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## The impact of chemical pollution on the resilience of soils under multiple stresses: A conceptual framework for future research

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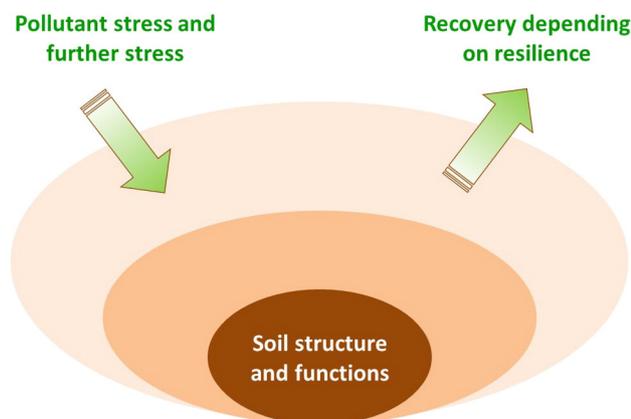
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### HIGHLIGHTS

- Many soils are impacted by chemical pollution and further natural or man-made stressors.
- Resilience of polluted soils against further stress is different than that of non-contaminated soils.
- Mechanisms controlling the interdependencies of several stress factors are largely unknown.
- The concept offers a new interdisciplinary area of research on ecosystem health.

### GRAPHICAL ABSTRACT



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### ABSTRACT

Soils are faced with man-made chemical stress factors, such as the input of organic or metal-containing pesticides, in combination with non-chemical stressors like soil compaction and natural disturbance like drought. Although multiple stress factors are typically co-occurring in soil ecosystems, research in soil sciences on this aspect is limited and focuses mostly on single structural or functional endpoints. A mechanistic understanding of the reaction of soils to multiple stressors is currently lacking.

Based on a review of resilience theory, we introduce a new concept for research on the ability of polluted soil (xenobiotics or other chemical pollutants as one stressor) to resist further natural or anthropogenic stress and to retain its functions and structure. There is strong indication that pollution as a primary stressor will change the system reaction of soil, i.e., its resilience, stability and resistance. It can be expected that pollution affects the physiological adaption of organisms and the functional redundancy of the soil to further stress. We hypothesize that

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Resistance  
Pollutants  
Natural stress  
Copper  
Compaction

the recovery of organisms and chemical-physical properties after impact of a follow-up stressor is faster in polluted soil than in non-polluted soil, i.e., polluted soil has a higher dynamical stability (dynamical stability = 1 / recovery time), whereas resilience of the contaminated soil is lower compared to that of not or less contaminated soil. Thus, a polluted soil might be more prone to change into another system regime after occurrence of further stress. We highlight this issue by compiling the literature exemplarily for the effects of Cu contamination and compaction on soil functions and structure.

We propose to intensify research on effects of combined stresses involving a multidisciplinary team of experts and provide suggestions for corresponding experiments. Our concept offers thus a framework for system level analysis of soils paving the way to enhance ecological theory.

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## 1. Introduction

Soils are an essential basis for human life and provide a large number of essential ecosystem services. However, it is obvious that changing climate as well as human activities like increase in land use intensity or pollution induce significant disturbance of soils and changes of ecosystem services (Smith et al., 2016; Veresoglou et al., 2015). Therefore there is a strong ongoing debate how to protect soils and how mitigation strategies should look like to maintain ecosystem functions of soils also for future generations (Adhikari and Hartemink, 2016; Schmidt et al., 2011). The reactions of soils to stress at the system level are differing and can include no response, adaptation to the stressor, or a sustainable loss of ecosystem functions. Terms in this context, such as resistance, stability, and resilience, will be discussed in detail in the following (for a summary, along with further terms, see Table 1).

Soils have been increasingly contaminated by inorganic and organic xenobiotic substances and other pollutants, but little is known on the long-term impact and side effects of such loads. Based on the concepts of Holling and Johnston (1973) and others, stability of an ecosystem is a function of time to respond and potentially to recover to a stable regime after stress impact, whereas resilience is the capacity to maintain or return to its essential functions and structure. Many articles have been published since then dealing with stability, resistance, resilience, adaption, vulnerability of ecosystems and human related systems. Resilience is considered a fundamental property of ecological systems such as soils (Lal, 1997; Lopez et al., 2013), protecting them against,

e.g., long-term changes in land use (N-deposition, grazing intensity), pollution (sulfur deposition) and climate change (1.5 °C mean increase) as demonstrated by 40 years of monitoring of soil acidity, cation exchange capacity, and C/N ratio in a large set of sites across a range of soil types (McGovern et al., 2013). Yet, little research has been performed on the combined impact of stressors on the resilience of soils, and how resistance against one stressor affects the resilience against the other.

Chemical pollution as one of the planetary boundaries (Steffen et al., 2015) may impact biological functions and structures of soils. The presence of simultaneously occurring stress factors is a typical situation in environmental systems, but knowledge on their interactive effects on soil functions, dynamics and structural development is very limited. We propose to intensify this neglected area of soil research involving experts from ecology, microbiology, physics, chemistry, and ecotoxicology and provide suggestions for new experimental approaches.

The objective of this discussion paper is to understand the effects of co-occurring stress factors on soil, structure, functions and services, a setting which is for instance typical for agricultural soils disturbed by pesticide contamination and compaction by heavy machinery. We will identify research gaps in this area and focus on the question whether the concept of resilience is adequate in its present form to describe the response of soils to disturbance. For instance, high microbial diversity and high levels of functional redundancy may challenge the concept of resilience in soils. Most existing studies in this context focus on the investigation of single endpoints (see definition of terms, Table 1) in

