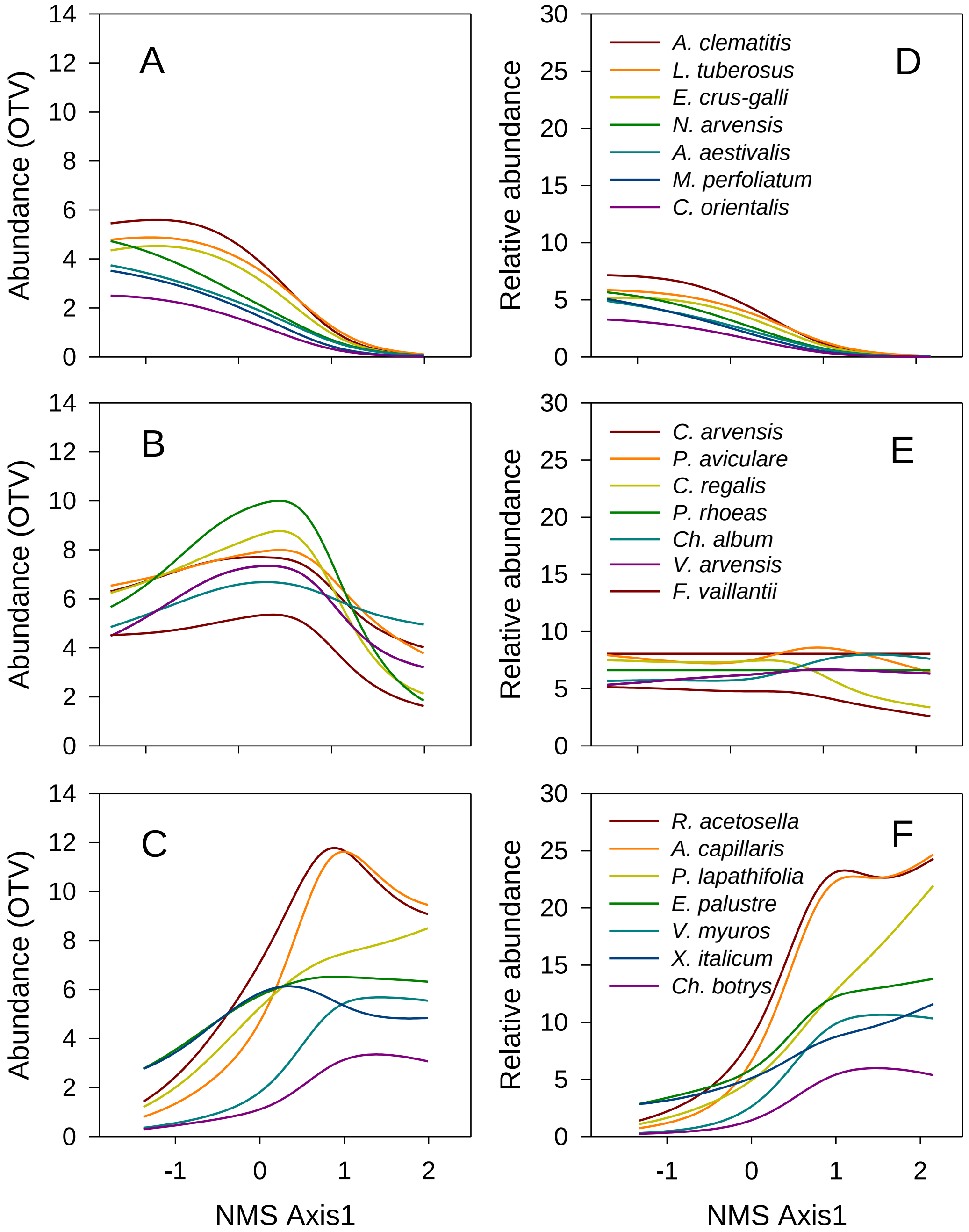

Fig. 4.6: Response of the major groups of weeds to the pollution-induced soil gradient. Species envelope curves along the main ordination axis after NMS ordination of untransformed (A, B, C) and relativized (D, E, F) abundances are shown. Groups are defined after Indicator Species Analysis (IV > 30%, P < 0.01) and subsequent classification (see Fig. 4.3). A, D – species indicating relatively unaltered calcareous soil; B, E – species of broad valence dominant in the middle portions of the soil gradient. C, F – species indicating most severely altered, nutrient-poor acidic soils. NMS axes are scored in standard deviations from the centroid in a normalized configuration. Relative abundance – % of the sum of OTV values in a sample.

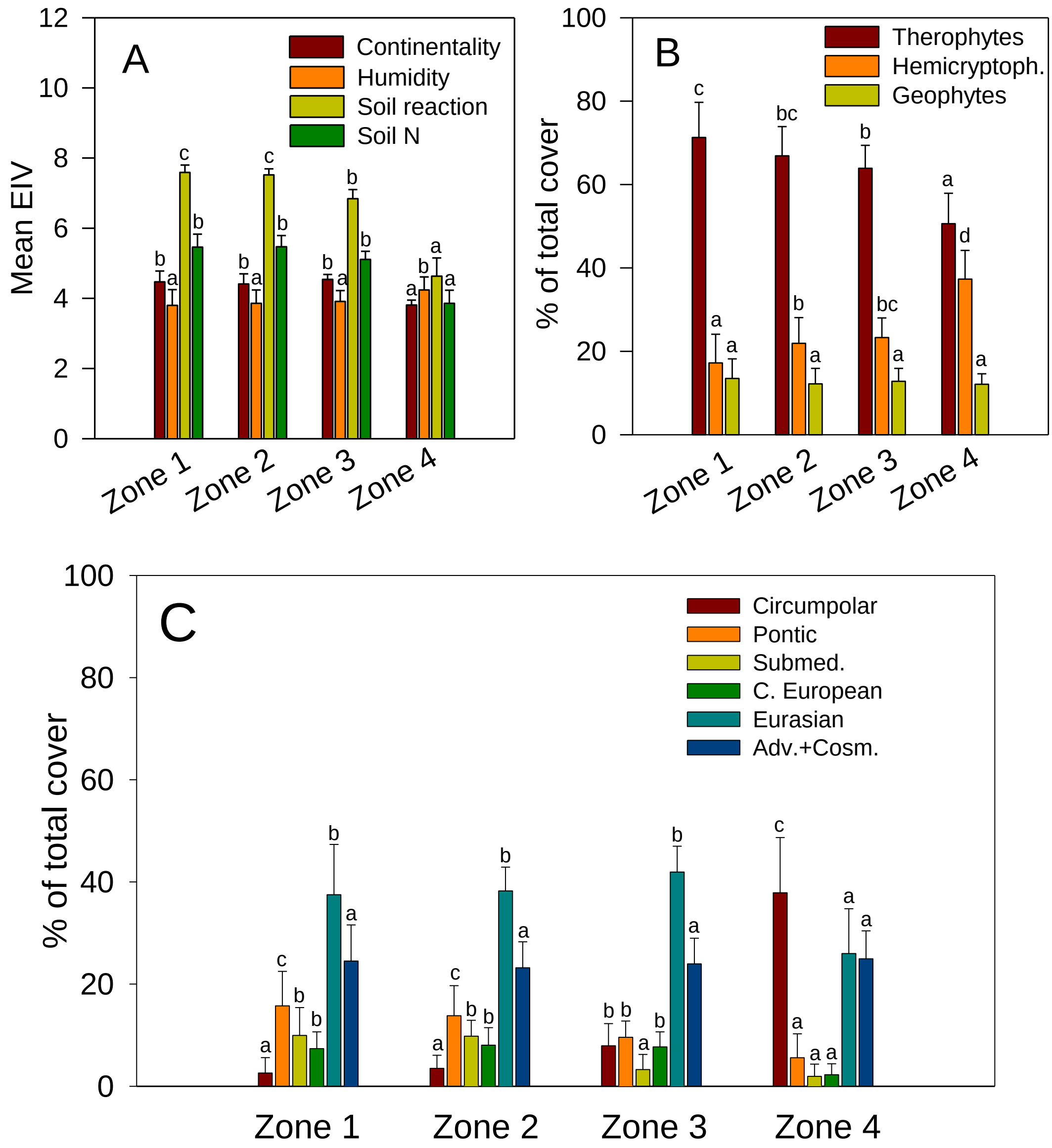

Fig. 4.7: Major ecological adaptations of cereal weed vegetation along the soil gradient. A– weighted Ellenberg Indicator Values; B – life form spectra; C – chorological spectra. Mean values ± SD marked by the same letter in one colour-coded category are not different at P=0.05.

Despite a 7-fold increase in plant available soil Cu concentrations (Table 4.4), soil Cu seems not to be correlated with any structure in the weed communities along the gradient (Fig. 4.5). The range of $Cu_{\text{DTPA-extractable}}$ concentrations in altered soils (Zone 3 and 4, Table 4.4) are rather high, but there is no trend of significant increase in leaf Cu concentrations along the gradient (Fig. 4.8). Only one species (*Chenopodium botrys*) is found to commonly have markedly elevated leaf Cu (over 100 mg kg^{-1} DW), but it never reached an OTV > 3 or a share in a total biomass (per m^2) larger than 5%. Occasionally, somewhat elevated Cu concentrations are found in populations of other indicators of the most polluted soils in Zone 4 (*Persicaria lapathifolia* and *Agrostis capillaris*), but in, general, the weed vegetation successfully maintains a homeostasis of leaf Cu concentrations along the soil gradient (Fig. 4.8).

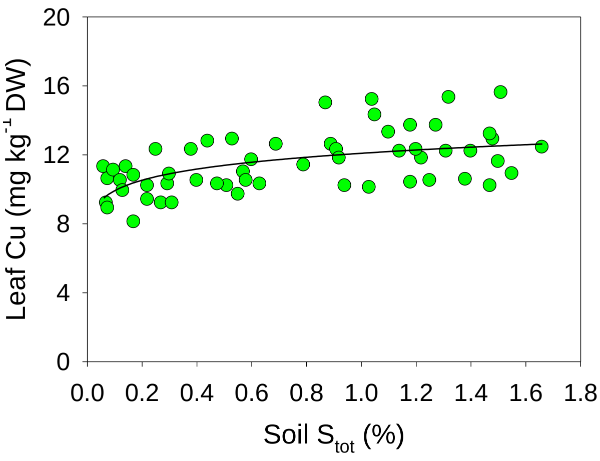

Fig. 4.8: Cu in weed vegetation along the soil pollution gradient. Leaf Cu concentrations are weighted by the relative proportion of a species in total biomass per m^2, sampled in crop milky ripeness phase (Z71-75).

Plant available fraction of Ca prominently decreases (about 3-fold) with the soil acidification (Table 4.4) and it is highly correlated with the major gradient in weed vegetation (Fig. 4.5). The cereal crops and the weed vegetation have, however, markedly different responses to a decrease of plant available Ca along the gradient (Fig. 4.9).

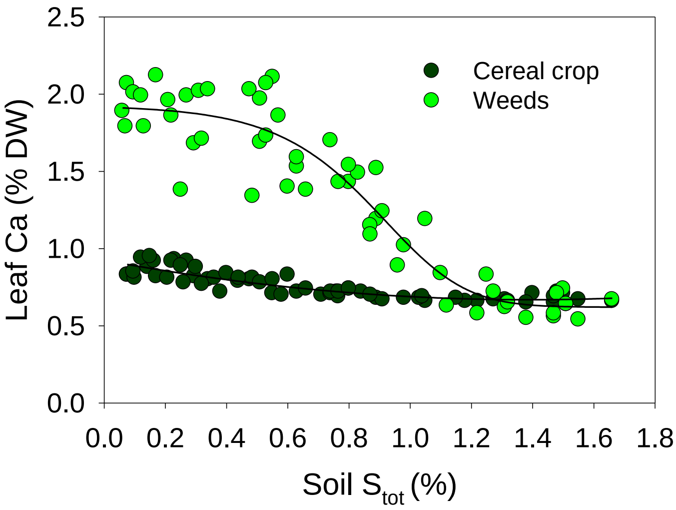

Fig. 4.9: Ca in weed vegetation and cereal crop along the soil pollution gradient. Leaf Ca concentrations are weighted by the relative proportion of a species in total biomass per m^2, sampled in crop milky ripeness phase (Z71-75).

Wheat and barley maintain a very narrow range of leaf Ca concentrations (up to 19% decrease in the most severely altered acidic soils) despite a drastic (about 6-fold) decrease in growth (Fig. 4.2) along the gradient. Spontaneous weed vegetation, however, shows a 3-fold decrease of the average leaf Ca concentrations along the soil pollution gradient (Fig. 4.9) without any trend of growth reduction (Fig. 4.2). This indicates a major succession of different physiotypes for Ca nutrition in weed vegetation along the gradient.

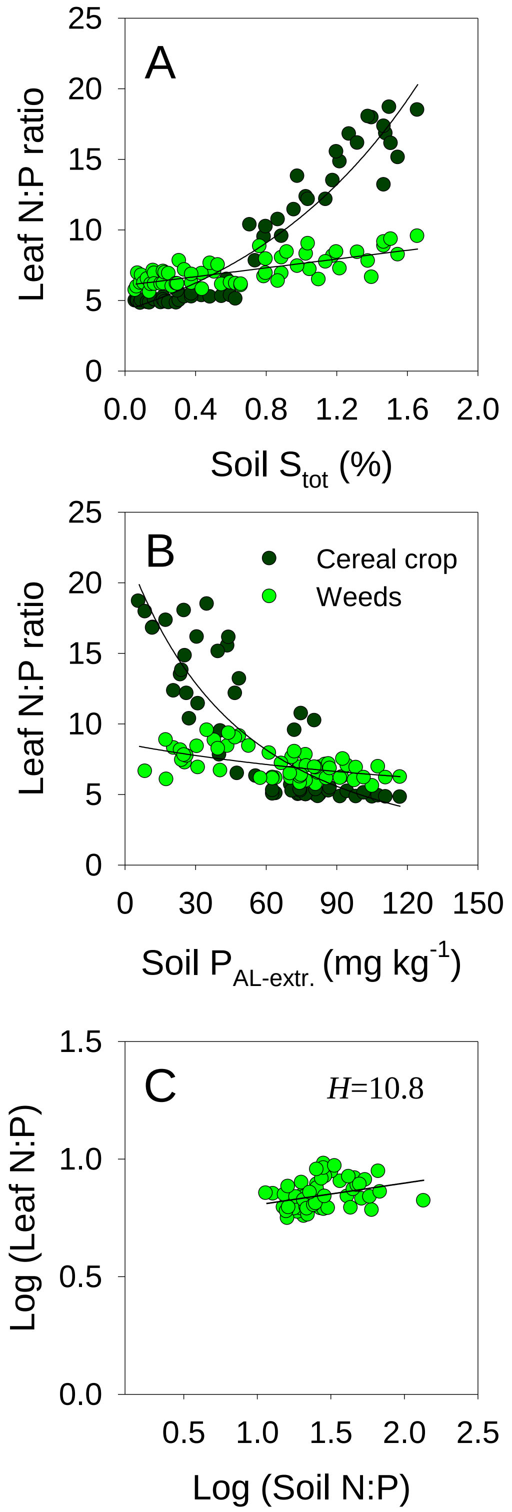

Fig. 4.10: N : P ratio in weed vegetation and cereal crops along the soil pollution gradient.

