Do permaculture's perennial polycultures promote microbial soil-health?

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Abstract. Some types of polycultures have been known to be able to increase crop production, pest resistance and overall agroecosystem stability. If important ecosystem services can be maintained and improved by increasing crop diversity, it is likely that some of these effects are measurable in the soil. Since many soil processes are performed by microbial actors, this thesis hypothesizes that polycropping should be measurable as an improvement of microbial indicators of soil-health, in particular microbial diversity and stress-resilience. To test this hypothesis, the literature was surveyed for studies that experimentally compare monoculture with polyculture yield, while keeping track of these soil-health indicators. Although insufficient studies were found for a definite answer, the studies that were found point in the same direction, so that a tentative answer can be formulated: selective crop diversity can lead to a healthier soil that is both more stable and more productive. Likely, some of the productivity improvements in polycultures are mediated by microbial factors, but more research is needed to make more confident statements on causality.

Contents

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1 Introduction

As a child brought up around ecological gardens, I remember telling my parents how great it would be to apply these same techniques to the growing of food. I imagined a forest edge system with a rich herbal layer below a mixture of berry shrubbery above which fruit trees bloomed. They told me that it wouldn't work; growing your own food had to remain a fight against nature. Since then, a whole movement has sprung up that doesn't agree with this vision. These forest gardeners (or vertical farmers, or *permaculture* designers), as they call themselves, attempt to imitate the workings of natural ecosystems. They claim decreased inputs in terms of fertilizers and pesticides (de Waard, 1996), and, unlike conventional organic farmers (Seufert et al., 2012), most also claim increased outputs, while some even claim decreased inputs in terms of human labor(Shepard, 2013).

Permaculture.   *Permaculture* (derived from the words 'permanent' and '(agri)-culture') is a design philosophy which applies broadly to any type of sustainable design.[1] In the narrow sense, permaculture refers to agricultural practices which are non-destructive to the soil and overall ecosystem to the extend that vital ecosystem services and fertility are left intact, so that the practice can continue indefinitely into the future. Although permaculture is a relatively new term (Mollison and Holmgren, 1978), traditional forms have existed and continue to exist, such as the Kendyan Gardens of Sri Lanka (Jacob and Alles, 1987), and *taungya* on Java (Weersum, 1982) and in south China (Menzies, 1988).

The growing body of literature on permaculture is mostly written for a lay audience and lacking in scientific rigor, despite the overlap of the subject matter with the emerging scientific field of *agroecology* (Ferguson and Lovell, 2014). This present paper investigates the microbial ecological characteristics of selected permaculture design practices through an agroecological lens, trying to highlight what permaculture has to gain by more fully embracing ecological science and what agroecology could learn from empirical experience gathered by permaculture experimentalists. This is done in 4 parts, (1) by first taking a critical look at the productivity of polycultures, (2) including perennial polycultures, (3) after which microbial indicators of soil health are investigated, (4) and finally, whether these microbial measures of soil health are affected by polyculture.

Polyculture.   A universal permaculture design feature—one that is well-established in agroecology (Ferguson and Lovell, 2014)—is the replacement of monocultures with *polycultures* (also called *poly-* or *intercropping*), where more than one crop is grown on a given plot. The purpose is increased productivity, resistance and resilience. In forest garden designs (a subset of perennial polyculture), this principle is taken to the extreme by adding a vertical dimension, which creates additional niches (for e.g. vines and shade-tolerant species).

A significant body of research has been performed to compare various outcomes of polycropping and monocropping strategies; yield, soil health, and

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[1] When the term 'permaculture' was originally coined by Mollison and Holmgren (1978), it meant just 'permanent *agri*culture'. Since then, the term has come to more broadly apply to sustainable life-styles and cultures.

ecosystem services could all be influenced. The first question addressed in this paper is an old one (Hart, 1975a,b): (1) whether productivity is increased by implementing polyculture (1a) in terms of crop yield and (1b) in terms of essential ecosystem services that are supportive of long-term sustainability.

Perennial polyculture. Even when intercropping is implemented, continuous tilling and resowing of annuals will limit the extend to which agroecosystems can increase in structural complexity and provide ecosystem services beyond yield of the (primary) crop. Therefore, permaculture adherents have long advocated replacing (bi-)annuals with perennial species for the bulk of our calories (Mollison and Holmgren, 1978; Mollison et al., 1991; Hart et al., 1991; Shepard, 2013). Whereas most calories in contemporary diets are derived from wheats and other grains (FAO, 2015), popular permaculture literature encourages us to replace grains as a source of carbohydrates with nuts, in particular hazelnuts and chestnuts (Shepard, 2013).

Large-scale genetic engineering efforts are underway to mitigate malnutrition around the world by making grains more nutritious. Golden Rice, for example, has been engineered to produce a vitamin A precursor not normally found in rice Ye et al. (2000), promising a future for the 667,771 children who now die annually as the result of dietary vitamin A deficiency because they have to subsist largely on a rice-only diet (Black et al., 2008, tbl. 6). An additional 20,854 deaths are attributed to iron deficiency (Black et al., 2008, tbl. 6)—iron which, together with vitamin C, is plentiful in chestnuts (Borges et al., 2008). *Cedrella sinensis* trees could be interspersed to provide vitamin A (Yang et al., 2007). With chestnuts in the upper story to provide for carbohydrates (Borges et al., 2008), along the (windy) edges of the food forest ecosystem, hazelnuts could provide dietary fat and protein (Monagas et al., 2009).

The second question that will be addressed is (2) whether there are qualitative and quantitative differences in (2a) crop yield and in (2b) overall ecosystem performance when annuals are substituted with perennails in polyculture.