**Table 1**  
Terminology in our conceptual framework.

Term	Definition
Disturbance	Natural or man-made stress events impacting soil functions or structure; pulse disturbance occurs as a relatively discrete event in time, eventually resulting in long-lasting consequences; more gradual or cumulative pressure on a system, lasting for some time, is referred to as press disturbance.
Dynamical stability	Function of time a system needs to respond and potentially recover to a stable regime after stress impact; the faster the system returns to equilibrium after a disturbance, the higher is its dynamical stability (dynamical stability is a function of the reciprocal of recovery time; see Fig. 2).
Ecological stability	Function of the energetic potential of an ecosystem with respect to a certain testable ecological endpoint, dominated by potential barriers (resilience) and the resistance (see Fig. 3).
Endpoint	Physical, chemical and biological characteristic or quality, tangible and measurable, with place- and time-specific experimental results. Measuring ecological endpoints, i.e., traits and functions, allows assessing the impact of stressors on ecosystems (examples in chapter 4).
Pollutant	See comments under "Xenobiotic". The term pollutant is the superordinate comprising natural products – if produced by anthropogenic activity at concentrations that might affect ecosystems – and xenobiotics.
Resilience	Ability of a system to return to its initial state after a disturbance; if a certain threshold of disturbance is surpassed the system state may change. Our concept for research refers to the ability of a polluted soil (pollutant as primary stress) to maintain or return to its function, structure, feedbacks, and therefore prior identity after impact of a second natural or anthropogenic stressor following a recovery phase. (see Figs. 1 and 3).
Resistance	Ease or difficulty of a soil's reaction upon a stress event. Resistance is defined by the inverse height of a response of soil upon stress: the higher the resistance, the smaller its response or reaction on a given stress intensity (see Fig. 3).
Response	Reaction of a soil's function and/or structure upon a stress event; the smaller the response, the higher its resistance (response is a function of the reciprocal of resistance; see Fig. 2).
Stability	Function of time that a polluted ecosystem needs to respond and potentially recover to a stable regime after stress impact; the faster the polluted system returns to equilibrium after a disturbance, the higher is its stability (see Fig. 2). See also "Ecological stability" and "Dynamic stability".
Stress factors	Either of natural (e.g., drought) or anthropogenic (e.g., chemical pollution) origin; primary and follow-up stress is an artificial discrimination because in nature a clear distinction which stress occurred first is often impossible. Different sequences of stress may lead to different consequences in the impacted ecosystem. Under laboratory conditions, however, a defined sequence of stressor impacts can be studied.
Xenobiotic	A chemical synthesized for a specific purpose or formed as a byproduct during synthesis, which does normally not occur as natural product. However, some products typically designated as xenobiotics may also be formed by natural processes, such as dioxins, carbon nanotubes, halogenated compounds. In our concept we also consider other pollutants, e.g., natural products, if they are formed by anthropogenic activity at concentrations high enough to have an impact on ecosystems, for instance elevated concentrations of nitrate by agricultural fertilization.

stressed soils, but we argue that a complex system like soil requires the study of several endpoints to be addressed by interdisciplinary research.

We here propose a concept for research to evaluate the ability of a polluted soil (chemical pollutant as one stressor) to resist further natural or anthropogenic stress and to retain its functions and structure, as well as to discuss the potential mechanisms that may control the underlying interactions. We will focus on Cu pollution as an example for chemical stress, first because many soils are polluted by Cu, e.g., by using fungicides in vineyards and biological farming, and second, because Cu in soils will persist and cannot be degraded. As non-chemical stressor we selected compaction, a typical scenario for soils in agriculture with long lasting impact.

Beside to soils, our concept is in principle applicable also to other ecosystems or environmental compartments, for example, at benthic water-sediment interfaces, thus offering a new broad area of research with respect to environmental pollution.

## 2. Resilience: linking ecosystem resistance to stability

In the pioneering article by Holling in 1973 resilience was defined as the dynamics of ecosystems close to equilibrium and the time required for a system to return to an equilibrium point following a disturbance event (Holling and Johnston, 1973). Walker et al. and the Resilience Alliance (<http://www.resalliance.org/>) sharpened this concept and specified resilience as the capacity of a system to experience stress while retaining essentially the same structure, function, feedbacks, and therefore identity instead of changing into another state (Walker et al., 2006a; Walker et al., 2006b). This definition includes the ability of the system to adapt to disturbances. According to the theory of adaptive cycles, ecosystems do not tend toward some stable or equilibrium condition. Instead, after stress impact they go through a spirocyclic development comprising the four phases exploitation, conservation, creative destruction, and renewal (Gunderson et al., 2002; Gunderson and Holling, 2001). Based on these definitions three categories of resilience have been defined: (1) the amount of change a system can undergo while retaining the same controls on structure and processes; (2) the degree to which the system is capable of self-organization; and (3) the degree to which the system expresses capacity for learning and adaptation (Walker et al., 2006a; Folke, 2006).

Later on the term resilience was expanded to dynamics far from equilibrium steady state and has been defined as the amount of disturbance that a system can stand before it changes into another stable regime defined by a different set of variables and with a different structure, termed ecological resilience. This definition resulted in another set of categories: (1) the latitude or the maximum amount the system can be changed before losing its ability to recover; (2) the resistance, which matches the ease or difficulty of changing the system; (3) the precariousness, i.e., the current trajectory of the system and proximity to a limit or threshold; and (4) cross-scale relations, or how the above three aspects are influenced by the dynamics of the systems at scales above and below the scale of interest (Folke, 2006; Walker et al., 2004).

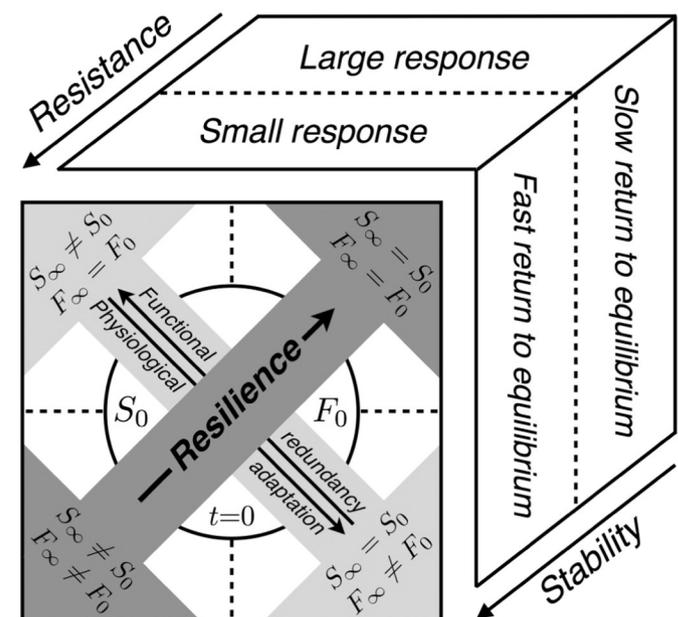
There is general agreement that resilience can be regarded as a non-linear phenomenon strongly connected to population dynamics (Ottermanns et al., 2014). Thus, age-classed populations exposed to environmental stress exhibit less chaotic dynamics with increasing chemical disturbance. Such non-linearly decreasing dynamics are directly connected to reproductive capacity, an organismic trait that triggers the population's ability to respond to further stress. Above certain thresholds of disturbance, qualitative changes in system dynamics (phase transitions or regime shifts) seem to occur (Costantino et al., 1995). They can be explained by a modification of the stability landscape (Walker et al., 2004) which may even be irreversible, depending on strength and duration of the environmental stress (Cline et al., 2014; Dakos et al., 2015).