A – Leaf N:P along the complex soil gradient indicated by the pollution load;
B – Leaf N:P along the decreasing plant available P concentrations in polluted soils.
C – Biomass N:P in weed vegetation as a function of soil N:P ratio. H – regulatory coefficient, slope of the linear trend line.

Leaf N and P concentrations are weighted by the relative proportion of a species in total biomass per m^2, sampled in crop milky ripeness phase (Z71-75).

The P availability becomes a severe constraint in the polluted soils; at the same time, soil N concentrations are low, but they change very little along the gradient (Table

4.4). The N:P stoichiometry in the leaves, however, is maintained in the spontaneous weed vegetation, while in cereal crop the strong N:P increase indicates severe decrease of leaf P concentration on the altered, acidified soils (Fig. 4.10). This pattern is very clear along the gradient of increasing pollution load, which is coarsely indicated by soil S_{tot} concentrations (Fig. 4.10A). Decreased leaf P status of cereal crops can be a “black-box” result of other rhizotoxic effects which might additionally decrease P uptake (Al, Cu, low pH *per se*, etc). If leaf N:P ratio is plotted against the decreasing $P_{AL\text{-}extractable}$ along the pollution gradient (Fig. 4.10B) the same patterned difference between crop plants and weed vegetation can be observed. Finally, Fig. 4.10C shows that a very strong stoichiometric plasticity of biomass N:P ratio is only very weakly affected by the external supply of N and P (in the range of soil N:P values measured in this study). This is also indicated by very high value of the regulatory coefficient *H* of 10.8; estimated as: log (leaf N:P) = 1/*H* × [log (soil N:P)] + log (b), Fig. 4.10C. The value of the regulatory coefficient *H* for cereal crops (wheat and barley) is drastically lower (1.56, not shown).

4.4 Discussion

4.4.1 Methodological issues

There are some minor methodological issues to be explained. The effect of biotic constraints (competition, most importantly with the cereal crop) is partially accounted for in this study by relativizing the abundance values by plot totals. It might further be speculated that on the relatively unaltered soils (Zones 1 and 2), P availability for weed species, towards the end of crop vegetation, might be somewhat less than measured in April due to a strong competition with cereal crop. Next, the apparent decrease of weed biomass from the Zones 3 and 4 recorded immediately post-anthesis (Fig. 4.2) is not so clear in Fig. 4.6, which is based on maximal abundance values during 3-months survey. The majority of biomass in Zone 4 is contributed by *A. capillaris* and *R. acetosella*; in this study they were not observed to reproduce from seeds, but only vegetatively, and the ploughing has visibly delayed their regeneration compared to the emergence of other weed species. Thus the decrease of the weed biomass after Zone 3 would probably be less prominent if the sampling was done later in the season. Furthermore, the effect of pollution gradient along the transects (transects clearly coincide with microelevation gradient) cannot be disentangled from the effect of hygric gradient, since the pollution was deposited by the same flooding process. For example, hygrophilous *Equisteum palustre* in Zone 4 might be partially brought about by increasing soil humidity; however, the rather xerophytic *Vulpia myuros* is also confined to this zone. Our results indicate that the importance of species' adaptations to the major soil chemical constraints have an overriding effect over the gradient; possible interaction of soil pollution and soil humidity can not be assessed from this study and we considered only their joint effect. Finally, the standardized sample size of 30 m^2 might not have been adequate for the relevées on the unaltered soils (Zone 1). It was chosen as the maximal area applicable over the whole transect. The sample size might thus have somewhat increased the spread of the Zone 1 (and Zone 2) samples along the second ordination axis (Fig. 4.5), but it does not essentially affect any finding.

4.4.2 Large scale "field laboratory"

Soil pollution by the fluvially deposited mining waste has led to the existence of the two drastically contrasting soil types at the field scale: calcareous soils (pH often > 7.5) are substituted by very acidic, leached, nutrient-poor soils (pH as low as 4, Table 4.4) over transect lengths of usually only up to 8-12 m (Fig. 4.4). In the nature, such contrasting soils: calcisols (warm, semiarid or arid regions) and podsols (coniferous forests, moist and cool regions) are physically separated by a large-scale climatic gradient (Driessen et al., 2001). The species which strongly indicate unaltered calcareous soils (cluster A in Fig. 4.3) are well described as typical/diagnostic species ("calcioles", "calciphiles") of the Caucalidion lappulae Tx 50 alliance of cereal weeds commonly found in warmer and drier climates on non-acidic soils (e.g. Ellenberg 1988a). In the same field (Fig. 4.4), the opposite end of the soil gradient is dominated by the species indicative of nutrient-poor, acidic soils (most prominently *Apera spica-venti*, *Vicia hirsuta*, *Rumex acetosella*, *Agrostis capillaris*, cluster C in Fig. 4.3), commonly termed "calcifuges" or "acidophytes", which (except *A. capillaris*) are typical for the order Aperetalia spicae-venti Tx & Tx 1960. The major distinction in cereal weed communities, between those on the lowland, drier, basic soils, and more acidic soils in moister areas, is usually observed over large geographical gradient (e.g. south – warmer, north – cooler; west – atlantic, east – continental) and likewise over altitudinal gradient (e.g. Holzner, 1978; Lososova et al., 2006; Šilc et al., 2009). In this study, the consistent assemblages of the representatives of these two distinct groups have the spatially explicit appearance along an extreme man-made gradient of soil properties (primarily of pH) in the same field. On the large area affected by mine effluents, and over sufficiently long period of 50 years, very acidic soils created in a pronouncedly non-acidic context represent a strong filter which cannot be observed under normal conditions. This research locality represents hence a large scale “field laboratory” where the possibility to control other factors which are likely to modify weed – soil relationship (altitude, climate, season, year, agricultural practices, surrounding vegetation, and landscape context) has resulted in an extremely high proportion of the “variance explained” (Fig. 4.5). Explanatory power of a main factor of interest in an average ecological experiment is much lower (commonly less than 10%; Møller and Jennions, 2002).

4.4.3 Filtering and weed species

Environmental filters are processes and conditions which constrain the establishment of species with unviable physiological limitations (Keddy, 1992). Recent research (de Bello et al., 2012) emphasizes the hierarchical nature of environmental filters, from large scale (regional flora, climate) to field scale (e.g. soil properties). In our study, regional environmental filters (climate: semiarid conditions, hot dry summers, Fig. 4.1; carstic geology and calcareous soils and xerothermic natural vegetation) do limit acidophytic species which would have naturally be associated with the type of soils induced by the pollution (i.e. with acidic, leached, nutrient-poor soils of cool and moist regions). However, some patches of natural acid arable soils do exist in the region (albeit at distances > 20 km). These are heavy, clayey soils of a gleysol type, with pH (in H_2O) 5 - 6, and a dominant weed association *Anthemis cotula – Gypsophila muralis* of the Aperetalia order (Milijić, 1980). In particular, the four species observed on highly acidified soils in our study (*Rumex acetosella*, *Persicaria lapathifola*, *Vulpia myuros* and *Vicia hirsuta*; Fig. 4.3) are important, but never dominant, constituents of that association (Milijić, 1980).

Two other factors operating on a landscape level are: the rather low level of filed management; and the surrounding vegetation matrix of predominantly fallow land in different successional stages. The former has enabled the maintenance of some species which have become rare in more intensively managed agricultural landscapes in Europe as, for example, *Agrostemma githago, Centaurea cyanus, Chondrilla juncea, Lactuca serriola*; and specialists like *Adonis aestivalis, Ajuga chamaepitys, Caucalis platycarpos, Conringia orientalis, Myagrum perfoliatum* (based on Šilc et al., 2009; Pinke et al., 2009). The latter has brought about a rather high proportion of non-segetal species, predominantly from the classes Molinio-Arrhenatheretea (hygrophilous, herbaceous communities affected by river floods: *Vicia hirsuta*, *V. cracca*, *Centaurea jacea*, *Equisetum palustre*, *Plantago lanceolata*); Artemisietea (nitrophilous ruderal vegetation: *Berteroa incana*, *Barbarea vulgaris*, *Daucus carota*, *Cichorium intybus*, *Linaria vulgaris*) and Festuco-Brometea (xerotherimic communities in the non-flooded surrounding: *Agrostis capillaris*, *Eryngium campestre*, *Chondrilla juncea*; class affiliations according to Kojić et al., 1997).

On the field level, the available regional species pool is further filtered by soil constraints and competition effects (Table 4.6). In general, the major constraints on calcareous soils for non-adapted species are the bioavailability of micronutrients (Fe, Zn and sometimes Mn) and of Ca-bound P, while on acidic soils plants have to cope with Al toxicity, H^+ toxicity, and Fe/Al – bound P (Neumann and Römheld, 2002). In a popular concept of weed distribution along pH gradient based on “calcicoles” and “calcifuges”, it is however still not clear how far it is a purely species-specific physiological feature, and how far it is modified by a complex climatic gradient (Holzner, 1978).

Table 4.6: Environmental filters for weed vegetation at the field scale.

Transect position (visual growth zone)	Major filters for cereal weeds	
	Abiotic	Biotic
Zones 1 & 2	Calcareous soils	Strong competition with crop
Zone 3	Nothing severe	Strong competition among weeds
Zone 4	Acidic soils, low P	Nothing severe

Weed vegetation changes dramatically over the soil gradient (Fig. 4.3; Fig. 4.4; Fig. 4.5; Fig. 4.6; and Fig. 4.7), yet, it shows no trend of growth reduction (Fig. 4.2). The peak of weed abundance is in the middle portions of the transects (Fig. 4.2 and Fig. 4.6). Soils are nutrient-impoverished and acidified in this zone (Zone 3), but no soil factor is so severely altered to limit plant growth (Table 4.4). Indeed, well over 70% of all the species recorded in this survey along the whole transect gradient are present in Zone 3 (Table 4.3). Indicators of both the calcareous and the highly acidic soils (Fig. 4.3; clusters A and C, respectively) do occur under these conditions of Zone 3 (Fig. 4.6), but the dominance is shared among a number of rather common species (Cluster B, Fig. 4.3). This zone thus provides a “potential niche” for the majority of the recorded weed species, but only a limited number has their “realized niche” in this zone. The weed vegetation in this zone is thus primarily determined by the species competition. Some of the dominant species (e.g. *Chenopodium album*, *Polygonum aviculare*, *Convolvulus arvensis*, *Cirsium arvense*, *Fumaria vaillantii*) were shown to be relatively independent of the large scale climatic gradient in Europe (Radics et al., 2004; Glemnitz et al., 2006).

The dominant species of the most severely acidified soils in Zone 4 (*Agrostis capillaris*, *Rumex acetosella* and, less apparently *Persicaria lapathifolia*) are frequently found pioneer colonizers on post mining, acidic and nutrient-poor substrates which are often also constrained by high concentrations of plant available fractions of heavy metals (Thompson and Proctor, 1983; Kelepertsis et al., 1985; Prach, 1987; Wiegleb and Felinks, 2001a). *A. capillaris* and *R. acetosella*, which dominate these assemblages (Fig. 4.6) are hemicryptophytes, what is also a trait common for pioneers on post-mining sites (Wiegleb and Felinks, 2001b). Surprising combinations of species as occurring in Zone 4, which bear little resemblance to the assemblages on non-polluted soils, are also a feature common for drastically altered post-mining sites (e.g. Wiegleb and Felinks, 2001a; Hobbs et al., 2006). Cropping appears to have arrested a post-mining succession in the Zone 4.

The variability of weed relevées along the soil gradient decreases (Table 4.2, Fig. 4.5), and the association among the major species is the strongest in Zone 4. Petit and Fried (2012) have shown that increasing environmental heterogeneity can increase the degree of association of specialist weed species at the field scale. Our study shows that soil filtering increases spatial co-occurrence of weeds which are not “specialists” as defined by their niche width in Europe (Fried et al., 2010), but are certainly specialized for the particular and severe soil conditions caused by mine waste deposition.

4.4.4 Adaptive traits

Surprisingly, soil Cu (Table 4.4), though rather high (for example, concentrations of only about 30 mg kg^{-1} were toxic for cereals; Fageria, 2001) was not found to be significantly correlated with any observed pattern in weed vegetation (Fig. 4.5). One possible cause of this low correlation might be the fact that in the surveyed fields, which are all at the outer edge of the polluted floodplain soils, high plant available Cu is invariably linked to high soil organic matter, as a clear consequence of land use history (as discussed previously, Nikolic and Nikolic, 2012). Soil organic matter might thus somewhat ameliorate Cu rhizotoxicity. Furthermore, Cu was shown to have a hump-shaped trend along the soil transect, and is thus poorly linearly correlated with the pollution load, i.e. with the main soil gradient (Nikolic and Nikolic, 2012). However, though soil Cu was not found to coincide with the major gradient in weed vegetation, possible rhizotoxic effect of deposited Cu cannot be excluded. Moreover, as Cu translocation to shoots at elevated soil concentrations is highly restricted in most plant species (Broadley et al., 2012), the relatively constant leaf Cu concentration in weed vegetation along the gradient (Fig. 4.8) is not surprising.

