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 heath [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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References
1. Bender, S.F. *et al.* (2016) An underground revolution: biodiversity and soil ecological engineering for agricultural sustainability. *Trends Ecol. Evol.* 31, 440–452
2. Kowalchuk, G.A. and Stephen, J.R. (2001) Ammonia-oxidizing bacteria: a model for molecular microbial ecology. *Annu. Rev. Microbiol.* 55, 485–529
3. Machado, A.A.S. *et al.* (2016) Metal fate and effects in estuaries: a review and conceptual model for better understanding of toxicity. *Sci. Total Environ.* 541, 268–281
4. Myers, J.P. *et al.* (2016) Concerns over use of glyphosate-based herbicides and risks associated with exposures: a consensus statement. *Environ. Health* http://dx.doi.org/10.1186/s12940-016-0117-0
5. Shi, S. *et al.* (2016) The interconnected rhizosphere: High network complexity dominates rhizosphere assemblages. *Ecol. Lett.* http://dx.doi.org/10.1111/ele.1263
6. Veresoglou, S.D. *et al.* (2015) Extinction risk of soil biota. *Nat. Commun.* http://dx.doi.org/10.1038/ncomms9862
7. Schwartz, M.W. *et al.* (2006) The promise and the potential consequences of the global transport of mycorrhizal fungal inoculum. *Ecol. Lett.* 9, 501–515
8. World Health Organization (1999) *Environmental Health Criteria 217: Microbial Pest Control Agent Bacillus thuringiensis,* WHO
9. Vályi, K. *et al.* (2016) Community assembly and coexistence in communities of arbuscular mycorrhizal fungi. *ISME* http://dx.doi.org/10.1038/ismej.2016.46
10. Green, R.E. *et al.* (2005) Farming and the fate of wild nature. *Science* 307, 550–555
11. Peay, K.G. *et al.* (2016) Dimensions of biodiversity in the Earth mycobiome. *Nature* 14, 434–447

Letter

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

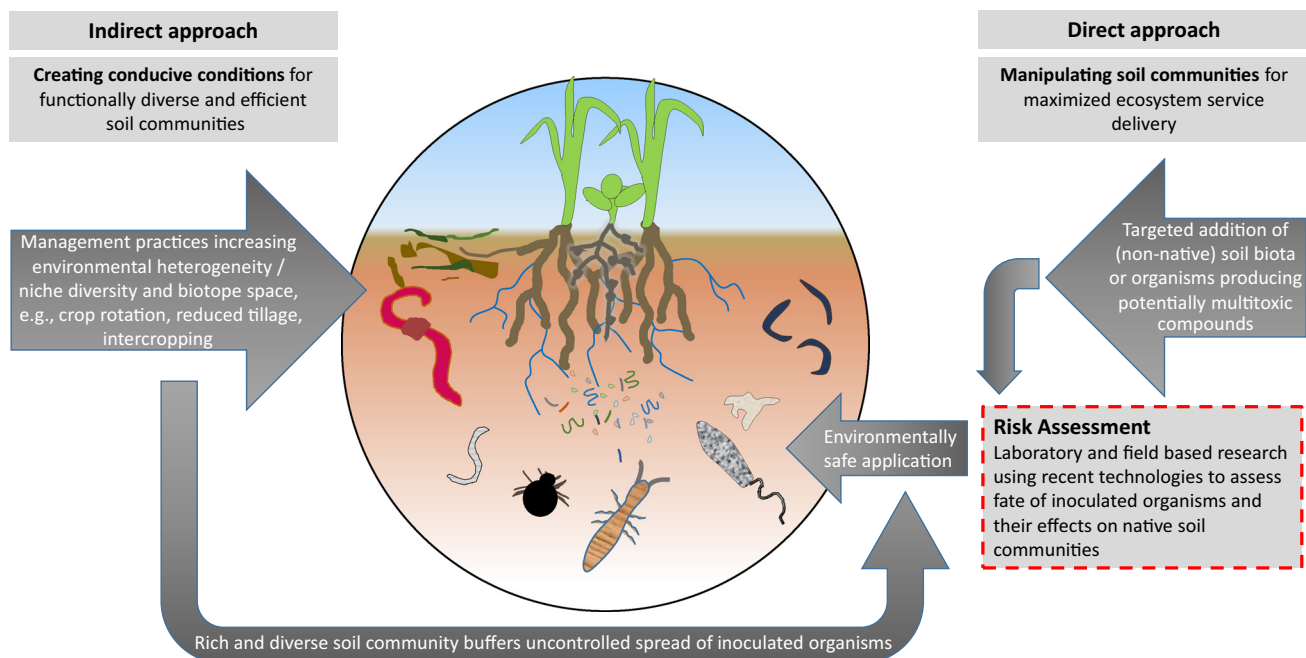S. Franz Bender,[1,*] Cameron Wagg,[2] and Marcel G.A. van der Heijden[2,3,4]