Many disciplines agree that the degrees of diversity and redundancy are relevant for resilience because they provide a wide range of degrees

of freedom for responding to disturbance. Thereby, response diversity and functional redundancy are particularly important. Response diversity refers to the variety of ways in which different species respond to a disturbance, whereas functional redundancy refers to the capacity of functionally similar elements or species to partly or fully substitute each other. Both aspects work in combination to enhance the resilience of ecosystems. Biggs et al. (2012) pointed out, that also connectivity between organisms, e.g. in remnant communities after impact of a stressor, is important to enable resilience of the ecosystem. However, highly connected systems may also increase the potential for disturbances to spread. Consequently, there is a trade-off in costs and benefits with increasing levels of connectivity, so that the resilience of ecosystems appears to be highest in moderately connected systems, especially when heterogeneity is high.

Resilience of soil may differ when comparing various traits and functions. Corresponding comprehensive experiments are missing so far. For instance, after stress impact the structural composition of a microbial community may change entailing that resilience of soil against the stressor was not strong enough to prevent the structural change. However, a functional parameter, such as the potential of the soil to degrade a specific substrate, may not change after the stress. Therefore, in any resilience investigation a clear endpoint-related hypothesis based on the general hypothesis needs to be provided. Furthermore, resilience must be defined in terms of “what to what” (Carpenter et al., 2001), i.e., what system state and endpoint will be considered (“resilience of what”), and what stressors are of interest (“resilience to what”), where the latter is defined by the boundaries of a corresponding experimental study.

A summary of our perception of the terms resilience, resistance and stability is presented in Fig. 1 showing the possible reactions of an

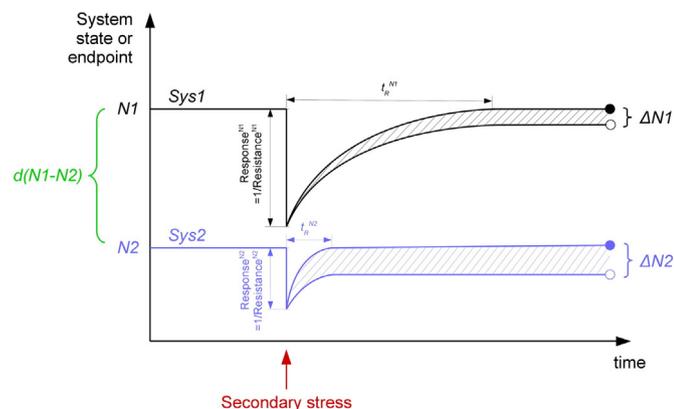


**Fig. 1.** The interplay of stability, resistance and resilience of ecosystems. The graph shows the possible reactions of an ecosystem to a stress applied at time zero ( $t = 0$ , center) by four extreme scenarios: (a) Full resilience: the system returns to the initial state in terms of both structure ( $S$ ) and functions ( $F$ ),  $S_{\infty} = S_0$ ,  $F_{\infty} = F_0$ ; (b) Full physiological adaptation: the structure is conserved but the functions have changed,  $S_{\infty} = S_0$ ,  $F_{\infty} \neq F_0$ ; (c) Full functional redundancy: the structure has changed but the functions are retained,  $S_{\infty} \neq S_0$ ,  $F_{\infty} = F_0$ ; (d) No resilience: both structure and functions have changed,  $S_{\infty} \neq S_0$ ,  $F_{\infty} \neq F_0$ . The resilience of the system is independent of the time needed to return to equilibrium. The third dimension illustrates further aspects of reactions: The higher the stress a system can withstand without turning into a new regime, the higher its resilience (cf. Fig. 3). The smaller the response of a system the higher is its resistance. The faster the return to equilibrium after a disturbance, the higher is the systems stability as defined by Holling (1973) (cf Fig. 2). Note that this definition of stability is different from that used in systems analysis (Ljapunov stability).

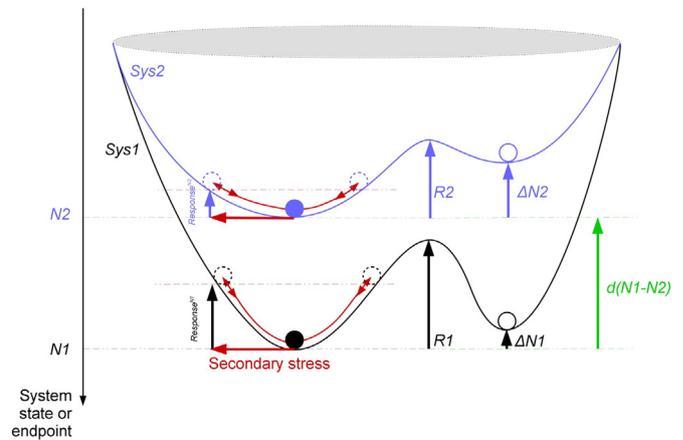
ecosystem to an applied stress. The higher the stress a system can resist without turning into a new system state, the higher is its resilience. The reaction of a system to stress can result in both structural and functional responses. The smaller this response, the higher is the resistance of the system against this disturbance. Four potential scenarios may occur: two extremes (complete recovery to the initial state or no resilience with changes of both structure and functions) and two intermediate states (with either recovery of the structure but not of the function and vice versa). Whereas resilience is independent of the time to reach the equilibrium after stress, the stability defines how fast the system returns to equilibrium after a disturbance. Later, we will differentiate between dynamical stability (Fig. 2) and ecological stability (Fig. 3).