Contrary to Cu, decrease of the plant available Ca with increasing soil acidification (see Table 4.4) is accompanied by a remarkable shift in the leaf Ca level in weed vegetation (Fig. 4.9). Crop biomass decreases more than 6 times along the transect (Fig. 4.2), but leaf Ca levels drop by only up to 20%. The very conspicuous weed species turnover along the gradient (Fig. 4.4; Fig. 4.5; and Fig. 4.6), however, is accompanied by a 100% decrease of leaf Ca levels, without any effect on decreasing biomass. Three distinct physiotypes of Ca–nutrition have been described (White, 2005). The most prominently contrasting responses to Ca supply between “calciotrophes” (i.e. calcicoles) and “oxalate plants” (i.e. calcifuges) and the range of the respective leaf Ca concentrations (White, 2005) are comparable to our results at the unaltered calcareous, and highly altered, acidic soils, respectively (Fig. 4.9).

Our results show that a clear succession of the three groups of dominant weed species over the induced soil gradient (Fig. 4.4; Fig. 4.5; and Fig. 4.6) has resulted in the maintenance of a constant leaf N:P ratio in the weed vegetation biomass (Fig. 4.10). N:P ratios have been proposed as a diagnostic tool for nutrient limitation in natural vegetation. The average reported mass N:P ratios for terrestrial plants in their field sites of 12-13, and the critical N:P of < 10 and > 20 for the respective N and P limitations (Güsewell, 2004) might not be always applicable. For example, in natural semiarid grasslands N:P ratio in biomass can be 5 – 6 (Li et al., 2011). Also, cereal crops are found to have a narrow N:P ratio in the same range of 5 – 6 (Sadras, 2006). In *Agrostis capillaris* communities Güsewell et al. (2005) found N:P ratio to be under 10. In our study soil N, though rather low, did not change drastically over the gradient, but the available P fraction drastically decreases with soil pollution by mining waste (Table 4.4).

Interspecific variations of N:P ratio were shown to be primarily caused by varying leaf P concentrations (Güsewell, 2004). Plants cope differently with N and P limitations. The internal N use efficiency is a trait which enables plants to maintain the critical N tissue concentrations by regulating growth rate (e.g. Barker and Bryson, 2007); hence, leaf N concentrations below the critical level are not likely to be recorded in viable spontaneously growing plants. On the other hand, root-induced chemical modification of the rhizosphere might be involved in mobilization and acquisition of sparingly soluble P sources in soil. The physiological mechanisms for mobilization of Ca-bound P in calcareous soils, and Al/Fe-bound P in acidic soils are very different, and species specific (e.g. Neumann and Römheld, 2002).

On the fields included in this study, P - deficiency was demonstrated to be the major limitation for wheat growth (Nikolic et al., 2011). The present work shows that P deficiency is likely the major driving force underpinning the observed floristic change in cereal weed vegetation along the pollution gradient. Indeed, leaf P concentration in the species which indicate calcareous soils (Cluster A, Fig. 4.3) exhibited the same pattern of change along the soil S_{tot} concentration gradient, as their abundances (Fig. 4.6, A and D) along the first ordination axis (not shown). Along the soil gradient, thus, N:P varies in individual species, but remains rather constant at the vegetation relevé level. Highly patterned species turnover along the soil gradient (Fig. 4.5 and Fig. 4.6) is accompanied by the extremely high value of the regulatory coefficient *H* (Fig. 4.10C) at the plot level (so far, H reported for individual species was below 5; Güsewell, 2004; Elser et al., 2010). Species' ability to maintain leaf P homeostasis along the induced soil gradient appears thus to be the major filter underlying the soil-induced vegetation structure.

4.5 Conclusions

This "field laboratory" study demonstrates the systematic variation in cereal weed vegetation as a function of the explicit soil gradient. Two interesting phenomena are shown: Firstly, the spatially explicit substitution of typical calcicole weed assemblages by a well-defined group dominated by typical acidophytes has, under extreme man-made soil filtering, occurred on a field scale. Secondly, this patterned change in species composition can be clearly linked with the ecophysiological adaptations of weed vegetation to the multiple soil constraints, most prominently to the low available P. Severe environmental filtering has brought about unusual combination of species with strong pattern of association, though little regard for the surrounding vegetation and the well-established phytosociological units. However, these novel associations showed a set of functional adaptations which have enabled the leaf N:P homeostasis and thus the continuous growth of weed vegetation along the soil gradient.

Acknowledgement

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Supplement 4.1: Visual symptoms in grain crop as a basis for sampling along the spatial gradient on soils affected by pyritic Cu tailings.

Zones	Visual symptoms				
	Growth reduction	Chlorosis	Translucent ears	Fungal shoot infestation	Weed infestation
1	–	–	–	–	–
2	–	–	+	+	+
3	++	++	++	++	+++
4	+++	+++	+++	+++	+++

–, no symptom; +, low; ++, moderate; +++, severe

Supplement 4.2: Weed list (84 species included in the analyses). Of all the species recorded during the 3-month survey only those with overall frequency >10% or, frequency in at least one sampling zone ≥ 20%, are included. Nomenclature follows the W3 TROPICOS database of the Missouri Botanical Garden.

Species	Reference	Family
Adonis aestivalis	L.	Ranunculaceae
Aethusa cynapium	L.	Apiaceae
Agropyrum intermedium	(Host) P. Beuv.	Poaceae
Agrostemma githago	L.	Caryophyllaceae
Agrostis capillaris	L.	Poaceae
Ajuga chamaepitys	Schreb.	Lamiaceae
Amaranthus retroflexus	L.	Amaranthaceae
Anagallis arvensis	L.	Primulaceae
Anchusa arvensis	M. Bieb.	Boraginaceae
Anthemis arvensis	L.	Asteraceae
Apera spica-venti	(L.) P. (Beauv).	Poaceae
Aristolochia clematitis	L.	Aristolochiaceae
Artemisia vulgaris	L.	Asteraceae
Avena fatua	L.	Poaceae
Barbarea vulgaris	W. T. Aiton	Brassicaceae
Berteroa incana	(L.) DC.	Brassicaceae
Bifora radians	M. Bieb.	Apiaceae
Bilderdykia convolvulus	(L.) Dumort.	Polygonaceae
Bromus sterilis	L.	Poaceae
Caucalis platycarpos	L.	Apiaceae
Centaurea cyanus	L.	Asteraceae
Centaurea jacea	L.	Asteraceae
Chenopodium album	L.	Amaranthaceae
Chenopodium botrys	L.	Amaranthaceae
Chondrilla juncea	L.	Asteraceae
Cichorium intybus	L.	Asteraceae
Cirsium arvense	(L.) Scop.	Asteraceae
Conringia orientalis	(L.) C. Presl.	Brassicaceae
Consolida regalis	Gray	Ranunculaceae
Convolvulus arvensis	L.	Convolvulaceae
Crepis tectorum	L.	Asteraceae
Daucus carota	L.	Apiaceae
Echinochloa crus-galli	(L.) P. (Beauv).	Poaceae
Equisetum palustre	L.	Equisetaceae
Erigeron canadensis	L.	Asteraceae
Eryngium campestre	L.	Apiaceae
Euphorbia falcata	L.	Euphorbiaceae
Euphorbia helioscopia	L.	Euphorbiaceae
Fumaria vaillantii	Loisel.	Papaveraceae
Galium aparine	L.	Rubiaceae
Kickxia spuria	(L.) Dumort.	Plantaginaceae
Lactuca serriola	L.	Asteraceae
Lamium purpureum	L.	Lamiaceae
Lapsana communis	L.	Asteraceae
Lathyrus aphaca	L.	Fabaceae
Lathyrus nissolia	L.	Fabaceae
Lathyrus tuberosus	L.	Fabaceae
Lepidium draba	L.	Brassicaceae
Linaria vulgaris	Mill.	Plantaginaceae
Mentha arvensis	L.	Lamiaceae
Myagrum perfoliatum	L.	Brassicaceae

Species	Reference	Family
Nigella arvensis	L.	Ranunculaceae
Papaver rhoeas	L.	Papaveraceae
Persicaria lapathifolia	(L.) Gray	Polygonaceae
Persicaria maculosa	Gray	Polygonaceae
Plantago lanceolata	L.	Plantaginaceae
Poa pratensis	L.	Poaceae
Polygonum aviculare	L.	Polygonaceae
Ranunculus arvensis	L.	Ranunculaceae
Rorippa sylvestris	(L.) Besser	Brassicaceae
Rumex acetosella	L.	Polygonaceae
Rumex crispus	L.	Polygonaceae
Scandix pecten-veneris	L.	Apiaceae
Senecio rupestris	Waldst.	Asteraceae
Setaria glauca	(L.) P. Beauv.	Poaceae
Silene latifolia subsp. *alba*	(Mill.) Greuter & Burdet	Caryophyllaceae
Silene noctiflora	L.	Caryophyllaceae
Sinapis arvensis	L.	Brassicaceae
Solanum nigrum	L.	Solanaceae
Sonchus oleraceus	L.	Asteraceae
Stachys annua	L.	Lamiaceae
Thymelaea passerina	(L.) Coss. & Germ.	Thymelaeaceae
Torilis arvensis	(Huds.) Link	Apiaceae
Vaccaria pyramidata	Medik.	Caryophyllaceae
Valerianella rimosa	Bast	Caprifoliaceae
Verbena officinalis	L.	Verbenaceae
Veronica persica	Poir.	Plantaginaceae
Veronica polita	Fr.	Plantaginaceae
Vicia cracca	L.	Fabaceae
Vicia grandiflora	Scop.	Fabaceae
Vicia hirsuta	(L.) Gray	Fabaceae
Viola arvensis	Murray	Violaceae
Vulpia myuros	(L.) C.C. Gmel.	Poaceae
Xanthium italicum	Moretti	Asteraceae
Xeranthemum annuum	L.	Asteraceae

Chapter 4

5 Gradient analysis reveals a copper paradox on floodplain soils under long-term pollution by mining waste

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Abstract

Arable alluvial soils are a globally important resource under increasing pressure from both industrial pollution and intensified agricultural land use. Quality of agricultural soils is ultimately defined by crop yields; it is however seldom feasible to study the consequences of complex soil pollution on crops under field conditions. This work analyses the long term effects of two gradients: spatial (relative distance from the water channel) and land use intensity (cropping frequency) on soil properties and model crop (barley) response. On an exceptional model locality in Eastern Serbia, degraded by fluvial deposition of sulphidic copper tailings during 50 years, multivariate analysis shows that land use accelerates the substitution of high plant available Cu by nutrient deficiency (primarily P and microelements) and excessive exchangeable Al. Though agronomic soil quality might not differ along the land use gradient, the environmental consequences do drastically change. The observed apparent "paradoxes" (e.g. soil Cu decreases towards the pollution source; higher yields might coincide with higher soil and leaf Cu concentrations; and leaching of Cu does not restore soils agronomic quality) can be explained by a) the Cu retention patterns along the transects, b) importance of higher SOM and nutrient availability for modifications of Cu toxicity, and c) the existence of plant adaptation mechanisms which can considerably counteract the adverse soil conditions. Land use-induced nutrient deficiency can counteract the positive effects of decreased Cu levels. In a long run, accelerated Cu mobilization is likely to increase vulnerability of these soils to further environmental hazards. This study demonstrates the clear and consistent patterns in soil properties andplant response

along the gradients and points out the probable long-term environmental trends in a "would be" scenario for agricultural use of similar polluted soils.

Keywords: plant/soil interactions; land use; soil quality; pyrite; soil trace elements dynamics; multivariate analysis

Highlights

- Floodplain soils: 50 years of pollution by sulphidic Cu tailings;
- Multivariate analysis of soil and plant growth parameters along two gradients
- Land use intensity: drastic succession of major soil constraints
- The same soil productivity, very different environmental consequences
- Plant adaptations: yield and leaf minerals do not reflect the induced soil changes

5.1 Introduction

Arable alluvial soils constitute an important global resource, estimated to provide food for as much as 25% of the world's population (Gerrard, 1987). These soils are currently under increasing cropping pressure: more than 90% of floodplains in Europe and Northern America are already cultivated (Tockner and Stanford, 2002). In the last decades, however, major failures of tailings dams reported worldwide, followed by a discharge of metal mining waste into the local river systems, have severely degraded large areas of agricultural floodplain soils via the lateral accretionary sedimentation processes (Macklin et al., 2006). Almost half of the sediment-associated contaminant input in the river could be deposited in the floodplain soils of the river basin annually (Walling and Owens, 2003). Yet, the long term fate of these contaminants and their actual impact on soil quality, particularly when no remediation is undertaken, has surprisingly rarely been studied. So far, the major focus in ecological risk assessment has been the toxicity issue of contaminated soils (Suter, 2000). Concomitantly, environmental assessments of contaminated soils commonly look either at its chemical properties or at single bioassay indicators (e.g. soil organisms or spontaneously growing "bioindicator" plants), and thus often fail to provide a full enough picture of soil quality (Bone et al., 2010). Quality of agricultural soils (including the contaminated ones) is nevertheless ultimately determined by crop yields. Unfortunately, it is seldom feasible to study the direct long-term effects of complex soil pollution (as for example by sulphidic metal tailings) on crops under field conditions and even less so to investigate the major trends on these soils by means of gradient analysis. In the largest and most thoroughly studied comparable tailings dam failure in Europe (Aznalcóllar case, more than 250 articles published so far), for instance, the layer of spilled sludge was physically removed immediately following the accident, and subsequent agricultural land use was officially banned (Grimalt et al., 1999).

