

[5]). This might often be the higher-rainfall or the warm boundary.

- (ii) Species boundaries are more likely to be determined by competition when travelling in the direction of longer intervals between disturbances such as fires.
- (iii) The regeneration phase is often a good guide to population-level outcomes. For example, species able to establish under shade are expected to dominate forests eventually. (This idea is important because the full demographic characterization recommended by Alexander *et al.* [1] will often be difficult.)

Each of these working hypotheses may or may not be true (e.g., [6,7]), or may be true under some circumstances but not others. For instance, there is debate about whether competition has more influence at sites with more intense competition or with less intense disturbances [5,6]. This is exactly why reliable field-based knowledge about these hypotheses is needed as quickly as possible. Ideally, the aggregate of beyond-boundary experiments worldwide would be distributed in such a way that they added up to strong tests of working hypotheses such as these. Plant biology has done well since the 1990s in building global networks and collaborations (examples include Angiosperm Phylogeny Group [8], Glonnet [9], the TRY trait database [10], and NutNet [11]). We suggest there could be value in developing a network for transplants beyond boundaries. It could discuss and coordinate priorities, share protocols, and advocate for the importance of these field experiments.

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Letter

Potential Environmental Impacts of an “Underground Revolution”: A Response to Bender *et al.*

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Bender *et al.* [1] discussed the promising idea of ecological intensification to positively influence agricultural practices through soil ecological engineering. Analysis of impacts is essential for the sustainability of environmental technologies, therefore, we complement their discussion by considering possible negative outcomes of manipulating soil biodiversity and composition. From an environmental

health perspective, we argue why ecological intensification *per se* does not guarantee absence of impacts. Then, we discuss how microorganisms differ from traditional agrichemical contaminants, thus requiring new ecotoxicological frameworks for proper regulation. Finally, we explore potential threats for soil biodiversity of an ecological intensification.

Ecological intensification could deliver agricultural benefits while causing unwanted biogeochemical effects. For instance, Bender *et al.* suggested interference with the microbiome using inhibition of denitrification to increase nitrate availability to plants [1]. This could cause problems similar to mineral fertilizers because nitrate is mobile and transferable to aquatic systems, causing eutrophication of surface waters and compromising aquifer potability. In fact, impacts in surface and groundwater have been noticed as adverse consequences of misuse of nitrogen cycling bacteria [2]. Modifications of soil community composition must be carefully done so that increased nutrient availability is coupled with fast uptake and minimal leaching. Additionally, microbiome management implies the need to control not only the target metabolite (e.g., nitrate) but also the populations that could disperse and impact elsewhere. However, the environmental fate of microorganisms is not easily predictable.

According to Bender *et al.* [1], to achieve maximum effects, management should cover multiple scales, including microbiomes, soil and plant communities, and genetics. Nonetheless, the introduction of microorganisms as prospective contaminants adds risks and complexity (Figure 1). For example, the environmental behavior and toxicity of metals are reasonably understood [3]. Cu interacts with the target organisms causing desired effects or with non-target organisms and potentially causes undesired effects. As Cu toxicity mechanisms are fairly described [3], estimation of effects provides solid risk assessments. For more complex compounds,