### 3. Stress impacts on soils

Soils are faced to several man-made and natural stress factors. Disturbance is defined as an event that affects ecosystems functions and community or population structures and changes resources, substrate availability or the physical environment (Pickett and White, 1985). Two different types of disturbance can be distinguished. On the one hand press disturbance will last for a certain period of time. The disturbance may cause long-term or permanent changes in species abundance and possibly the loss of some taxa with the establishment of an alternative community composition and structure, e.g., by increased temperature associated with climate change or continuous input of toxic chemicals or acidification of soils. As long as the soil is under the influence of a stressor, a community under continuous disturbance will presumably not return to its prior condition but rather to a different state (Lake, 2013). On the other hand, pulse disturbance from a single event results in prompt changes. Effects of pulse disturbances are generally assumed to recede after some time, so that the system can return to its initial equilibrium state, e.g., by soil compaction due to heavy traffic loads. Taking compaction as representative example, it can be easily seen, however, that there is no clear-cut distinction between pulse and press disturbances in real soil systems, but that they have to be



**Fig. 2.** Dynamical stabilities of an unpolluted and of a polluted soil after action of a secondary stressor. Black lines refer to an unpolluted (Sys1), blue lines to a polluted soil (Sys2). N1: Initial state of Sys1 without contamination, N2: Initial state of Sys2 with long-term contamination.  $d(N1-N2)$ : Difference in initial system states of Sys1 and Sys2.  $t_R^{N1}$ : Recovery time of uncontaminated (control) soil Sys1;  $t_R^{N2}$ : Recovery time of contaminated soil Sys2. After the secondary stress occurred (red arrow on the x-axis) the recovery time of the polluted soil  $t_R^{N2}$  is shorter than  $t_R^{N1}$  and the reaction of the contaminated system ( $Response^{N2} = 1 / Resistance^{N2}$ ) is lower compared to the uncontaminated system ( $Response^{N1} = 1 / Resistance^{N1}$ ) due to stress adaptation of soil organisms. The dynamical stability of Sys1 and Sys2 is a function of the respective recovery times,  $(t_R^{N1})^{-1}$  and  $(t_R^{N2})^{-1}$ . Filled circles (●) stand for a complete recovery to the state before the secondary stress, open circles (○) stand for alternative recovery states (lower or higher than the initial state), i.e., the system may not fully recover but reach an equilibrium state different from the state before the secondary stress occurred ( $\Delta N1$  and  $\Delta N2$ ). The diagram exemplarily describes the level of the system state, the response and the recovery time but it has to be kept in mind that these may change depending on the investigated endpoint. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)



**Fig. 3.** Ecological stability landscapes of polluted and unpolluted soils. Note the opposite direction of the y-axis compared to Fig. 2. Resilience of an unpolluted (black lines, R1 of Sys1) and of a Cu-polluted soil (blue lines, R2 of Sys2) after compaction. Symbols of parameters and colors are identical to those in Fig. 2. The stable states of systems N1 and N2 before compaction are pictured by the position of the filled bowls in the bottom centers of the potential wells. Upon compaction (red arrow on the x-axis) the unpolluted system will be disturbed and the bowl will move and fluctuate with a certain rate to return either to the bottom center (full recovery) or fall into a second minimum of the potential well if a certain potential barrier (resilience R1) is passed (open bowls). Difference in system states is defined as  $\Delta N1$ . Resistance, defined by the inverse heights of the responses ( $Response^{N1}$  and  $Response^{N2}$ ) of the unpolluted soil to withstand the secondary stress by compaction would be lower than that of the polluted soil. The absolute level of the system state of Sys2 (N2) is lower than that of Sys1 (N1), but the Cu-polluted system is more stable and returns faster to an equilibrium state after compaction than the unpolluted system (i.e., its dynamical stability is higher as shown in Fig. 2). We hypothesize that the potential barrier R2 (= resilience) of the polluted soil is smaller than that of the unpolluted system, i.e., the bowl may enter the alternative system state ( $N2-\Delta N2$ ) more easily after compaction, in other words, the resilience of Sys1 is higher than that of Sys2. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

regarded as extremes on a continuous time scale. Of course, the mechanical force on a soil, leading to compaction, will last only for seconds, but the resulting compaction will last considerably longer until natural processes (e.g. bioturbation or freeze-thaw cycles) and anthropogenic activities will recover the soil structure again. So, compaction may be considered as a stress factor persisting for some time. Thus, the rather theoretical differentiation in pulse and press disturbance depends obviously on the point of view, the time scale, and the endpoints to be tested.

In the following, two single stress factors, i) soil pollution with copper and ii) soil compaction, will be exemplified, a scenario typical for vineyard agriculture.

#### 3.1. Single stress factors

##### 3.1.1. Example for chemical stress: Cu pollution

It has been reported that Cu-based fungicides, used in viticulture and organic farming, can accumulate in top soils over time and concentrations from 77 mg/kg and in extreme cases up to 3200 mg/kg have been reported (Ruyters et al., 2013; Komarek et al., 2010; Wightwick et al., 2008; Wightwick et al., 2013; Mirlean et al., 2007). In addition, Cu contaminations are also related to the mobilization of horizontal gene transfer by mobile genetic elements that are mostly also containing antibiotic resistance genes (Sen et al., 2011; Pereira et al., 2006; Ng et al., 2009). Cu concentrations in vineyard soils are often above the European legislative limits and above the predicted no effect concentration (PNEC) of 20 to 200 mg/kg depending on the soil type (Smolders et al., 2009). In comparison, typical Cu background concentrations of unpolluted soils are in the range of 30 mg/kg. This accumulation of inorganic fungicides may also affect non-target fungal diversity in the soil whereas bacterial communities may remain unaffected (Adetutu et al., 2008). The effect on soil microbial communities, however, is also largely

controlled by physical and chemical soil properties controlling the bio-availability and bioaccessibility of the accumulated fungicides in the soil.