The present work sets out from the hypothesis that in a long run the major constraints for plant production on severely polluted alluvial soils (no remediation undertaken) are likely to change. Temporal trends on alluvial soils polluted by sulphidic tailings include weathering and transformation of minerals, acidification, and metal retention and leaching (Álvarez-Ayuso et al., 2008; Du Laing et al., 2009). Moreover, Cu retention is shown to depend strongly on land use history, i.e. on soil organic matter (Graf et al., 2007). The overall impact of these changes on agricultural land use, however, is not known. This survey was undertaken on an exceptional model locality where pyrite-rich tailings from a copper mine had been deposited over alluvial fields during almost 50 years of regular annual flooding (Nikolic et al., 2011). The objective was to investigate the trends in the major soil limiting factors for cropping (using barley as a model crop) along the spatial gradient (relative distance from the watercourse) at different land use intensities (i.e. different ploughing frequencies). Our work should enhance understanding of long-term pollutant dynamics in floodplains in conjunction with actual land use, and provide insight into a “would be scenario” on similar mining-affected sites.

5.2 Material and methods

5.2.1 Research locality

Copper mines of the Bor metallogenic zone are one of the four environmental hotspots in Serbia and the key environmental issue in the Timok river (a tributary to the Danube) watershed. They affect not only Serbia, but also the neighbouring countries, Bulgaria and Romania. Copper ore occurs as massive sulphide (predominantly pyrite) deposits. The major previously reported polluting agents were, Cu, As, Pb, Zn and the acidity generated by sulphide oxidation (Wolkersdorfer and Bowel, 2005).

In the alluvial flatland of the Timok watershed the climate makes a transition from submediterranean to subcontinental, with average annual rainfall up to 600 mm and average temperature of 11.6 °C. The bimodal rainfall distribution and the mountainous surrounding landscape with lasting snow cover have commonly been the cause of spring and autumn flooding of the cropped fields in the lower course of the Timok, particularly at meander locations. The soils of these fields were originally of loamy texture, calcareous, and poor in available nitrogen, phosphorus and soil organic matter, with the regular floods providing an important nutrient input for local cropping practices.

In the early 1940's, however, heavy rainfalls and snow melting caused the tailings slurry to finally overtop the dam crests of the already overfilled sedimentation ponds containing highly sulphidic (mostly pyrite) copper ore flotation waste. This untreated tailings slurry was directly received by the two tributaries of the Timok ("passive dispersal" event), and carried through its floodplain to the Danube. During the next four decades nothing was done to take the problematic sedimentation ponds out of use, and the subsequent flooding of the downstream cropland with tailings slurry became a regular event every spring and autumn. As a result of these lateral accretionary sedimentation processes, about 10,000 ha of agricultural land was destroyed, and far more damaged; the depth of the tailings material deposited over the fields ranged from few centimetres to over a metre. Due to interplay of historical and political circumstances, no rehabilitation measures had been undertaken; pollution ceased about 17 years ago, when the problematic sedimentation ponds were finally taken out of use.

5.2.2 Field trials

The survey included 17 fields of winter barley (*Hordeum vulgare* L. cv Novosadski 313, used as fodder), located within the 5 km radius from the N 44º 04′34″, E 22º31′10″ (about 70 km from the pollution source), at meander positions where the Timok flow slows down, creating a braided channel pattern with large medial and lateral bars. Satellite image of the research area (fields belonging to the villages Rajac, Braćevac and Tamnič is available at:
http://maps.google.com/maps?ll=44.082508,22.519602&z=13&t=h&hl=en).

All the fields were at the outer edge of the affected area, up 200 m distance from the water channels, and partially damaged by the fluvially deposited tailings. Despite the very visible delineation of the soil severely affected by tailings deposition, the whole area of a field is nevertheless uniformly cropped (local cultural issue). In the predominant low-input system, rainfed barley yields between 2 and 4 t ha^{-1} on non-polluted soils in the research area. Fields were selected on the basis of detailed participatory surveys with local farmers (similar land use histories, loamy soil texture, extensive crop management and the absence of waterlogging in the year of the trial). The surveyed fields were fertilized with 50-75 kg ha^{-1} of NPK (15:15:15) at sowing, followed by about 50 kg N ha^{-1} early in spring; also, low doses of manure (up to 10 t ha^{-1} each 2-4 years) were usually applied.

5.2.3 Sampling design

The samples of soils in partially damaged barley fields (and concomitantly the samples of the barley crop for the analysis of biomass, root/shoot allocation and foliar diagnosis) were two-way stratified. Firstly, according to the land use intensity, i.e. according to the duration of the fallow period preceding barley cropping: long fallow (> 25 years, A), intermediate (7-15 years, B), and fallow shorter than 5 years (C). The preceding land cover in a long fallow group was dense bushland/sparse forest, showing that some natural soil productivity restoration had already taken place. Based on the detailed participatory surveys with local farmers, we have assumed that there was no systematic difference between the soils of the three land use intensity groups with respect to either intrinsic soil quality (prior to the onset of pollution) or flooding frequency. Secondly, subsequent sampling in each land use intensity group was carried out according to the flexible systematic model (Smartt, 1978), a form of stratified sampling where samples were allocated on the basis of the visual symptoms in the barley crop (chlorosis, translucent ears, fungal and weed infestation; Supplement 4.1). Relative yield reduction was calculated a posteriori, and found to closely correspond to the observed visual differentiation of the crop field. In each field, it was possible to clearly distinguish two to four (depending on the site-specific topography) physiognomically uniform areas (zones) along the transect perpendicular to the river channel previously described by Nikolic et al. (2011). In each zone (sample) three 1 m × 1 m quadrates (replications) were laid for the measurements, and mean values of these replications were used for further analyses. Soil concentrations of S_{tot}, which *per se* have no direct effects on plant growth, are used as indicator of pollution intensity in perpendicular direction to the water channel (Nikolic et al., 2011). The sampling of the fluvially deposited tailings, where no plant growth occurs, was opportunistic.

5.2.4 Plant sampling and analyses

Plant measurements (root and shoot biomass) and tissues sampling were carried out at the onset of milky development, immediately after the anthesis (Zadoks growth stage Z70-71), except for grain yield which was recorded in the full maturity of barley crop. In each sample, three 1 m × 1 m quadrates (replications) were laid for destructive sampling; biomass was clipped, air dried and weighted; mean values of the 3 replications were calculated per sample. Root biomass was measured in the excavated soil volume 0.5 m x 0.5 m x 0.5 m (3 replications per sample).

For elemental analyses, flag (youngest fully emerged) leaves from 20 plants were taken for a composite sample per quadrate. Plant tissue samples were thoroughly washed with deionised water, dried at 70 ^{o}C and digested with a mixture of HNO_3 and H_2O_2 (3:2) in a microwave oven. Upon digestion, the concentrations of nutrients (P, K, S, C, Mg, Fe, Cu, Zn, Mn, B, Mo, and Ni) and other elements (Al, As, Pb, Cd, and Cr) were determined by inductively coupled plasma optical emission spectrometry (ICP-OES; SpectroGenesis EOP II, Spectro Analytical Instruments GmbH, Kleve, Germany). The concentrations of C and N in leaf samples were determined by the CHNOS elemental analyzer (Vario ELIII, Elementar Analysensysteme GmbH, Hanau, Germany). The reference sample SRM 1547 Peach Leaves (NIST, Gaithersburg, MD, USA) was used to assess the accuracy and precision of plant analysis.

5.2.5 Soil and sediments sampling and analyses

Two types of substrate were sampled: barren fluvially deposited Cu-tailings where no plant growth occurs, and alluvial soils partially damaged by the deposited tailings. In total, 24 samples of barren tailings deposits (10 “fresh” and 14 “weathered”) were collected from the depth of 0-30 cm (Table 5.1). “Weathered” tailings deposits were light-yellow coloured, acidic, well drained, and, according to the local land users, at least 20 years before this survey without any vegetation. “Fresh” tailings deposits were dark grey coloured, non-acidic, at microdepression positions. “Fresh” tailings sediments were preserved from intensive oxidative weathering because they were buried under layers of other fluvially deposited material, and only very recently (few weeks before the sampling) got exposed by the river erosion.

Soil (Fluvisols, IUSS Working Group WRB, 2006) sampling in the barley fields was done together with flag leaf sampling (onset of milky development immediately after the anthesis). A composite soil sample was obtained by mixing the subsamples from the depth of 0-30 cm, taken in each of the three 1 m × 1 m quadrates where the leaves were sampled (i.e. in each visually distinguished zone of barley growth appearance). In total for all three land use intensity groups in 17 fields, it makes 15 samples in Zone I, 14 in Zone II, and 17 in Zones III and IV each.

Analyses of soil and tailings samples were done in fine earth fraction (< 2 mm), after drying and sieving through a 2 mm mesh screen. Particle size distribution was determined by the pipette method and pH was measured in water (soil : water = 1:2.5). Water soluble salts were determined conductometrically (soil : water = 1:5); the percentage of total soluble salts was approximated by multiplying the measured electrical conductivity (dS/m) by 0.34. Soluble $CaCO_3$ was determined by calcimetry, and the concentrations of total C, N and S by the CHNOS elemental analyzer. Organic carbon (C_{org}) was calculated from total C and $CaCO_{3.}$ Potential CEC was determined by ammonium acetate extraction buffered at pH 7 (with ethanol treatment adjusted for salty and carbonate samples). Pseudototal fractions of metals and As in soil and tailings sediments samples were extracted by hot conc. $HNO_{3.}$ The precision and accuracy of the analysis was assessed with the reference sample NCS ZC 73005 Soil (CNAC for Iron and Steel, Beijing, China). Different extraction procedures were applied to determine plant available concentrations of elements (Allen, 1974): ammonium lactate – ammonium acetate (AL) for P and K, ammonium acetate (NH_4-Acc) for Mg and Ca, KCl extraction for available Al, and hot water (70 °C) for B. Plant available fractions of metals (Fe, Cu, Zn, Pb, Cd, Cr, Ni and Mn) were extracted by the DTPA-TEA solution (buffered at pH 7.3), with the soil : solution ratio of 1:5 (Norvell, 1984). Although the DTPA extraction has been primarily developed for non polluted calcareous soils (Lindsay and Norvell, 1978), the increase of soil to solution ratio from 1:2 to 1:5 was found to extend the effectiveness of the extractant also to acidic and metal-contaminated soils (Norvell, 1984). The concentrations of chemical elements in soil samples subjected to different extraction procedures were determined by ICP-OES, with the exception of the P concentrations which were determined colorimetrically at 882 nm.

5.2.6 Statistical analyses

Upon discarding outliers, multivariate analyses were performed with the measured properties of 63 soil samples and 63 barley leaf samples; data on barley biomass (63 samples) was also included. Regression analyses (ANOVA and multiple linear regression) were done by STATISTICA 6 (StatSoft Inc., Tulsa, USA). Variables with approximately lognormal distribution were log transformed prior to analyses. A posteriori comparison of means was done by Tukey's test, at p=0.05. Multivariate gradient analyses was performed by Nonmetric Multidimensional Scaling (NMS) by PC-ORD5 software (MjM Software Design, Gleneden Beach, USA), a technique well suited for nonnormal and nonlinear data (McCune et al., 2002). After 250 runs with real and randomized data (Euclidean distance measure), a 2-D final solution with instability $< 10^{-4}$ and final stress 6.02 (Monte Carlo test for stress in relation to dimensionality) was selected.

5.3 Results and discussion

5.3.1 Weathering of the deposited mining waste

Oxidative weathering is shown to be the major process in soils affected by sulphidic mine waste (Hüttl and Weber, 2001). The false time series of the fluvially deposited pyritic Cu-tailings (not mixed with soils, no anthropogenic influence) is shown in Table 5.1. The weathering of the tailings sediments is a "black box" process, which comprises different transformations and leaching. The trends of this aging process are however very clear: strong acidification and increase of exchangeable Al, and drastic loss of other elements. Only pseudototal As, though also diminished during the ageing process, is occasionally found in elevated concentrations in the highly weathered tailings sediments. The least change is in S_{tot} (up to 30% leached), while only total soluble salts and plant available B (which is extremely low) do not change.

The amounts of pseudototal Cu, mobilized and leached below 30 cm are about 8 t ha^{-1} (calculated from the data in Table 5.1), what is extremely high considering the rather limited Cu mobility in normal alluvial soils (Graf et al., 2007). Though with the values in a far less drastic range, similar trend of acidification, decrease of Cu and increase of Al concentrations was reported after only three months of oxidation of comparable tailings in Sweden (Stjernman Forsberg and Ledin, 2006). Plant growth is found to be impossible at both ends of the weathering gradient of the tailings sediments. Table 5.1 shows that the major limiting factors for plant survival on the deposited tailings change drastically with the advance of the weathering process: extremely high plant available Cu concentrations on the fresh sediments are substituted by the extreme nutrient deficiency, lack of soil organic matter, increased acidity, and increased exchangeable Al.

Table 5.1: Selected chemical characteristics of the two extreme stages in the aging process of fluvially deposited pyritic Cu-tailings where no plant growth occurs. Plant available concentrations of elements (obtained by different extractions) and pseudototal (HNO_3 -extractable) are shown. Mean values ± SD followed by the same letter in a row are not different at P=0.05.

Parameter	"Fresh"tailings deposits (n=10)	"Weathered" tailings deposits (n=14)	Limits*
pH (in H_2O)	6.4 ± 1.4a	3.2 ± 0.4b	-
S_{tot} (%)	3.4 ± 1.1a	2.4 ± 0.6b	-
Soluble salts (%)	0.36 ± 0.25a	0.42 ± 0.23a	-
C_{org} (%)	1.7 ± 0.4a	0.3 ± 0.2b	-
N_{tot} (%)	0.19 ± 0.03a	0.04 ± 0.02b	-
P_{AL} (mg kg^{-1})	94 ± 51a	0.9 ± 1.7b	-
K_{AL} (mg kg^{-1})	246 ± 112a	38 ± 11b	-
$Ca_{NH4\text{-}Ac}$ (mg kg^{-1})	176 ± 69a	60 ± 13b	-
$Mg_{NH4\text{-}Ac}$ (mg kg^{-1})	16.2 ± 0.7a	11.2 ± 1.1b	-
$B_{hot\ water}$ (mg kg^{-1})	0.2 ± 0.2a	0.6 ± 0.7a	-
Cu_{HNO3} (mg kg^{-1})	1933 ± 169a	211 ± 33b	< 100
Cu_{DTPA} (mg kg^{-1})	532 ± 41a	22 ± 16b	-
Zn_{HNO3} (mg kg^{-1})	337 ± 83a	7.5 ± 4.1b	< 300
Zn_{DTPA} (mg kg^{-1})	60 ± 47a	0.83 ± 0.81b	-
Cd_{HNO3} (mg kg^{-1})	1.7 ± 0.4a	0.3 ± 0.1b	< 3
Ni_{HNO3} (mg kg^{-1})	28 ± 6a	3.5 ± 1.9b	< 50
Cr_{HNO3} (mg kg^{-1})	33 ± 6a	9 ± 2b	< 100
Pb_{HNO3} (mg kg^{-1})	53 ± 13a	21 ± 9b	< 100
Al_{KCl} (mg kg^{-1})	0.53 ± 0.45a	73 ± 42b	-
As_{HNO3} (mg kg^{-1})	74 ± 38a	35 ± 11b	< 25

*Maximal allowed values for agricultural soils in Serbia (Serbian Ministry of Agriculture, Forestry and Water Resources, 1994).