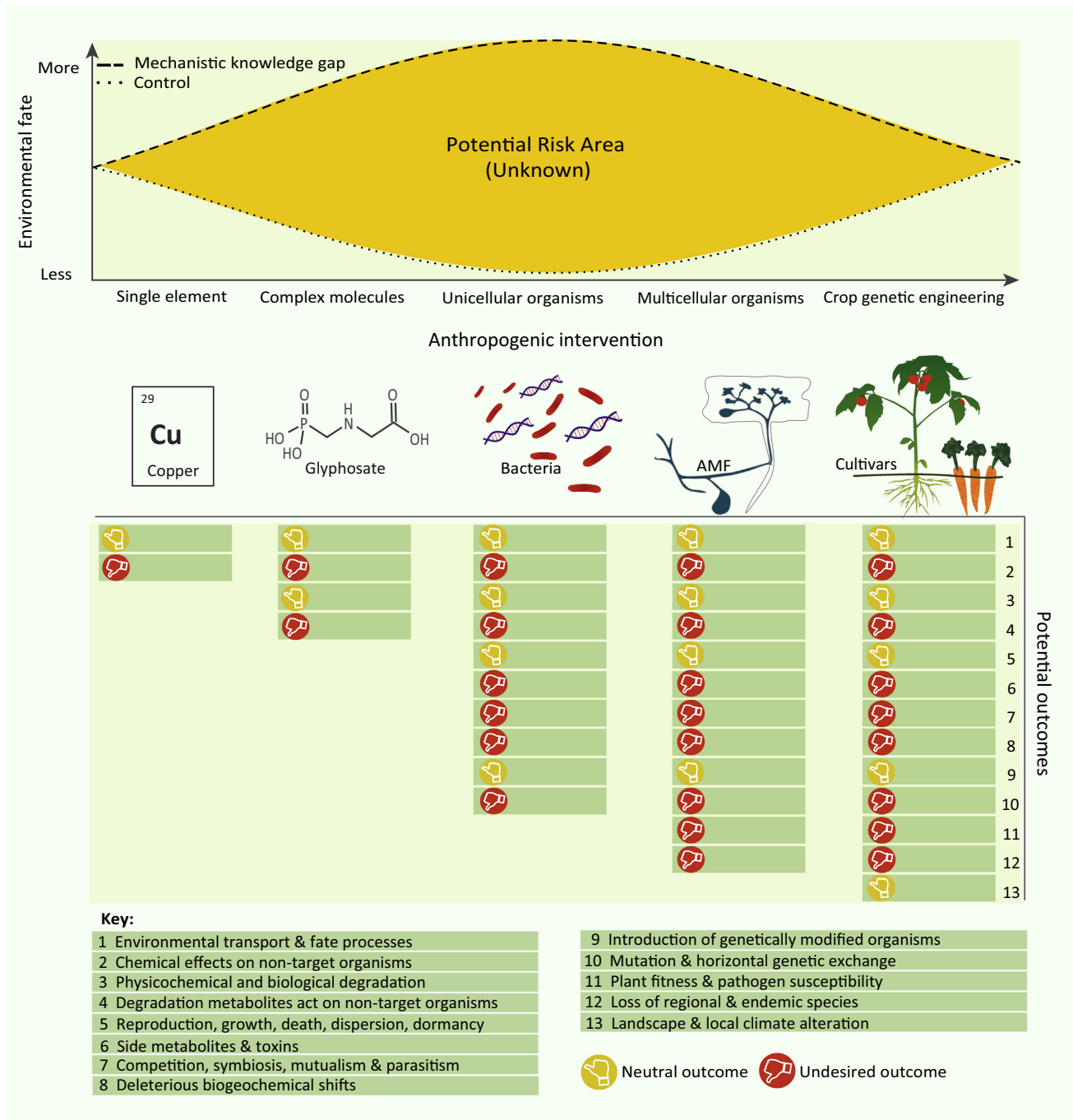


Figure 1. Environmental Fate and Effects of Selected Entry Points for Management of Crops and Soils. The anthropogenic interventions (x axis for both upper and lower panels) are compared regarding technology to control the outcome of such interventions (short-dashed line, upper panel), current knowledge gap on mechanisms of action (long-dashed line, upper panel), and the potential outcomes (lower panel). The examples of anthropogenic interventions at various entry points for management in the lower panel are Cu (micronutrient, molluscicide, and fungicide), glyphosate (herbicide), entomopathogenic bacteria (insecticide), arbuscular mycorrhizal fungi (AMF-plant symbiont), and cultivar genetic selection. Note that the suggested underground revolution focuses on entry points with larger potential risk area. We omitted the multiple possible positive effects on the interaction of microorganisms causing the desired outcome since they were properly addressed in Bender *et al.* [1]. Also, adverse outcomes are not exhaustively explored.

degradation additionally generates metabolites with diverse physicochemical properties that drastically hamper estimation of fate and effects. Glyphosate, for instance, designed as a specific enzymatic inhibitor for weed germination, is currently accepted as a broad-spectrum toxicant, endocrine disruptor, and human carcinogen [4]. Glyphosate metabolites have even broader toxicity [4]. Comparatively, our ability to forecast the fate [5,6] and effects [7] of organisms lags far behind predicting chemical effects. Lack of mechanistic knowledge denotes low environmental control and unknown risks [7], that is, a single organism can attain unknown population sizes, produce unpredicted metabolites, and give rise to complex ecological interactions. Consider the soil entomopathogenic bacteria *Bacillus thuringiensis* (Bt), a successful biopesticide. It produces thousands of different toxins and metabolites that synergistically account for its insecticidal activity [8]. Predicting the fate or monitoring each compound is currently impossible because they are not fully described and their production varies with environmental conditions [8]. Bt is toxic to ~25% of non-target organisms studied, shows vertebrate *in vitro* cytotoxicity, and it horizontally exchanges genetic material with other populations. Also, evolutionary pressure by Bt caused trophic rearrangements in heavily treated areas. Notwithstanding these effects, modern Bt biopesticides fail to show toxicity in old standardized ecotoxicological assays [8], and manufacturers have assured that Bt biopesticides are globally marketed without application limits. New ecotoxicological tools are required to properly establish safe limits for usage of pathogenic microorganisms. Ecological indirect effects might be less explicit. For instance, uncontrolled populations of beneficial mycorrhizal fungi might impact below- and above-ground communities because they selectively influence the fitness of better host plants [7,9]. In contrast to microbes, better mechanistic knowledge and advantages of controlling larger size organisms [7] make less likely unforeseen risks of engineered crops.

Unregulated ecological intensification might also exacerbate risks of biodiversity loss and extinction. The main anthropogenic causes for extinction are habitat loss and introduction of invasive species [6]. Traditional agriculture can cause the first [1,10], whereas the inoculation of soil organisms and genetic manipulation as proposed by Bender *et al.* [1] and others might foster the latter. Important soil organisms like fungi may show low intercontinental genetic exchange and high regional endemism [11]. The assembly of such organisms is limited by dispersal [9,11]. Therefore, their global biogeography can change rapidly if few strains are made commercially available, with undesirable wide-ranging effects on plants, biodiversity, and ecosystem functions [7]. Moreover, Bt genes inserted in different below- and above-ground organisms provide the agricultural market with booming genetically modified multipesticidal organisms that require fewer interspecies interactions to deliver desired outcomes. Thus, it is sensible that commercialization of competitive transgenic hybrid plants or microbes proposed [1] could affect soil biodiversity.

We believe that techniques from Bender *et al.* [1] are encouraging. Agriculture must be adjusted to secure future food production and environmental health [9]. However, there are biogeochemical, ecotoxicological, and biodiversity threats associated with soil microbiome management. Without the proper mechanistic knowledge, it is not prudent to assume that the consequences are strictly positive [7]. To help to achieve the sustainability goal from Bender *et al.*, we propose that such an underground revolution must be accompanied by a dedicated consideration of its potential impacts on soil biodiversity and its function.

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Letter

Strategies for Environmentally Sound Soil Ecological Engineering: A Reply to Machado *et al.*

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Recently, we proposed that soil ecological engineering can be used to enhance