Cu sensitivity has been demonstrated for various fungal groups (Guillen and Machuca, 2008; Macdonald et al., 2011; Gadd, 1994; Baath et al., 1998). It is evident that members belonging to all major phylogenetic groups of Mycobionta (i.e. Eumycota), which also include soil fungi, may respond sensitively to Cu (and Zn as well) (Baath et al., 1998; Hartikainen et al., 2012; Gadd, 1986) as do the Peronosporomycetes (i.e. Oomycetes), the major targets of the Cu bearing spraying agents. However, resistance against Cu stress is also known for many representatives of true fungi. Therefore, at high concentrations, Cu pollution may select for fungal organisms that possess resistance mechanisms (Gadd, 1994) like extra-cellular complexation by exoenzymes or intra-cellular complexation by metallothioneins (Cervantes and Gutierrezcorona, 1994) and phytochelatins (Collin-Hansen et al., 2007), biosorption and biomineralisation capabilities (Gadd, 2009). In such metal polluted areas copper and other metals function as a selective pressure and entail the dominance of certain fungal species (Gadd, 1986). In contrast to fungi, bacterial communities are able to adapt to heavy metal stress by gathering the required resistance genes through horizontal gene transfer mainly mediated by plasmids (Sen et al., 2011; Pereira et al., 2006) or transposons that are carrying also antibiotic resistance genes (Ng et al., 2009). The frequency of these mobile genetic elements in nature is high (Top et al., 1994) and is often a stress response. However, the transfer of genetic information and the expression of the related genes require larger amounts of energy which is often lacking in bulk soil. Thus, if the microbial strain is not resistant to the heavy metal per se, it can be postulated that bacteria may not be limited by their potential to adapt to heavy metal stressors, but by carbon and energy limitations which preclude the expression or efficiency of the related functional traits. Investment into these functional traits may nevertheless be awarding for resistant organisms because the heavy metal resistance opens a niche with only few competitors and predators for them. In addition to acquired resistance a large number of isolates from soil has been described that are generally able to tolerate high concentrations of heavy metals based on their natural ecophysiology (Karnachuk et al., 2003). Although Archaea have been described as microbes that can tolerate a large number of stressors and have often been isolated from extreme environments (Gribaldo and Brochier-Armanet, 2006), no studies have been performed so far investigating their role in promoting resilience in soils to maintain its structural and functional properties after heavy metal contamination. Copper-adapted soil microbial communities show a distinct tolerance against additional “fresh” Cu exposure compared to non-adapted soils due to selection and increased proportion of Cu-resistant strains (Brandt et al., 2010; Deng et al., 2009a; Deng et al., 2009b). This is in agreement to Li et al. (2014) who showed that initial Cu stress strengthens the resistance of soil microorganisms to a subsequent Cu stress, e.g., by learning mechanisms mediated by gene transfer or adaption.

High Cu concentrations can also reduce the efficiency of soil microorganisms to enzymatically degrade xenobiotic compounds, as observed for the metabolization of the primary metabolites of atrazine and indoxacarb (Dewey et al., 2012). However, Lebrun et al. reported that variations in the activities of acid and alkaline phosphatases, beta-glucosidase, *N*-acetyl-beta-glucosaminidase, urease and dehydrogenase in soils were dominated by the natural spatiotemporal variability rather than by the effect of contamination with Cu at 2–200 mg/kg (Lebrun et al., 2012). Girvan et al. showed that Cu exposure had little if any effect on litter decomposition when soil microbial diversity was high, however, the mineralization of the pollutant 2,4-dichlorophenol, i.e., a niche function, was impaired (Girvan et al., 2005). Soils with a higher microbial diversity showed a higher structural resilience (maintenance of genetic diversity) than mineral soils with lower diversity.

Griffith et al. proposed that the resilience, tested by the ability of inoculated *Pseudomonas fluorescens* to decompose added plant residues and by monitoring the development of extracted microbial communities inoculated in sterilized soil, under heat stress (40 °C, 18 h) combined with Cu-intoxication is governed by the physico-chemical structure of a soil (its texture) (Griffiths et al., 2008). In another study on Cu stress in soils, earthworms were the most sensitive investigated organisms, followed by bacteria, nematodes, and fungi as the least sensitive organisms. For all these species, community compositions were changed by the long-term pollution. At low Cu concentrations (ca. 20 mg/kg) the soil did not show any decrease in biodiversity, bioactivity, and the above mentioned soil functions, whereas at extremely high Cu concentrations above 3000 mg/kg a severe decrease of these parameters was observed (Naveed et al., 2014). Degraded soils showed lower resistance against several forms of stress (Cu, heat, drought) than the same soils that had been restored over years by fertilizers when comparing the stress effects on nematode abundance and richness as well as on the soil's ability to degrade plant biomass (Liu et al., 2012). This can be explained by the large number of different biogeochemical interface structures in a natural soil, which allows microbes to develop or colonize a variety of different habitats, depending on their particular ecophysiology (Vogel et al., 2014).

These examples show the difficulties to investigate pollutant mediated impacts on soil functions. Inconsistent results are obtained because these effects – and also the recovery from them – may be multifactorial and interdependent. Noteworthy, not in all cases a full recovery of the system is achieved. The effect of pollutant stress on soil organisms (Alguacil et al., 2012; El Azhari et al., 2012) may also result in a severe decrease in bioturbation, which can subsequently lead to structural changes in the soil, such as clay dispersability, bulk density, porosity, and air permeability, as demonstrated for Cu polluted sites (Arthur et al., 2012a). Above a certain Cu contamination level in soil the toxic effect on plants and micro-, meso- and macrofauna leads to formation of more compacted soil due to reduced bioturbation. Such soil has fewer large pores and, thus, reduced air and water permeability and nutrient cycling compared to uncontaminated soil. In this regard, the concept of resilience relies on threshold levels, which may not be an easily determined constant but depend on environmental factors like the prevailing temperature and moisture conditions.

Soils with high SOM stocks and abundance of microorganisms were more resistant against compaction, heat or Cu stress than soils with lower SOM when considering the recovery of the soils void ratio and substrate induced respiration after disturbance (Gregory et al., 2009). The SOM content correlated positively with the recovery of soil functions (plant litter degradation potential) after Cu- or compaction stress (Kuan et al., 2007). Hence, it seems likely that the key soil properties commonly aimed to maintain by good agricultural practice, e.g., SOM conservation, also control the resilience of soils against environmental stress in particular by maintaining functional redundancy.

Little is currently known on long-lasting effects of organic pollutants on microbial performance. Yet, as organic pollutants will degrade and form non-extractable residues, there are natural detoxification mechanisms, rendering non-persistent organic pollutants usually to perform rather like a pulse stressor than as a permanent one. This is different to inorganic pollutant loads such as metals – with Cu as focus of our article – that may change their bioavailability and may be leached, but cannot be degraded in time. However, to advance the research on the impact of multiple stressors on soil, persistent organic pollutants should be investigated as well. Here, our example of chemical pollution with Cu may be used as a proof of concept in forthcoming research alliances.