5.3.2 Gradients in soil properties

The analysed soil parameters are exceptionally well ordered along the two distinct axes (Fig. 5.1) which represent 98% of all the variance encountered. This regularity is accounted for by the two groups of factors. Firstly, by the nature of fluvial soil pollution: sediments deposition regularly decreases in the lateral direction (Asselman and Middelkoop, 1995) and follows the lateral hydraulic sorting of particles (Miller, 1997). Secondly, very efficient sampling was possible due to the very high correlation between the visual symptoms in the crop field and the intensity of soil pollution (as previously shown for this locality, Nikolic et al., 2011). Ordination of soil samples (Fig. 5.1, A and B) shows the two major soil gradients: spatial gradient (Axis 1) of increased pollution level (indicated by S_{tot}), and land use intensity gradient indicated by increasing soil C_{org} and plant available metals (primarily Cu) concentrations with less frequent ploughing (Axis 2).

The pollution gradient clearly ordinates the continuous yield reduction (Fig. 5.1A), but has no correlation with plant available soil Cu concentrations. On the other hand, high values of Cu and C_{org} (Axis 2) clearly separate samples from the least frequently ploughed soils, but are not linearly correlated with yield reduction (Fig. 5.1B). Soil organic matter has been shown to prevent pyrite oxidation (Walker et al., 2004; Rigby et al., 2006), but it also exerts a main control of soil functioning at localities polluted by heavy metals (Balabane et al., 1999). Moreover, soil organic matter can decrease phytotoxicity of metals in soils (Walker et al., 2004).

While C_{org} does not change much along the spatial gradient i.e. the pollution intensity gradient (Fig. 5.2C), the soil Cu concentrations follow a distinct spatial pattern (Fig. 5.2, A and B). The well-established trend of decreasing metal deposition in flooded alluvial soils with growing distance from the watercourse (Miller, 1997; Martin, 2000, 2004; Middelkoop, 2000; Wyzga and Ciszewski 2010) could not have been demonstrated in this study (Fig. 5.2A). On the contrary, decrease of pseudototal Cu is found at locations nearest to the watercourse, with the highest amounts of deposited tailings (indicated by S_{tot}). Moreover, this decrease was the most pronounced with frequently ploughed soils (“B” and “C” series). In “A” series soils this paradoxical decrease of pseudototal Cu with the most intensive Cu-tailings deposition has not been observed. Since our survey was not done in “time zero” after the pollution, but more than 50 years later, the possibility that the “A” soils had just as well been affected by the oxidative weathering and Cu leaching can not be excluded. It is thus possible to speculate that the Cu plateau in “A” soils is also the consequence of historic Cu loss by leaching.

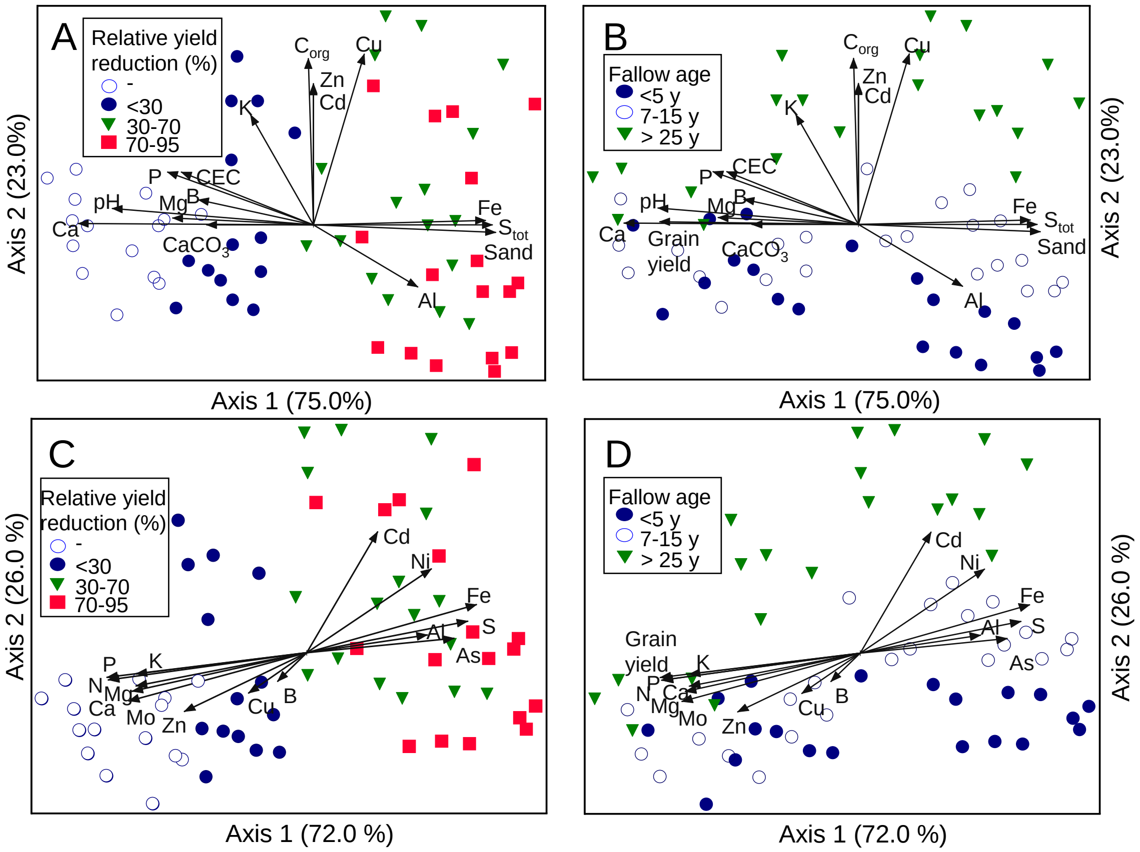

Fig. 5.1: Multivariate analysis (NMS) of soil properties and plant response. Concentrations of plant available fractions (except for N and S) of soil mineral elements were used for the NMS. The values in parenthesis denote the proportion of variance represented by each axis. The angles and lengths of the radiating lines indicate the direction and strength of relationships of the variables with the ordination scores.

A and B – NMS of soil properties. Ordination solutions rotated by 30°; variables correlated with ordination scores by > 30% are plotted.

C and D – NMS of barley leaf chemical composition overlaid on the ordination of soil parameters. Ordination solutions rotated by 20°; variables correlated with ordination scores by > 15 % are plotted.

A, C – samples according to the visual symptoms in barley crop (spatial gradient); B, D – samples according to the fallow length (land use intensity gradient).

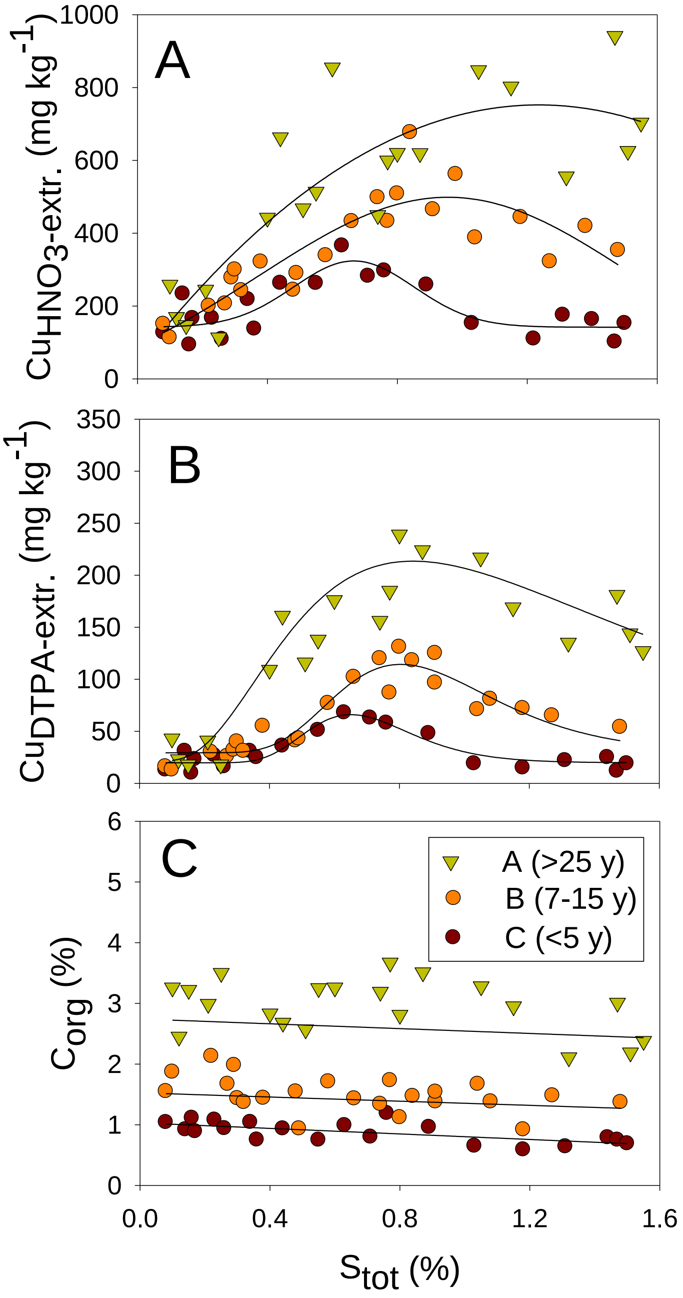

Fig. 5.2: Long term trends of Cu and C_{org} along the soil pollution gradient at different land use intensities.
A – pseudototal Cu (HNO_3-extractable);
B – plant available Cu (DTPA-extractable); C – organic carbon concentrations.

We had no reason to assume any systematic difference in historic pollution deposition among the three land use intensity groups: firstly, no difference was found in particle size distribution along the pollution gradient (Fig. 5.3A), and secondly, no difference was found in S_{tot} (Fig. 5.1B). It can therefore be assumed that the encountered patterns of pseudototal Cu concentration (Fig. 5.2A) are the outcome of the long-term "black box" processes involving oxidation of deposited pyritic tailings, and Cu mobilization by leaching, rather than a consequence of any intrinsic difference in Cu deposition among the soils in different land use intensity groups. The patterns of pseudototal soil Cu fraction (Fig. 5.2A), with the assumptions mentioned above, show: Firstly, along the land use intensity gradient, the overall, long-term retention of Cu has been stronger in least frequently ploughed ("A") soils, which also have had significantly and consistently higher C_{org} (Fig. 5.2C), CEC (Fig. 5.3B) and pH (Fig. 5.3C) along the transects. Secondly, along the spatial gradient, Cu leaching has been most prominent in the severely polluted soils (high S_{tot}), which had the lowest clay content and the lowest CEC (Fig. 5.3A and B) in all three land use intensity groups. This is essentially in accordance with the findings of Graf et al. (2007).

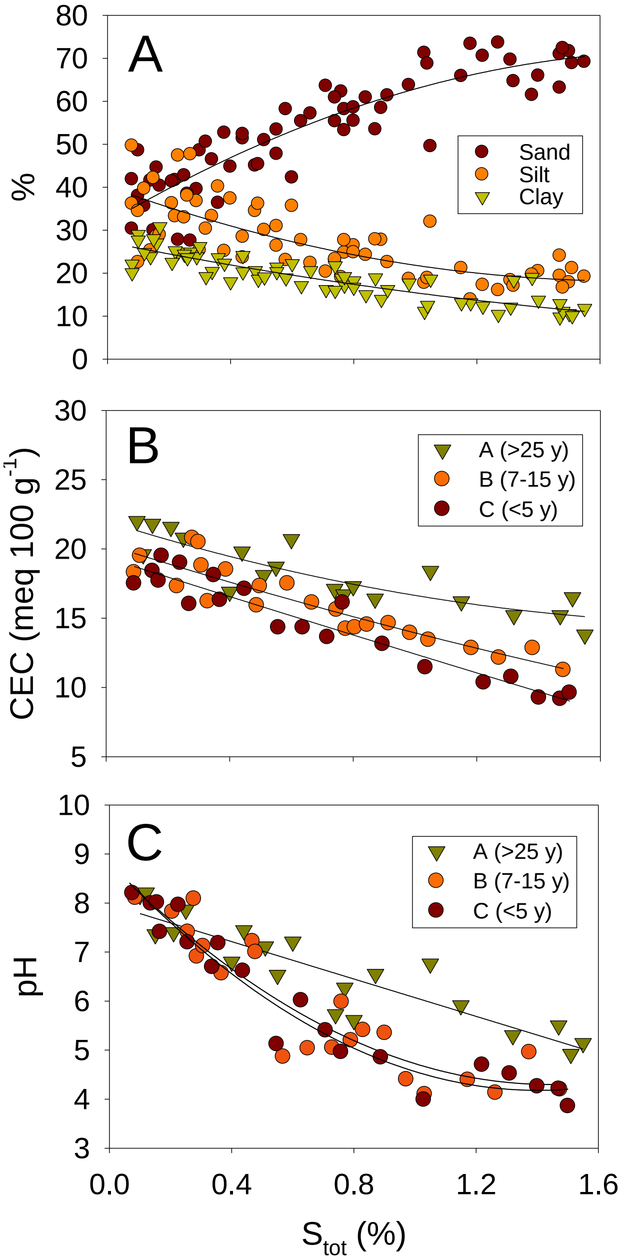

Fig. 5.3: The change of soil physico-chemical properties along the pollution gradient at different land use intensities.