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

agricultural sustainability [1]. In their response to our article, Machado *et al.* [2] stress the point that potential undesirable side effects of soil ecological manipulations have to be considered and carefully investigated. We fully agree with their general appeal to consider ecological consequences of ecosystem manipulations (see also p. 447 in [1]). However, we want to recall that there are no human activities without a certain impact on ecosystems. Agricultural management of soils generally has strong adverse impacts on the structure and functioning of soil communities. The impacts of the ecological manipulations proposed in our article [1] therefore have to be considered in the light of the already existing management system to which they are applied.

We categorized the soil ecological engineering approaches proposed in Bender *et al.* [1] in two groups, the 'direct' and the 'indirect' approaches (Figure 1). The indirect approach serves to foster, optimize, and use the inherent potential of soil ecosystems to maximize ecosystem functioning by appropriate management. For instance, crop rotation and the inclusion of grass–clover pastures/ley in rotations can be used to promote beneficial soil life and soil ecosystem services. Grass-clover pastures can fix substantial amounts of nitrogen (up to 300 kg N/ha year [3]), sequester carbon [4], contribute to pest and disease control (including effective control of the corn rootworm *Diabrotica virgifera* [5]), and a one- or two-year grass–clover pasture in a rotation can enhance populations of beneficial soil biota such as earthworms and arbuscular mycorrhizal fungi [6,7]. These strategies affect soil communities indirectly by increasing environmental heterogeneity, niche diversity, and biotope space, providing conducive conditions for well-developed and functionally robust soil food-webs (Figure 1). The breeding of crops efficiently interacting with native soil communities through specific root exudation patterns also falls in this group. As long as no non-native soil organisms are introduced to the system or crops releasing multitoxic compounds are planted (see the example of *Bacillus thuringiensis* biopesticides described in Machado *et al.* [2]), we consider these approaches as relatively unproblematic for soil ecosystems and their biodiversity. We agree with Machado *et al.* [2] that these approaches might lead to unbeneficial outcomes under some circumstances. If properly managed, however, these effects can be minimized and are unlikely to cause more negative side-effects than those caused by 'mainstream' agricultural practices such as pesticide application, fertilization, and intensive tillage. For example, if crops with denitrification-inhibiting traits [8] were planted and nitrate availability to plants increased, the amounts of external N fertilizer could be accordingly reduced. Such approaches could lead to more efficient nutrient cycling (from an agronomic perspective), as plant

Indirect approach

Creating conducive conditions for functionally diverse and efficient soil communities

Management practices increasing environmental heterogeneity / niche diversity and biotope space, e.g., crop rotation, reduced tillage, intercropping

Direct approach

Manipulating soil communities for maximized ecosystem service delivery

Targeted addition of (non-native) soil biota or organisms producing potentially multitoxic compounds

Risk Assessment
Laboratory and field based research using recent technologies to assess fate of inoculated organisms and their effects on native soil communities

Environmentally safe application

Rich and diverse soil community buffers uncontrolled spread of inoculated organisms

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Figure 1. Indirect and Direct Approaches of 'Soil Ecological Engineering' [1]. The indirect approach comprises management practices creating conducive conditions for rich and diverse native soil communities. The direct approach comprises inoculations with soil biota possessing desired traits to optimize ecosystem service delivery, or the planting of engineered crops producing compounds toxic for some members of soil communities (e.g., as a biocontrol strategy). The direct approach potentially bears the risk of unwanted environmental risks as addressed by Machado *et al.* [2] and requires careful risk assessment before large-scale application. Through beneficial biodiversity effects, the indirect approach can also help to buffer unwanted side-effects of the direct approach.

nutrition is maintained, while fertilizer inputs and gaseous N losses, including the strong greenhouse gas N_2O, are reduced. Thorough scientific investigations have to develop proper management guidelines to assure that such approaches lead to the desired increase in nutrient cycling efficiency.