### 3.1.2. Example for physical stress: Soil compaction

Soil compaction due to agricultural traffic leads to rapid changes of the soil by structure deformation with an immediate impact on physical, chemical and biological soil properties controlling its function as habitat (Weisskopf et al., 2010). There is a strong dependency of microbial and

biogeochemical processes in soils on its structure and vice versa on the microbial regulation of soil structure dynamics (Chenu and Cosentino, 2011) demonstrating the intimate link and feedback mechanisms between biotic and abiotic factors in soil. The highest compaction and therefore largest effects occur in the upper part of the soil profile, while soil functions below 20 cm are less impaired (Kim et al., 2010). Soil fauna and soil microorganisms and their respective functions on, e.g., macropore formation, respiration and nutrient turnover are affected most strongly by compaction (Keller et al., 2013; Nawaz et al., 2013). However, a general trend of soil compaction on biological activities cannot be derived. Both stimulation and impairment of several functions have been reported after slight or strong compaction, although above a certain threshold value (bulk density > 1.7 g cm<sup>-3</sup>), microbial biomass and C-mineralization rates are more frequently reduced than enhanced (Beylich et al., 2010). The apparent discrepancies in the available literature linking soil compaction and biology are in part due to the fact, that the studies comprise a large variety of experimental conditions and situations, such as different land management, climate, and soil properties all interacting with the investigated stress by soil compaction (Beylich et al., 2010). For example, Hartmann et al. (2014) showed that the deterioration of soil structure by compaction did not only decrease soil porosity, air permeability and water conductivity, but also resulted in a significant reduction in abundance, an increased diversity and a persistent alteration of the community structure of microbiota. Soil compaction destroys to a large extent the biopores in the soil, which are hotspots of microbial activity (Uksa et al., 2014; Worrlich et al., 2016) and are the basis of microbial networks like so-called fungal highways (Banitz et al., 2013; Banitz et al., 2014) as well as for syntrophic interactions between organisms (Laksmanan et al., 2014). They are also major pathways of air and water transport.

The response of fungi and bacteria to soil compaction are different. Soil fungi are known to readily respond to compaction by population decrease (Kara and Bolat, 2007). Only fungi with smallest hyphal and conidial diameters are able to survive in the reduced pore sizes of compacted soils. In such situations, compaction may even have an inverse effect by reducing the population of fungal- and bacterial-feeding antagonists like nematodes with higher body diameters (Bouwman and Arts, 2000; Duijker, 2004). Besides the effect of compression shear forces may additionally entail severe suppression of the soil mycota after strong compaction events and when excessive horizontal shear displacement occurs (e.g. due to slip; this is, however, not relevant for spores and conidia).

Although most prokaryotes are much smaller than fungi and may tolerate the reduced pore space better than fungi, the changes in redox conditions and reduced oxygen availability related to compaction can result in significant changes in bacterial and archaeal community structure and the related functional traits, favoring the growth of facultative anaerobic and strictly anaerobic microbes (Radl et al., 2007). Overall a decrease in the kinetics of most turnover processes and thus nutrient cycling must be expected due to reduced availability of oxygen as electron acceptor (Wang et al., 2014) and the lower energy gain supported by alternative electron acceptors. However, possible negative impacts of compaction on individual groups of microbes can be often outbalanced by other less affected groups. An increasing number of studies indicate that many bacteria and fungi form dense networks, including the above mentioned fungal highways, and that these network structures of organisms matter more than the potential function of single organisms since they maintain functional redundancy, an important factor for the resilience and the resistance of soils to environmental stresses (Banitz et al., 2013).

Compacted soils can be also structurally regenerated by physical (e.g., shrinking-swelling, freezing-thawing), and biological (e.g., macrofaunal and microbial activity) processes on a time scale ranging from weeks over months to years depending on soil texture, organic carbon content and dynamics of environmental boundary conditions (Capowiez et al., 2009; Arthur et al., 2012b; Arthur et al., 2013).

These soils have been shown to be colonized by earthworms creating macropores (Yvan et al., 2012), but, reasonably, to a lesser extent than non-compacted ones (Muller-Inkermann et al., 2013). However, earthworm density in compacted soil (Coll et al., 2011) recovers much more rapidly after the initial decrease than soil structural (e.g. macroporosity) and functional properties (e.g. water infiltration capacity) (Yvan et al., 2012). Nevertheless, bioturbation by earthworms as visualized by non-destructive X-ray tomography (Capowiez et al., 2011) has been shown to slowly regenerate compacted soil zones (Capowiez et al., 2009; Dorner et al., 2012; Langmaack et al., 2002). Earthworms in compacted soil (bulk density 1.4 g cm<sup>-3</sup>) may re-transform the soils to a similar state as in less compacted soil (1.1 g cm<sup>-3</sup>) in terms of cast production, water infiltration, ammonium and nitrate leaching, and soil respiration (Jouquet et al., 2012).

In summary, there are a number of studies on the effect of single stressors, e.g., pollution or compaction on soil processes, functions and soil communities. The results show clear indications of strong effects of both chemical and physical stressors on soil functions. However, the vast majority of studies addressed only the effects of a single stressor, with very limited knowledge on the history of other stresses. Thus, there is still a significant gap of knowledge if soil communities under the environmental stress of chemical pollution will be less or more sensitive to another, additional environmental stress compared to those that have not previously been stressed.

### 3.2. Combined effects of chemical pollutants and follow-up stressors

There is increasing evidence that there are legacy effects of multiple stressors on soil functions.

Using a relative soil stability index, indicating the proportion of six enzyme activities the soil retains after a perturbation compared to the activities of an unperturbed soil, it was shown that 2,4-D contamination reduced the ability of three enzyme classes, arylsulfatase, glucosidase and urease, to resist a 60 °C heat perturbation as secondary pulse stress compared to non-contaminated soil (Becaert et al., 2006). As an example for another temperature effect, freezing of soil significantly reduced the tolerance of earthworms against elevated Cu concentrations and, vice versa, Cu exposure reduced their tolerance against soil freezing (Bindesbol et al., 2005).

In contrast to harsh stresses discussed above, pre-exposure to mild stress, such as minor heat, Cu or herbicide contamination, may lead to increased microbial community stability against severe stress by other pollutants, such as mercury intoxication (Bressan et al., 2008; Philippot et al., 2008). Earthworms in Cu polluted soils (150 mg/kg) showed lower drought resilience than worms in unpolluted soils (LC50<sub>drought</sub> at pF 4.48 vs pF 4.09) because the development of estivation cells in Cu-exposed worms has been significantly depressed (Friis et al., 2004). Enchytraeid population density and species composition were highly affected by Cu concentration in soils (300–500 mg/kg) but drought did not have an additional effect (Maraldo et al., 2006).