The soil concentrations of DTPA-extractable Cu (Fig. 5.2B) are very high; for example, concentrations of only about 30 mg kg^{-1} were found to be toxic for cereals (Fageria, 2001). Furthermore, plant available Cu roughly reflects the pseudototal Cu concentrations (Fig. 5.2A). The share of plant available Cu in pseudototal Cu, however, has a clear and consistent trend along the transects. In the relatively unpolluted soils (up to about 0.3 % S_{tot}), this share is about 12 % in "B" and "C" soils. With the gradual acidification (Fig. 5.3C) the share of DTPA-extractable Cu in HNO_3 extractable Cu increases, as expected, to about 20-23 % in "B" and "C" soils (approximately up to 1% S_{tot}), but afterwards it again decreases to about 15 %. The same trend is also very clear in "A" soils, only the share of DTPA-extractable Cu is consistently higher than in "B" and "C" soils; at the same time, C_{org} concentrations in "A" soils (Fig. 5.2C) are 2-3 times higher than in "B" and "C" soils. This study points out the following apparent "paradox": the share of plant available Cu fraction in pseudototal Cu fraction decreases in the most acidic soil samples. These most acidic soils are at the same time nearest to the pollution source (i.e. to the water channel), but also apparently most intensively affected by leaching. Future work on Cu fractionation along the spatial gradient is expected to explain this phenomenon.

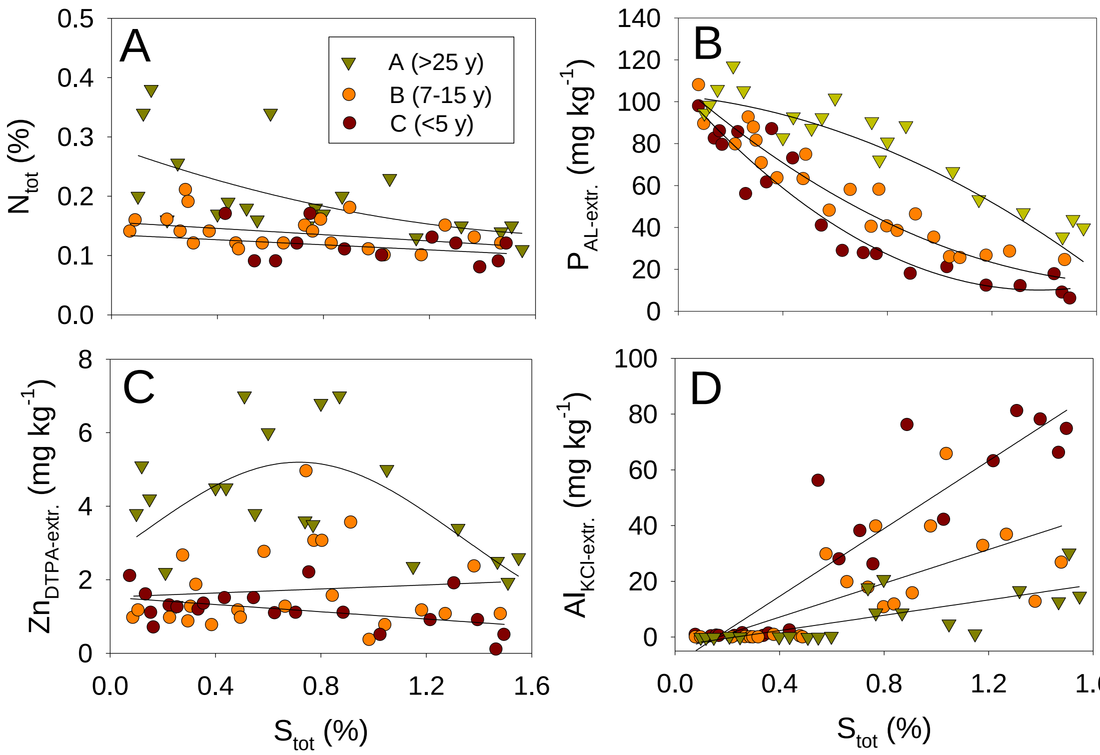

Fig. 5.4: Trends in selected soil constraints for barley growth along the pollution gradient at different land use intensities.

Table 5.2: The change in soil concentrations of essential and harmful elements (not shown in the Fig. 5.2 and Fig. 5.4) along the spatial gradient. Plant available concentrations (obtained by different extractions) and/or pseudototal concentrations (HNO_3 - extractable) of elements are shown. Results are means ± s.d. in every visually delineated sampling zone, averaged for all the three land use intensity groups.

Element	Relative yield reduction* (%)			
	n.a.	< 30	30-70	70-95
	Macronutrients (%)			
Ca $_{NH4-Ac}$	336 ± 37	271±36	170±44	145±47
Mg $_{NH4-Ac}$	21.4±2.0	18.9±2.1	16.2±1.8	14.7±3.1
K $_{AL}$	209±33	174±25	164±16	145±41
	Micronutrients (mg kg^{-1})			
Zn $_{NHO3}$	20.1 ± 4.2	31.5 ± 7.2	52.1 ± 11.2	23.4 ± 8.1
Mn $_{DTPA}$	14.9 ± 2.9	12.4 ± 3.0	14.3 ± 6.5	10.8 ± 6.1
B $_{NHO3}$	155 ± 18	124 ± 32	187 ± 31	117 ± 14
B $_{hot\ water}$	0.9 ± 0.1	0.7 ± 0.3	0.42 ± 0.08	0.35 ± 0.09
Ni $_{NHO3}$	3.2 ± 2.0	6.3 ± 2.8	9.5 ± 5.6	4.5 ± 2.1
Ni $_{DTPA}$	0.7 ± 0.4	0.5 ± 0.4	0.6 ± 0.3	0.4 ± 0.3
Mo $_{NHO3}$	0.3 ± 0.1	0.8 ± 0.6	2.7 ± 0.4	1.8 ± 0.6
Mo $_{DTPA}$	0.003 ±0.006	0.008 ± 0.01	0.009 ± 0.01	0.02 ± 0.03
	Harmful elements (mg kg^{-1})			
As $_{NHO3}$	19.6 ± 5.3	25.3 ± 3.8	46.1±4.8	26.1 ± 2.7
Cd $_{NHO3}$	0.3 ± 0.1	0.4 ± 0.21	0.7 ± 0.1	0.4 ± 0.2
Cd $_{DTPA}$	0.04 ±0.03	0.05 ± 0.04	0.05 ± 0.04	0.05 ± 0.06
Pb $_{NHO3}$	32.2 ± 15.3	42.1±11.0	65.9 ± 22.6	40.8 ± 13.5
Pb $_{DTPA}$	1.7 ± 0.9	0.8 ± 0.50	0.7 ± 0.9	0.3 ± 0.1
Cr $_{HNO3}$	20.3 ± 2.8	23.4 ± 6.3	27.3 ± 6.2	14.3 ± 4.1
Cr $_{DTPA}$	0.06 ± 0.04	0.04 ± 0.02	0.12 ± 0.03	0.06 ± 0.02
Co $_{NHO3}$	2.1 ± 0.3	2.8 ± 0.8	9.8 ± 2.0	4.1 ± 1.0
Co $_{DTPA}$	0.11 ± 0.05	0.11 ± 0.07	0.4 ± 0.2	0.11 ± 0.09

*Measured a posteriori, relative to the “no symptom” barley growth zones (no yield reduction, see Supplement 4.1).

Soil properties change in the same direction along the pollution (spatial) gradient in all three land use intensity groups (Fig. 5.2; Fig. 5.3; and Fig. 5.4). Along the land use intensity gradient, soil properties essentially reflect the type of change observed during the weathering of the barren tailings deposits (Table 5.1): soils under longer previous fallow (> 25 y, subjected in the lowest degree to oxidative weathering) are separated by higher Cu concentrations, but also higher nutrients, C_{org}, CEC, pH and lower plant available Al concentrations. On the other hand, frequently ploughed soils ("C" series) are constrained primarily by high concentrations of exchangeable Al (Fig. 5.4D); 50 mg kg^{-1} is Al_{KCl} toxicity threshold for barley (Adams and Pearson, 1967). This false time series (from "A" to "C" soils), based on land use history, demonstrates the succession of the soil constraints attributed solely to the cropping frequency.

Furthermore, P deficiency (Fig. 5.4B) is a severe constraint in all the land use intensity groups. Other available macronutrients (N, Ca, K, Mg) also decrease along the transects in all the soils (Fig. 5.1A and B; Fig. 5.4A; Table 5.2). Though HNO_3-extractable As was occasionally still elevated (up to 50 mg kg^{-1}, Table 5.2), DTPA-extractable As was very low (< 0.06 mg kg^{-1} in all the samples, not shown). Pseudototal (HNO_3-extractable) concentrations of other metals (Zn, Mn, Ni, Mo, Cd, Pb, Cr and Co, Table 5.2) essentially follow the same pattern as Cu (Fig. 5.2A): the highest concentrations are found in "A" soils, with a "peak" approximately between 0.6 - 1 % S_{tot} (i.e. zone of grain yield reduction of 30-70 %) in all the land use intensity groups (Table 5.2). However, concentrations of all metals were far below toxicity range (Table 5.2). Plant available B and Mo were occasionally deficient, while Mn was excessive. Plant available Zn is low in calcareous soils not affected by the tailings (up to approximately 0.3 % S_{tot}, Fig. 5.4C). Along the spatial transect, the increase in plant available Zn in "A" soils (Fig. 5.4C) occurs with slight soil acidification (up to about pH 6, Fig. 5.3C). In other fallow age groups Zn is already leached along the whole transect, and often deficient at high pollution levels; the pattern essentially reflect the plant available Cu (Fig. 5.2B).

5.3.3 Gradients in crop response

5.3.3.1 The "paradox" of yield and leaf mineral composition

Fig. 5.5A shows that the reduction of barley yield is strongly correlated with the relative distance from the river channel (i.e. with the pollution level), but it does not differ among the land use intensity groups. An exception is the significantly higher yield on only slightly polluted "A" soils (up to 0.3 % soil S_{tot}), attributed to the effect of long fallow *per se* on nutrient pool building (Fig. 5.2B and Fig. 5.4). However, the major soil constraints which cause the grain yield reduction along the spatial gradient do drastically change in different land use intensity groups (Table 5.3); only P deficiency is consistently significant in all the groups. To our knowledge, the only well-established constraint for crops on alluvial soils polluted by sulphidic metal tailings reported so far was low P in wild olives (Domínguez et al., 2010) and wheat (Nikolic et al., 2011).

Table 5.3: Multiple regression (stepwise forward) of barley grain yield on 11 selected soil parameters (concentrations of plant available fractions of chemical elements). Soil parameters were included when found in the range of nutrient deficiency (N_{tot}, P, C_{org}, Mg, Ca, B, Zn, and Mn) and/or metal toxicity (Cu and Al) in at least 20% of samples in each fallow age group and each visually distinguished zone. Severely correlated parameters are omitted. Only nominally significant predictors are shown.

Soil variable	β	Partial correlation	Semipartial correlation	Tolerance	P value
	A: Preceding fallow age > 25 years; $F(2,17) = 100.3$; $R^2=0.91$				
Cu	-0.39	-0.77	-0.33	0.71	0.000132
P	0.69	0.90	0.58	0.71	0.00000
	B: Preceding fallow age 7-15 years; $F(4,18) = 134.7$; $R^2=0.96$				
P	0.81	0.88	0.49	0.36	0.000000
C_{org}	0.18	0.47	0.14	0.59	0.037
	C: Preceding fallow age < 5 years; $F(4,15) = 85.34$; $R^2=0.94$				
Al	-0.39	-0.60	-0.15	0.15	0.01
P	0.44	0.67	0.19	0.17	0.03
C_{org}	0.20	0.58	0.14	0.53	0.014

Heavy metal content is commonly thought to be the major limiting factor for plant growth on soils polluted by metal tailings (Becker and Brändel, 2007). The preponderant effect of nutrient availability and soil organic matter over heavy metal concentrations on metal ecotoxicity has so far been implicated only for soil invertebrates (Anand et al., 2003; Lair et al., 2009). On the examined soils with long preceding fallows ("A" series) extremely high Cu concentrations (Fig. 5.2A) are likely the major constraint (Table 5.3). Frequent ploughing, however, seems to bring about an apparent paradox: soil Cu (Fig. 5.2, A and B) is leached to almost normal background values in "C" soils, but crop yields (Fig. 5.5A) are no better than initially, before Cu leaching ("A" soils). Land use did change the major soil constraints for cropping (Table 5.3) but not the final yield (Fig. 5.5A), i.e. the yield did not differ at DTPA-extractable Cu levels of approximately 200 and 60 mg kg^{-1}. Even more, at high levels of pollution (transect portions from approximately more than 0.6% S_{tot}) in all land use intensity groups, the highest crop yields (Fig. 5.5A) occur with the highest soil Cu concentrations (Fig. 5.2A). This "paradox" is apparently a consequence of a) the importance of higher

C_{org} and nutrient availability for modifications of Cu toxicity (as discussed by Domínguez et al., 2010); and b) the existence of other soil constraints which accompany the land use-induced Cu decrease (high Al, low P and Zn, Fig. 5.4) in these complexly polluted soils.

The main gradient in leaf mineral composition (decrease of Ca and Mg, increase of S, Fe and Al, Fig. 5.1C) clearly reflects the main soil spatial gradient (Axis 1 in Fig. 5.1A). However, just like the grain yield, the leaf mineral composition does not respond to the change of major soil constraints, i.e. does not respond to the increased plant available soil Cu (Fig. 5.1D, see Axis 2 in Fig. 5.1B). Overall, leaf chemical analysis (Fig. 5.6) shows: a) no phytotoxic concentrations of elements (with the exception of As on some most severely polluted soils); b) severe deficiency of N and P; and c) no toxic concentrations for animals (Chaney, 1989; Mortvedt et al., 1991; Bergman, 1992).