The direct approach (Figure 1) comprises the addition of non-native soil-organisms into agricultural field sites. We fully agree that this approach requires careful risk assessment, as such organisms could potentially become invasive and threaten native soil communities (Figure 1). As stated in Bender et al. [1], methodology is developing fast and allows deeper insights in the fate and the competitive abilities of microbes introduced into agricultural soils. For example, with a newly developed sequencing method, Schlaeppi et al. [9] were able to show that an inoculated strain of arbuscular mycorrhizal fungi (AMF) largely replaced a native, but similar AMF strain in wheat roots. As noted by Machado et al. [2], large scale application of such AMF in agriculture could lead to problems of invasiveness and in extreme cases potentially lead to the competitive exclusion and extinction of native organisms [10]. More research is indeed required to reveal whether these scenarios are realistic and more harmful for soil ecosystems than 'mainstream' agricultural practices.

Figure 1 by Machado et al. [2] adequately summarizes the potential environmental risks related to anthropogenic interventions of differing complexity. However, many of the presented potential outcomes of interventions related to soil ecological engineering would apply to natural ecosystems converted to agricultural land. In our article [1], we proposed to apply the principles of soil ecological engineering to existing agricultural systems. Existing systems are often already confronted with many of the potential risks of agricultural practices summarized in their Figure 1. The set of potential approaches summarized as 'soil ecological engineering' [1] addresses many of these negative outcomes of existing agricultural practices and tries to alleviate them. When focusing on 'soil ecological engineering', we consider the range of potential negative outcomes in Figure 1 of Machado et al. [2] as too extensive.

A hypothetical, optimized agroecosystem managed by soil ecological engineering principles would consist of a rich and biodiverse soil community. It has been shown that soil communities of higher biodiversity are more resistant towards pathogen invasions [11]. Therefore, management systems that maintain and promote a rich and diverse soil community are also likely to be more resistant against species invasions, as shown in more diverse plant communities [12]. By applying several principles of soil ecological engineering in conjunction, the overall benefits for agroecosystems are likely to be greater as if single approaches are applied in isolation.

We thank Machado et al. [2] for the important complementation of our discussion. Some approaches proposed by Bender et al. [1] and others require new ecotoxicological frameworks and careful considerations before being applied on a large scale (Figure 1). The goal of an 'underground revolution' is to optimize internal soil biological processes to generate sustainable agroecosystems producing and maintaining high yields, while minimizing environmental impacts. We have to make sure that unwanted side-effects are minimized, so that the 'underground revolution' will endure and aid in feeding the human population without causing new environmental problems.

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References

1. Bender, S.F. et al. (2016) An underground revolution: biodiversity and soil ecological engineering for agricultural sustainability. Trends Ecol. Evol. 31, 440–452
2. Machado, A.A.S. et al. (2017) Potential environmental impacts of an 'underground revolution': A Reply to Machado et al.. Trends Ecol. Evol. 32, 8–10
3. Nyfeler, D. et al. (2011) Grass–legume mixtures can yield more nitrogen than legume pure stands due to mutual stimulation of nitrogen uptake from symbiotic and non-symbiotic sources. Agric. Ecosyst. Environ. 140, 155–163
4. Albizua, A. et al. (2015) Crop rotations including ley and manure can promote ecosystem services in conventional farming systems. Appl. Soil Ecol. 95, 54–61
5. Kiss, J. et al. (2005) Western corn rootworm (Diabrotica virgifera virgifera LeConte) and the crop rotation systems in Europe. In Western Corn Rootworm: Ecology and Management (Vidal, S. et al., eds), pp. 189–220, CABI
6. Verbruggen, E. et al. (2010) Positive effects of organic farming on below-ground mutualists: large-scale comparison of mycorrhizal fungal communities in agricultural soils. New Phytol. 186, 968–979
7. Riley, H. et al. (2008) Soil structure, organic matter and earthworm activity in a comparison of cropping systems with contrasting tillage, rotations, fertilizer levels and manure use. Agric. Ecosyst. Environ. 124, 275–284
8. Bardon, C. et al. (2014) Evidence for biological denitrification inhibition (BDI) by plant secondary metabolites. New Phytol. 204, 620–630
9. Schlaeppi, K. et al. (2016) High-resolution community profiling of arbuscular mycorrhizal fungi. New Phytol. 212, 780–791
10. Veresoglou, S.D. et al. (2015) Extinction risk of soil biota. Nat. Commun. 6, 8862
11. van Elsas, J.D. et al. (2012) Microbial diversity determines the invasion of soil by a bacterial pathogen. Proc. Natl. Acad. Sci. U. S. A. 109, 1159–1164
12. Kennedy, T.A. et al. (2002) Biodiversity as a barrier to ecological invasion. Nature 417, 636–638