Heavy metal-polluted soils (Cd, Pb, Zn) differ in their reaction on temperature stress (42 °C) in comparison to unpolluted soils, which are showing higher resilience when measuring basal respiration, β-glucosaminidase activity and protease activity (Epelde et al., 2012). In an another study on Cu-polluted soils (750 mg/kg) with lead contamination as follow-up stress it was shown that soils not contaminated with Cu were more resistant to lead than those with Cu as measured by the respiration rate and the growth rate of bacteria and fungi as endpoints (Tobor-Kaplon et al., 2005). The researchers suggested that microbial communities in the unpolluted soil exhibited the highest functional stability. In contrast, using heat pulses (50 °C) and dry-wet cycles as stressors, soils without Cu load were less stable against additional stress than the Cu loaded soils (Tobor-Kaplon et al., 2006). Interestingly, Pan et al. (2014) showed that the recovery and succession of microbial communities is fairly independent of the stressors and followed a similar pattern across differently stressed soils. The composition of

the starting community was also not a major driver for the succession pattern. In this study, the recovery of community structure also followed recovery of functional traits.

Notably, Arthur et al. addressed the combined effect of Cu as pollutant and compaction as secondary stress on soil function and structure (Arthur et al., 2012a). They found that above a certain threshold of Cu (ca. 500 mg/kg) the soil biological activity has decreased resulting in a more compact soil structure with reduced air and water permeability. The increased density of the Cu polluted soils led to a greater resistance to a subsequent compaction pulse compared to non-contaminated soil. Detrimental impacts on ecosystem services of Cu polluted soil, such as the habitat for soil organisms, air and water regulation, and recycling of nutrients and organic waste, have also been observed by Naveed et al. (2014).

All these examples highlight that a principal prediction of interactive effects of several stress factors – as they usually occur in the environment – is a complex challenge. According to Tobor-Kaplon et al. (2005), there are two contrasting hypotheses about the behavior of stressed systems to an additional stressor. According to the first theory, non-stressed systems should be more resilient and stable, because they have large resources available to maintain their function and structure in case of stress. Second, stressed systems will be more stable and resilient, because the first stress led to adaptation and physiological biological structural changes. Thus, these systems can better cope with additional stress and maintain their structure and function, for example by gene transfer. The often inconsistent results published and our gap in understanding the underlying mechanisms are also partly due to the fact that experiments are designed frequently to elucidate solely the effects and mechanisms for isolated endpoints. However, such a design does not take into account multifactorial interdependencies of soil ecosystems, and strongly calls for a holistic multidisciplinary approach. There is still a lack of knowledge on how pollutants affect the ability of soils to react and cope with follow-up environmental stressors. The sequence of multiple stress, for instance first chemical pollution followed by a further stress or vice versa, may lead to a different response of the impacted ecosystem, which should clearly be a topic for research. In natural systems, the sequence of multiple stress impact cannot easily be determined, this can only clearly be defined (and mutually exchanged) in research projects.

#### 4. The impact of chemical pollution on the resilience of soils under multiple stresses

Referring to the ecological definitions we here propose a concept for research on the ability of a polluted soil (pollutants as primary stressors) to maintain its function, structure, feedbacks, and therefore identity after impact of a further natural or anthropogenic stressor following a recovery phase.

The notion of dynamical stability of polluted soils after follow-up stress is exemplary presented in Fig. 2. Both scenarios, an unpolluted (black lines) and a polluted soil (blue lines), are described by means of a system state (Sys2, Sys1), a generic term representing an individual investigated endpoint that can relate to population and community structures as well as to soil functions and structure. The existence of multiple stable states for functional traits as well as community composition especially as a result of anthropogenic disturbance has been observed in many ecological systems (Fung et al., 2011; Van De Koppel et al., 2001; Carpenter et al., 1999; Scheffer et al., 1993; Scheffer et al., 1997).

The absolute level of the system state of the polluted soil can be lower (for instance a lower degradation potential or lower enzymatic activities etc.) than the one from the unpolluted soil, due to the long term intoxication and adaptation of the soil organisms. After a follow-up stress impact (e.g., compaction) the system state of the polluted soil may change less, i.e., showing a smaller response (= higher resistance), or more, i.e., showing a higher response (= lower resistance),

compared to the unpolluted system. The change in a certain endpoint ( $d(N1-N2)$ ) between the unpolluted control system (N1) and the polluted system (N2) can be normalized to the control value:

Change from  $Endpoint_{control (N1)}$  (%)

$$= \frac{(Endpoint_{2nd\ stressor (N2)} - Endpoint_{Control (N1)})}{Endpoint_{Control (N1)}} * 100$$

In this way a system with a high resistance would be characterized by a low percentage change compared to the control value. In addition, the polluted and unpolluted soil may also differ how they respond to a follow-up stressor with respect to how fast they recover to equilibrium. For instance, the recovery time ( $t_R$ ) of the polluted soil can be shorter compared to that of the unpolluted soil, i.e., the dynamical stability (a function of the reciprocal of the recovery time,  $t_R$ ) of the polluted soil with stress-adapted organisms is higher. Both soils can completely recover to the prior system state of the endpoint or they can reach an equilibrium state which is different from the state before the further stress occurred. Characteristics of the recoveries (% recovery, recovery time, recovery rate) can be used to further describe and compare the impact of the follow-up stressor and the respective endpoint and to reveal underlying processes and their interdependencies in soil.

The concept of ecological stability can be viewed in form of a moving bowl diagram (Fig. 3). Therewith, the follow-up stressor (e.g., compaction) will deviate the soil that will either return to the prior state or move to another equilibrium state if a certain threshold, presumably equivalent to the resilience (R) of the soil, is surpassed. In a similar perception by Scheffer et al. resilience refers to the “size of the valley around a state, which corresponds to the maximum perturbation that can be taken without causing a shift to an alternative stable state” (Scheffer et al., 2001). In our simplified exemplary case, resilience of the non-polluted soil (R1) is higher than that of the polluted soil (R2), which may be more prone to shift to another stable system state after compaction. When confining strictly on pollution by xenobiotic chemicals (see definition of terms in Table 1) the resilience of polluted ecosystems could be defined as “xenoresilience” (or “primed resilience”), which would be a new term taking into account that the overall resilience of the system is influenced by an external and foreign, xenobiotic, impact.