Two interesting phenomena can be observed (possible physiological background discussed in details previously by Nikolic et al., 2011). Firstly, increase of leaf As (exceeding the phytotoxicity threshold of 3 mg kg^{-1}, Chaney, 1989; Fig. 5.6C) and drastic decrease of leaf N (Fig. 5.6A) with increased tailings deposition along the spatial gradient (represented by Axis 1 in Fig. 5.1). Leaf concentrations of As and N apparently do not reflect the respective soil concentrations: in the severely polluted transect portions (S_{tot} > 1 %, yield reduction > 70 %), the concentrations of HNO_3-extractable soil As actually decreases (Table 5.2) while soil N remains constantly low along the whole transect (Fig. 5.4A). However, leaf As and N are consistently and significantly related to the leaf P status and plant available soil P along the spatial gradient (Axis 1 of Fig. 5.1). Secondly, the pronounced concentration effect (due to the drastic growth inhibition) precludes micronutrient deficiency diagnosis on severely polluted soils (Fig. 5.6B). However, it is clear that Zn and B concentrations in the leaves are positively related to the grain yield (Fig. 5.1 C and D).

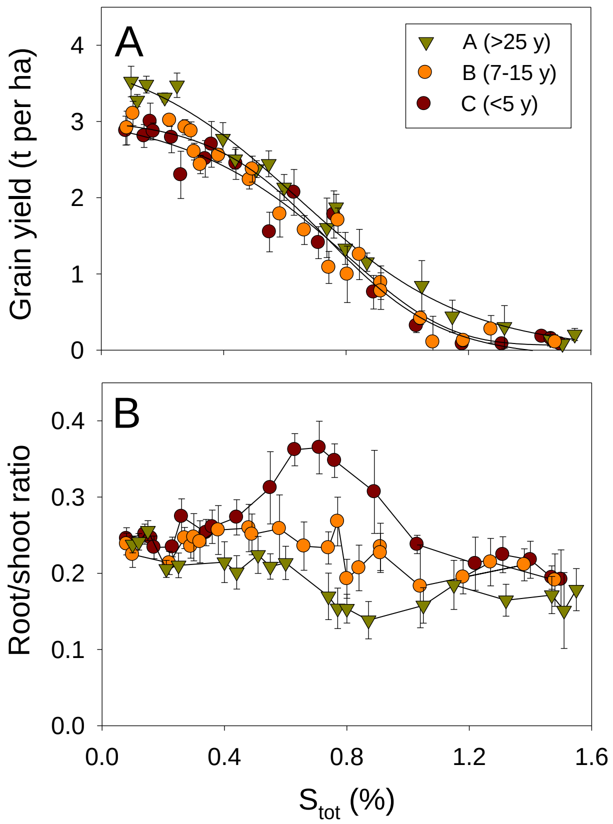

Fig. 5.5: Growth parameters of barley crop as affected by pollution gradient and different land use intensities.
A – grain yield at maturity;
B – root/shoot ratio
(post anthesis, Z70-71).

Surprisingly, even Cu appears to follows the same trend (though far from reaching deficiency range), i.e. higher yields are actually accompanied by higher Cu in leaves. On the lower soil Cu pollution levels (about 0.2 - 0.5 % S_{tot}, Fig. 5.2B) where the grain yield reduction is up to 30% (Fig. 5.5A), barley leaf Cu concentrations are by about 35 % higher than on the more severely polluted soils where yield reduction is 30-70% (Fig. 5.6B). In barley, as in most plant species, Cu translocation to shoots at elevated soil concentrations is highly restricted (Broadely et al., 2012), and leaf Cu concentrations can usually provide no information on soil Cu levels. The occurrence of higher leaf Cu status with higher yields seems to be rather a consequence of an overall restriction of nutrient uptake along the pollution gradient, caused by multiple and successive root constraints along the gradients, which include excessive Cu and Al and extremely deficient P. For instance, it has been demonstrated that nutrient uptake can be strongly restricted due to the Cu – induced root growth inhibition (Adalsteinsson, 1994).

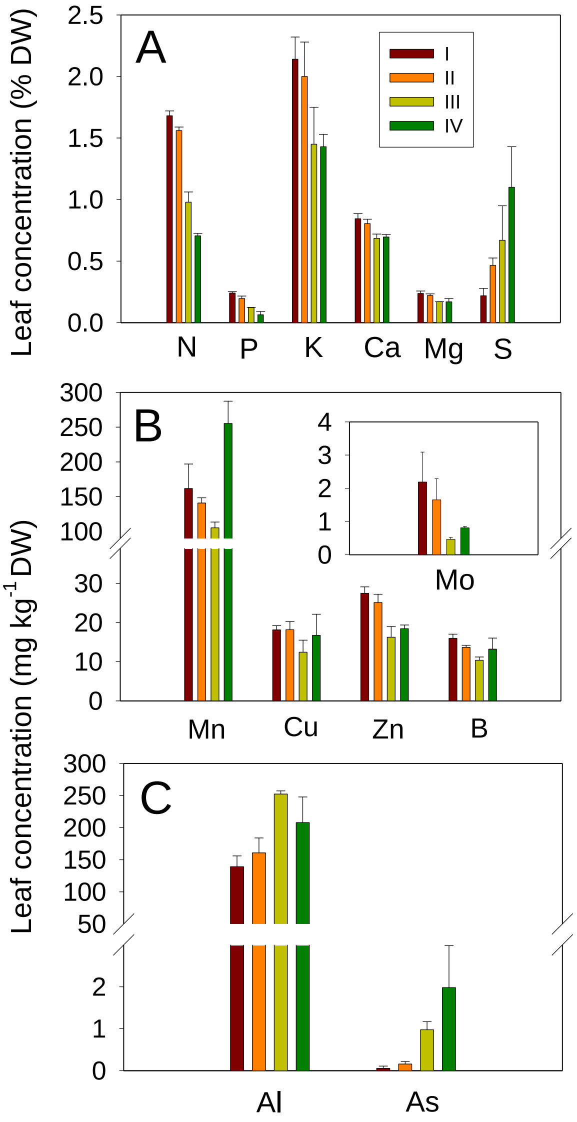

Fig. 5.6: Concentrations of mineral elements in barley leaves (post anthesis, Z70-71) along the pollution gradient.
A – macronutrients;
B – micronutrients;
C – harmful elements;
Relative grain yield reduction (%):
I: n.a.; II: <30; III: 30-70; IV: 70-95).

5.3.3.2 Plant adaptations as soil quality indicators

The model crop (barley) has shown differing root growth responses to changes of the dominant soil constraints along both the land use intensity gradient and the pollution level gradient (Fig. 5.5B); root/shoot (R/S) ratio gives an insight into a change of soil environmental quality which cannot be obtained by observing yield or leaf chemical composition alone. The complex interactions in the crop rhizosphere are treated as a "black box" in this study. Though in intermediate soils ("B" series) with both high Cu and low nutrients, multiple stresses preclude any distinct pattern of R/S change, the trends in very long-fallowed ("A") and very short-fallowed ("C") soils are very clear (Fig. 5.5B).

The R/S ratio commonly increases in response to the soil P and N deficiency (Fageria and Moreira, 2011; Lynch et al., 2012) and this can be seen in the "C" soil series (Fig. 5.5B). With the increased pollution level (S_{tot} approximately > 0.6%) however, both the extremely low P and the growing concentrations of the exchangeable Al and low pH (Fig. 5.3C and Fig. 5.4B) cause severe root growth inhibition and decrease of the R/S ratio in “C” soils. On the other hand, root response in the “A” series soils, where high concentrations of plant available Cu are assumed to be the major constraint (Table 5.3), shows a contrasting pattern along the spatial gradient (Fig. 5.5B): The R/S ratio decreases in response to the increasing plant available Cu (Fig. 5.2B), followed by increasing R/S ratio in P-deficient soils (Fig. 5.4B). Although plant available Cu concentrations are extremely high (Fig. 5.2B), they do not *per se* prove the Cu rhizotoxicity, which is highly plant specific and depends on many soil factors (such as soil organic matter, pH, alkali and alkaline metals, Thakali et al., 2006). Nevertheless, a strong Cu-induced root growth inhibition and a decrease of R/S ratio, occurring before any toxicity symptoms in upper plant parts, have been demonstrated (O'Dell et al., 2007).

5.3.4 Environmental implications

The same agronomic soil productivity (measured by grain yield) with locally affordable but modest fertilization levels is found both on the long fallowed soils where very high Cu levels had been phytostabilized with dense vegetation and increased soil C_{org}, and on regularly cropped soils with low Cu and intensive weathering. Likewise, no risks associated with using the fodder barley crop from the polluted soils as animal feed (according to the criteria of Chaney, 1989) were found. The environmental consequences of the two land uses, however, are drastically different. Regularly ploughed soils had retained up to 2 t ha^{-1} of pseudototal Cu fraction less than long-fallowed soils; considering the strong fluvial influences on groundwater and watershed transport processes, this amount presents a severe environmental risk. Furthermore, the clear Cu retention patterns (Fig. 5.2A and B) imply that after several decades, the evidence of the historic pollution is not likely to be found by the river channel where the pollution deposition was most intensive (indicated by the highest S_{tot}), but rather at some distance from the pollution source where the soil matrix (including clay content) had the capacity to retain the metal. Finally, land use *per se* can bring about a three-time drop in C_{org} (Fig. 5.2C), and decrease the already naturally low CEC (Fig. 5.3B); in a long run, the buffering potential of the affected soils to counteract any future environmental hazard becomes thus pronouncedly lower.

5.4 Conclusion

This study demonstrates for the first time that the long-term fluvial pollution by sulphidic Cu tailings can create very clear and consistent patterns in both the properties of alluvial soils and in response of cultivated plants. These patterns point out the probable long-term environmental trends in a "would be" scenario for agricultural use of similar soils polluted by spills of mine tailings.

Though soil characteristics change in the same direction (mainly decrease of nutrients) along the spatial gradient in all land use intensity groups, ploughing promotes a clear succession of the major identified constrains: from Cu toxicity in long fallowed, to Al- and low pH- toxicity and overall nutrient deficiency in regularly ploughed soils. The observed apparent "paradoxes" (both pseudototal and plant available concentrations of soil Cu decreases towards the pollution source; higher yields might coincide with higher soil and leaf Cu concentrations; and leaching of Cu does not restore soils agronomic quality) can be explained by a) the Cu retention patterns along the transects, b) importance of higher C_{org} and nutrient availability for modifications of Cu toxicity, and c) the existence of plant adaptation mechanisms which can considerably counteract the adverse soil conditions.

Using barley as an indicator of soil quality this study demonstrates that land use – induced nutrient deficiency can mask the positive effects of decreased Cu levels on complexly polluted arable alluvial land. While the soil productivity remains the same, environmental impacts of land use intensification are drastically different. In a long run, accelerated Cu mobilization in the watershed is likely to coincide with increases vulnerability of these soils to further environmental hazards.

Acknowledgement

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6 General conclusions

A unique combination of fluvial dynamics with a long-term sedimentation of mine tailings brought about very clear soil spatial gradients along the transects perpendicular to the river channel. Exceptionally strong structure in vegetation, extracted by multivariate analysis, was highly correlated with the measured soil properties. Moreover, strongly acidic, nutrient poor soils created amidst xerothermic surrounding with calcicole vegetation imposed a further strong environmental filter. Altogether, these conditions promoted the research locality to a true “field laboratory”, where some new patterns, which were not reported by other relevant studies so far, could be detected.

The main conclusions to the research questions addressed are:

1. Severe P deficiency is identified as the master limiting soil factor for grains under these multiple abiotic stresses. A drastic decrease of wheat growth (30 – 40 times) over a prominent soil gradient is a sum effect of different plausible soil constraints (excessive concentrations of available Cu and Al, deficiency of B and N; P deficiency-induced increase of As uptake; competition with superior hemicryptophytic weeds), which have, however, remained in domain of a “black-box”. Phosphorus availability was shown to have the highest unique contribution to predicting wheat yield, consistent correlation with growth and visual symptoms, and concentrations in the range of severe deficiency.

2. Spontaneous revegetation of the created barren land is a highly patterned process. Strong vegetation gradients and explicit spatial segregation of primary vegetation types are closely correlated to the main soil constraints. Moreover, one type of pioneer forests was shown to considerably affect the soils by slowing down the oxidative weathering of the tailings deposits and preventing Cu leaching. Pioneers species are the well-known colonizers of post-mining sites worldwide, with different phytoceonological affiliations, geographic origin, and major ecophysilogical adaptations, but they all maintain homeostasis of leaf P concentrations across the pollution-induced soil gradient. Xerothermic surrounding vegetation, adapted to calcareous soils, has a very limited role in spontaneous revegetation process on these severely altered soils. Instead, primary succession relies on novel types of early vegetation which comprise not only novel combinations of species, but also the key role

of species which are novel to the affected region, and do not survive outside of the polluted area. We demonstrate for the first time that pollution-induced severe nutrient deficiency can override the well-established importance of both surrounding vegetation and water level gradient for primary succession.

3. Highly systematic variation in weed assemblages along the soil pollution gradient at the field scale comprises a patterned substitution of calcicoles by acidophytes, increase of circumpolar chorotype, decrease of Ellenberg indices for pH and continentality, decrease of therophytes, succession of Ca-physiotypes, and homeostasis of leaf Cu concentrations. Yet, it's the species' ability to maintain leaf P homeostasis along the soil gradient which appears to be the key adaptation underlying the observed structure in the weed vegetation. Severe environmental filtering has brought about unusual combination of species with strong pattern of association, though little regard for the surrounding vegetation and the well-established phytosociological units. However, these novel associations showed a set of functional adaptations which have enabled the leaf N:P homeostasis and thus the uninterrupted survival of weed vegetation along the soil gradient.

4. Yield and leaf mineral composition of a model crop (barley) were highly correlated with the spatial soil gradient, while root/shoot ratio responded to the land use intensity gradient. Frequent ploughing accelerates the oxidative weathering of the deposited mine tailings and the substitution of high plant available Cu (in long-fallowed) by nutrient deficiency (primarily P and microelements), excessive exchangeable Al, and deteriorated CEC and soil organic matter (in frequently ploughed polluted soils). Because of this succession of soil constraints, agronomic soil quality indicated by crop yield does not improve even when Cu is leached to the background levels. Environmental consequences of cropping are however drastic; up to 2 t ha^{-1} is leached below 30 cm when fallow is shortened from over 25, to less than 5 years. In a long run, accelerated Cu mobilization is likely to increase vulnerability of these soils to further environmental hazards.