Soil as one of the most complex ecosystems presents an extremely high biodiversity resulting in a high redundancy for many functions. Disturbance of the system may lead to a community shift, but the overall function may remain stable. If stress is gradually increased, it may become strong enough to cause more and more subpopulations to fail resulting in gradual decrease of the function. Therefore, rather than a sudden transition to a new equilibrium, as it is implicitly assumed in Figs. 2 and 3, gradual transition may occur between a number of new equilibria. In addition, as different functions may have a different resilience, they do not get lost at the same time, but in a sequence.

#### 5. Future research needs

Based on Figs. 2 and 3 several exemplary hypotheses can be formulated, which may differ depending on the investigated endpoints (Table 2).

To prove such hypotheses and account for multifactorial interdependencies of both resilience effects and mechanisms in the soil ecosystem various endpoints should be investigated comprehensively covering a range of abiotic and biotic structural and functional parameters at different spatio-temporal scales. The following effects of stressor combinations on relevant sets of endpoints, which are essential indicators for soil functions and processes, need to be assessed in future research:

- Soil pore space architecture and link between pore network properties and physical soil functions (air permeability, oxygen diffusivity, retention and transport of water, soil mechanical stability and aggregate strength)

**Table 2**

Exemplary possible hypotheses which may differ depending on the investigated endpoints; the symbols < and > are relative measures referring to the comparison of polluted and non-polluted soils; other symbols refer to Figs. 2–3. The initial system state, measured by testing a certain endpoint such as an enzyme activity (other examples see listed in the text below), in a polluted soil is lower than in a non-polluted soil. The resistance of a pollutant adapted soil after an additional stress impact and its dynamical stability are higher, whereas its resilience is lower.

	Polluted vs unpolluted soil	Symbols
Initial system state	<	N1, N2
Response (= Resistance <sup>-1</sup> )	<	
Dynamical stability (= Recovery time <sup>-1</sup> )	>	( $t_{R1}^{N1}, t_{R2}^{N2}$ ) <sup>-1</sup>
Deviation from initial state if stress impact exceeds the systems resilience	>	$\Delta N1, \Delta N2$
Resilience	<	R1, R2

- Spatial distribution, binding forms, potential mobility, bioavailability/accessibility of the pollutant in the soil matrix, microbial hot spots and how these properties are altered by the follow-up stresses; fate and degradation of pollutants; nutrient cycling and organic matter turnover (respiration measurements) at different scales
- Litter availability and accessibility for soil organisms, community of litter decomposers and predators in the terrestrial food web
- Enzymatic activities
- Composition and behavior of soil bacterial and fungal communities along a pollution gradient; *ditto* for soil meso- and macrofauna
- Dynamics of fungal spatial distribution, growth, presence and amounts of propagules, and feeding behavior as well as the spatial distribution and function of motile and immobile bacteria and network performance according to matrix stress
- The response of any of the above endpoints to a certain sequence of two stressors, e.g., in our example first chemical pollution followed by compaction or vice versa
- The impact of the exposure time of an ecosystem to a certain stress before a further stress occurs

For the prediction of effects of (sequential or simultaneous) multiple stressors on soil a wide range of abiotic and biotic structural and functional endpoints have to be tested. This may involve methods

- to characterize soil structural properties by e.g. 3D X-ray microscopy;
- to characterize nutrient and organic matter turnover and the spatial distribution, speciation, bioavailability, and accessibility of pollutants using wet-chemical fractionation and extractions techniques, coupled to spectroscopic and spectrometric techniques such as spatial resolved DGT, SEM/EDX, XRF, NanoSIMS, or XANES spectroscopy and complemented by isotope tracing techniques (e.g., 13C, 14C, 15N, 18O, 2H);
- to quantify and map spatial distribution of enzyme activities (Spohn et al., 2013) and integrate and compare obtained results using e.g., a soil stability index (RSSI) in relation to physical and chemical soil parameters (Boyd, 2010);
- to disentangle effects of different stress factors by laboratory incubation experiments under controlled conditions;
- to investigate the gene coding for fungal exoenzymes and enzymes by metatranscriptome and metaproteome analyses. Additionally, the introduction of molecular techniques in (microbial) ecology allows for a phylogenetic description of community structures in soil (environmental barcoding, using Next Generation Sequencing) as well as fingerprinting fungal communities and identification of selected functional traits (Emmerling et al., 2002). Although these approaches have been used in a number of studies on contaminated soils, only few papers also address the effects of shifts in the soil microbiome for ecosystem functions (Ollivier et al., 2013).

The understanding of resilience mechanisms requires the involvement of different disciplines. By merging the results from endpoint analyses linkages within and across scales may be revealed – for instance from microorganisms to epigeic predators, from enzymatic activities to degradation potentials, and from biotic to abiotic properties. Therefore, individual multi-disciplinary endpoint analyses have to be integrated by an overarching mechanistic modeling of soil physical, chemical and microbial linkages and dynamics parameterized by calibration to experimental results from individual investigated endpoints as shown above.

## 6. Conclusion

More than 100,000 chemical substances are nowadays commercially available, which at least partly end up in the soil, either intendedly (i.e., pesticides, biocides) or unintendedly. The toxic input into soil becomes even larger if we consider products such as engineered biocidal nanoparticles (for instance silver nanoparticles) that may enter the soil by amendment with sewage sludge where these materials accumulate. Moreover, pollutants are heterogeneously distributed in soil, leading to local hotspots of pollutant loads on, e.g., the surfaces of litter, minerals, or aggregates. We can expect that a basic load of xenobiotic contaminants will pertain in many soils while other stresses occur, disturbing soil functions and structure. Thus, understanding resilience, stability and resistance of polluted soils to follow-up stress is of utmost importance to preserve their ecosystem services. Such research will contribute to providing information and forecasts about how soils impacted by pollutants will respond to additional natural and anthropogenic stresses.

So far most studies on polluted soils consider only the effects of single stressors on isolated endpoints and, thus, neglect multifactorial interdependencies in the soil. Interacting effects of multiple stress factors on various structural and functional endpoints are, however, the rule rather than the exemption. We believe that the resilience of polluted soils against further stress is a highly relevant research topic which hasn't been studied systematically in the past. It offers a framework for the comprehensive understanding of interactive effects of multiple stressors on soil structure and functions. It is evident that such studies can only be performed in a multidisciplinary research alliance comprising experts and methodologies from soil ecology, microbiology, physics, (geo)chemistry, ecotoxicology, and modeling. To our knowledge such synergistic investigation of biological, chemical and physical properties of contaminated soils in the presence of one or several other stress factors offers a new and broad area of highly relevant research with respect to environmental pollution and ecosystem health.

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