Overall, this study implies that severe nutrient deficiency, which is often a neglected issue in studies of metal-polluted sites, can override the effects of Cu toxicity, the role of surrounding vegetation, and even the role of water level gradients, on the process of spontaneous restoration of vegetation cover. It is highly unlikely that spontaneous restoration under the current conditions would allow the reestablishment of the original (or close to original) alluvial vegetation or pre-pollution agricultural land use. Weathering of the substantial residues of sulphides, further acidification and Cu leaching is the major environmental risk; ploughing (or any mechanical soil disturbance with similar effect) of this vast marginalized area should be avoided. On the other hand, pioneer forest vegetation has effectively phytostabilized substantial quantities of Cu. Maintenance of the natural flooding regime, which had, bizarrely, caused this vast degradation in the first place, is indicated important for the current spontaneous succession and warrants further research.

7 Summary/Zussamenfassung

7.1 Summary

Mining and extraction of metals generates huge amounts of tailings waste (a mixture of water, finely ground ore rock and processing effluents, which remains after removal of mineral concentrate). Unfortunately, accidental release of mine tailings into river systems and their further deposition in floodplains, often over arable land, has been reported from many parts of the world, with environmental implications difficult to predict. Mine tailings from sulphidic metal ores combine high potential for generating soil acidity, and high concentrations of plant available heavy metals, which are the two most intractable issues in restoration of post-mining sites. On the other hand, barren land degraded by mining waste provides an exciting opportunity to reveal some important ecological principles which might not be apparent under “normal” conditions. Understanding of the process of primary vegetation succession is in particular crucial for the practice of ecological restoration, which is increasingly seen as a preferable alternative to technical reclamation of land degraded by mining.

This work addresses the two major issues relevant for ecology and restoration of alluvial arable land polluted by mining waste, which have so far been very poorly understood: Firstly, the long-term fate of contaminants and their actual impact on soil quality and crop production under true field conditions. Secondly, the process of spontaneous revegetation of barren land under the extreme soil conditions and the environmental setup considerably different from the well-studied Central European. The explorative study was undertaken on an exceptional locality created by long-term and large scale-fluvial deposition of sulphidic copper (Cu) tailings over alluvial fields in Eastern Serbia. Comprehensive surveys of spontaneous vegetation, weed assemblages and cereal crops (species cover-abundance; biomass per m^2; and foliar mineral analyses), and concomitant surveys of rhizosphere soils (31 physical and chemical parameter) included 297 sampling locations throughout the polluted floodplain (flexible sampling scheme based on visual appearance of vegetation). Data were jointly analysed in a gradient approach framework by different multivariate statistical methods (ordination: NMS, PCA, CCA; classification: agglomerative clustering; group comparisons: MRPP, ISA; habitat modelling: NPMR; and, regression analysis).

The results revealed exceptionally strong structure in the vegetation which was highly correlated with the measured soil properties; the regular change of vegetation and

soil properties occurred along spatially explicit transects perpendicular to the river channel. The clear gradients observed in this “field laboratory” research brought forward some new ecological patterns which had not been reported by other relevant studies so far:

1. Severe P deficiency, most likely not amenable by fertilization, is identified as the master limiting soil factor for grains under the multiple abiotic stresses caused by deposition of sulphidic Cu mining waste. Other plausible soil constraints (low pH, excessive concentrations of available Cu and Al, deficiency of N and B; P deficiency-induced increase of As uptake; competition with superior hemicryptophytic weeds), have remained in domain of a “black-box”.

2. Frequent ploughing accelerates the substitution of high plant available Cu by nutrient deficiency (primarily P and microelements) and excessive exchangeable Al. Thus, agronomic soil quality indicated by crop yield does not improve even when Cu is leached to the background levels. The environmental consequences of intensive land use are however drastic, and increase vulnerability of these soils to further environmental hazards. On the other hand, one type of spontaneously occurring pioneer forests was shown to considerably slow down the oxidative weathering of the tailings deposits and thus prevent Cu leaching via phytostabilization.

3. Although many characteristics of cereal weed assemblages markedly change along the soil pollution gradient, the species’ ability to maintain leaf P homeostasis appears to be the key adaptation underlying the observed vegetation structure. The novel associations of unusual species combinations showed a set of functional adaptations which have enabled the leaf N:P homeostasis and thus the uninterrupted survival of weed vegetation along the soil gradient.

4. The proposed conceptual model describes a highly patterned process of spontaneous revegetation of the created barren land under the severe environmental filtering. In this process, the xerothermic surrounding vegetation adapted to calcareous soils has a very limited role. Instead, primary succession relies on novel types of early vegetation which comprise not only novel combinations of species, but also the key role of species which are novel to the affected region, and do not survive outside of the polluted area. We demonstrate for the first time that pollution-induced severe nutrient deficiency can override the well-established importance of both surrounding vegetation and water level gradient for primary succession.

Overall, this study implies that severe nutrient deficiency, which is often a neglected issue in studies of metal-polluted sites, can override the effects of Cu toxicity, the role of surrounding vegetation, and even the role of water level gradients, on the process of spontaneous restoration of vegetation cover. It is highly unlikely that spontaneous restoration under the current conditions would allow the reestablishment of the original (or close to original) alluvial vegetation or pre-pollution agricultural land use. Weathering of the substantial residues of sulphides, further acidification and Cu leaching is the major environmental risk; ploughing (or any mechanical soil disturbance with similar effect) of this vast marginalized area should be avoided. Maintenance of the natural flooding regime, which had, bizarrely, caused this vast degradation in the first place, is indicated important for the current spontaneous succession and warrants further research.

7.2 Zussamenfassung

Bergbau und Erzgewinnung erzeugen ungeheure Mengen an Abraum (eine Mischung aus Wasser, Grundgestein, Prozessabwässern, die nach dem Metallaufschluss verbleiben). Unglücklicherweise geschieht es immer wieder, dass Bergbau Schlämme in die Vorfluter gelangen, sich in den Auen absetzen oft auch auf fruchtbaren landwirtschaftlichen Flächen. Derartige Unfälle werden aus aller Welt immer wieder gemeldet mit nicht vorhersehbaren Folgen für die Umwelt. Schlämme sulfidischer Metalle haben ein hohes Potenzial für Bodenversauerung und hohe Konzentrationen pflanzenverfügbarer Schwermetalle. Das sind die größten Probleme bei der Restaurierung ehemaliger Bergbaugebiete. Auf der anderen Seite bieten durch Bergbau und dessen Abfälle verwüstete Gebiete die Möglichkeit ökologische Prinzipien zu entdecken, die unter „normalen“ Bedingungen nicht so offensichtlich erscheinen. Das Verständnis für die primäre Sukzession der Vegetation ist besonders wichtig für die Restaurations-Ökologie, die zunehmend als Alternative gegenüber rein technischen Wiederherstellungsmaßnahmen für degradierte Bergbaustandorte zu sehen ist.

Diese Arbeit behandelt vornehmlich zwei für die Restaurierung von Auenflächen, die mit Bergbauschlämmen bedeckt wurden, wichtige Feststellungen, die bisher kaum geklärt wurden: erstens, das Langzeitverhalten der kontaminierenden Substanzen auf Bodenqualität und Getreide-Produktion unter Feldbedingungen. Zweitens, den Entwicklungsprozess spontaner Vegetation auf Offenland unter extremen

Bodenbedingungen und Umweltbedingungen die beachtlich von den sehr gut bekannten Mitteleuropas abweichen.

Diese Untersuchung wurde auf einem außergewöhnlichen Standort durchgeführt, der sich durch langzeitliche und großflächige Auen-Deposition von sulfidischen Kupfer-Schlämmen (Cu) über Alluvionen in Ost-Serbien auszeichnet. Gründliche Untersuchungen der spontanen Vegetation (Arten-Gemeinschaften) und Getreide (Abundanz-Dominanz der Arten; Biomasse pro m2 und Blatt-Mineral-Analysen), Analyse der Kontamination der Rhizosphäre (je 31 physikalische und chemische Parameter) von 297 Aufnahme-Standorten in den belasteten Flussauen (Aufnahmen erfolgten nach dem Erscheinungsbild der Vegetation). Die Datenanalyse erfolgte Gradienten-basiert mit verschieden multivariaten statistischen Methoden (Ordination: NMS, PCA, CCA; Klassifikation: Agglomerative Clusterbildung; Gruppen Vergleiche: MRPP, ISA; Habitat Modellierung: NPMR; und Regressions-Analysen).

Die Ergebnisse zeitigten außergewöhnlich deutliche Strukturen der Vegetation die hoch korreliert mit den gemessenen Bodendaten waren; der regelmäßige Wechsel der Vegetation sowie der Bodenunterschiede zeigte sich entlang hin und herwechselnder Transekte im Bezug zum Gerinne des Flusses. Die eindeutigen Gradienten, die in diesem „Freilandlabor" ermittelt wurden, erbrachten Erkenntnisse zu neuen ökologischen Mustern, die bisher in der Literatur nicht beschrieben wurden:

1. Starke Phosphor-Unterversorgung, nicht aufhebbar durch Düngung, wurde als Hauptbegrenzungsfaktor für die Getreideproduktion unter diesem vielfältigen abiotischen Stress verursacht durch die Kupfer-Schlämme ermittelt. Andere plausible Boden-Stressoren (niedriger pH, stark erhöhte Konzentrationen pflanzenverfügbarem Cu und Al, Mangel an N und B; P Mangelinduzierter erhöhter As Aufnahme; Konkurrenz unter den hemikryptophytischen Kräutern) verblieben in einer Art "black-box".

2. Häufiges Pflügen beschleunigt den Austausch hoch pflanzenverfügbarem Kupfers durch Nährstoffarmut (in erster Linie P und Mikronährstoffe) und exzessiv viel austauschbarem Aluminium. Außerdem verbessert sich die landwirtschaftliche Qualität der Böden angedeutet durch die Getreideerträge nicht auch wenn das Kupfer bis auf das Hintergrundniveau ausgewaschen wird. Die Umweltkonsequenzen intensiver Landnutzung sind drastisch und erhöhen die Verletzbarkeit der Böden bei weiteren Umweltkatastrophen. Auf der anderen Seite hat ein Typ spontaner Pionierwälder zu beachtlicher Verlangsamung der oxidativen Verwitterung der Schlammablagerungen

geführt und so über die Phytostabilisation zur Verhinderung der weiteren Kupfer-Auswaschung geführt.

3. Obwohl die Charakteristik der Unkrautgemeinschaften sich entlang des Belastungsgradienten deutlich verändert ist die Pflanzeneigenschaft eine P-Blatt-Homöostasis herbeizuführen der Schlüssel für die beobachteten Vegetationsstrukturen. Neue Gemeinschaften ungewöhnlicher Artenkombinationen zeigten eine Gruppe funktionaler Anpassungen, die die Blatt N:P Homöostasis und damit das ununterbrochene Überleben der krautigen Vegetation im Bodengradienten ermöglichte.

4. Das dargestellte konzeptionelle Modell beschreibt den Prozess der Musterbildung in der Vegetationsregeneration auf dem Brachland unter extremen Umwelteinflüssen. In diesem Prozess hat die Flora der xerothermen Umgebungsvegetation auf Kalkböden so gut wie keine Auswirkungen. Im Gegenteil: Die primäre Sukzession basiert auf einem neuen Typ Primärvegetation, die nicht nur neue Artenkombinationen beinhaltet sondern auch veränderte Schlüsselrollen der Arten in der betroffenen Gegend, darüber hinaus überleben die Arten nicht außerhalb der belasteten Areale. Damit wird erstmalig gezeigt, dass belastungsinduzierter Nährstoffmangel sich stärker Auswirkt als es die Wirkung sowohl der Umgebungsvegetation als auch der Grundwassergradient für die Primäre Sukzession ist.

Schließlich zeigt diese Untersuchung, dass starker Nährstoffmangel, der oft auf von Metall belasteten Standorten außer acht gelassen wird, sogar die Wirkung der Kupfer-Toxizität übertreffen kann. Es ist höchst unwahrscheinlich, dass unter den gegebenen Bedingungen eine Reetablierung der Auenvegetation (oder entsprechender Vegetationstypen) oder der früheren Landnutzung stattfinden kann. Verwitterung der erheblichen Rückstände von Sulfiden und weiterhin die Versauerung und Cu-Auswaschung sind die Haupt-Umweltrisiken; Pflügen (oder irgendeine mechanische Bodenstörung mit vergleichbarem Effekt) dieser weiten degradierten Landschaft sollte vermieden werden. Die Aufrechterhaltung des natürlichen Wasserregimes, das ursprünglich – bizarrer weise – die großflächige Degradation der Aue bewirkt hat, ist von großer Bedeutung für die momentane spontane Sukzession und dieses bedarf weiterer Grundlagenforschung.

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- DAAD Award for the best foreign student at the University of Hohenheim (2001).
- IAESTE internship grant, Sementes Agroceres (Monsanto), Brazil (1996).

Research Interests

Vegetation ecology and natural resource management; ecophysiological adaptations of plants to marginal land; ecological restoration.

Selected publications

Nikolic N., Nikolic M. 2012. Gradient analysis reveals a copper paradox on floodplain soils under long-term pollution by mining waste. Science of the Total Environment 425: 146-154. (5-Year IF: 3.536)

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Affidavit

I hereby declare that the presented thesis, submitted to the Faculty of Agricultural Sciences, University of Hohenheim, Stuttgart, for the award of Dr.sc.agr./Ph.D. in Agricultural Sciences, is the result of original work carried out independently by myself under the supervision of Prof. Dr. Reinhard Böcker. Any assistance or citation of other work has been duly acknowledged. This work has not been supported by a commercial agent at any instance. I further declare that the results of this work have not been submitted for the award of any other degree or fellowship.

Stuttgart, 2013

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