Soil health and microbes. In popular permaculture literature, much emphasis is placed on soil health (e.g. Shepard, 2013), and whole book titles are devoted to the subject of restoring healthy soils that are "teaming with microbes" (Lowenfels et al., 2010). Indeed, it is well-established that microbes are a necessary part of a healthy soil (Lambers et al., 2009), dependent as important nutrient cycles are on microbial activity (e.g. Salamanca et al., 2002 on decomposition, Galloway et al., 1995; Peoples et al., 1995 on nitrification).

The literature is less definite on what precisely constitutes a microbially healthy soil: (3) Are there microbial indicators of a healthy soil? (3a) And what are they? (3b) Is soil richer in microbial diversity healthier?

Synthesis. This thesis reviews the literature on the effects of polyculture on soil quality. More specifically, (4) I try to address the question (4a) if microbial indicators of soil quality are improved by polyculture, and (4b) if perhaps, some of the positive plant-plant interactions in polyculture are mediated by the soil microbiome. The hypothesis for (a) is that "diversity begets diversity", so that if microbially more diverse soil are indeed healthier, this affects crop performance. Thus, the hypothesis for (b) is that microbial abundance, diversity and stability

are affected by plant diversity so that the microbially more healthy soil supports increased plant productivity.

2 Polyculture productivity

Biomass. In terms of total biomass, functional diversity in natural ecosystems has a positive effect on system productivity (Naeem et al., 1994; Tilman et al., 1996, 2001), although this effect is dependent on the temporal position in the successional sequence (Weis et al., 2007); in early succession, when growth rates are unaffected by intra- and interspecific competition, Weis et al. (2007) in fact observed a "negative sampling effect" in a microcosm experiment with freshwater algae, due to the less likely presence of the most productive species during early succession. Hooper (1998) found that it is not functional group richness *per se*, which boosts productivity, but rather "the functional attributes of, and interactions between, certain groups," confirming the conclusions of his group after an earlier experiment, that "composition (the identity of the functional groups present) explained much more variance than did richness (the number of groups present)" (Hooper and Vitousek, 1997).

Crop yield. Congruent with the aforementioned pattern for natural ecosystems, diversity in experimental agroecosystems has also often been reported to increase productivity in terms of useful yield. And, like with natural ecosystem, there's no one-to-one correlation of plant productivity with plant species diversity (Iverson et al., 2014). Where some studies find "a net benefit of intercropping with functionally diverse species in terms of both overall productivity and per plant productivity" (e.g. Franco et al., 2015), some studies find no benefit of intercropping or find even a decrease of productivity (e.g. Herrmann et al., 2014).

Thus, whether polycropping provides an increase in net yield depends much on the particular intercropping pattern and on the species selected for the experiment.

In a recent meta-analysis of 26 field studies, Iverson et al. (2014) found a 40% mean increase in per-plant productivity for substitutive polycropping designs. But, even in additive study designs, they found that per-plant productivity of the primary crop could be maintained if the secondary vegetation was leguminous (fig. 1). The study by Iverson et al. (2014) is limited in that it only looks at yield of the primary crop in the studies they included, because they're investigating the feasibility of improving ecosystem services while maintaining yield. Another limitation is that to be able to compare observations of additive treatments with observations of substitutive treatments, they used per-plant-unit yield as a measure of productivity instead of per-area-unit yield, which is likely a contributing reason why they found substitutive treatments to be more productive than additive treatments.

Mechanisms. There are three oft-proposed mechanisms by which plant species diversity could contribute to measures of ecosystem productivity: (1) the *complementarity* hypothesis predicts that differences in resource requirements among species (niche partitioning) leads to more complete resource utilization (Hooper et al., 2005); (2) *facilitation* occurs when one species favors the growth of a
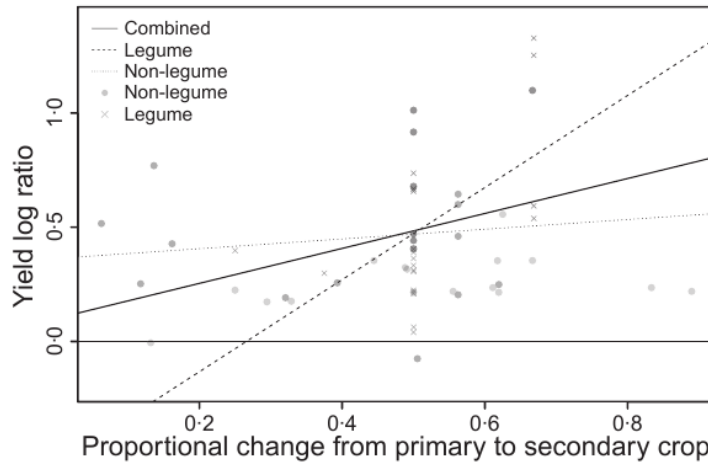
Figure 1. This figure, reproduced from a meta-analysis by Iverson et al. (2014) of 301 observations from 26 studies on the effect of intercropping on primary crop yield and pest resistance, shows that there's an increase of per-plant productivity (primary crop yield response ratio) when a larger proportion of primary produce is substituted with the secondary crop (legumous, non-legumous and combined). "Trendline for legume and non-legume combined: $R^2 = 0.1456(P < 0.001, N = 117)$; trendline for legume only: $R^2 = 0.310(P < 0.001, N = 50)$; trendline for non-legume only $R^2 = 0.0004(P = 0.31, N = 61)$. Sample size of combined values is larger than sum of subsets as some studies included a mix of both legumes and non-legumes as secondary crops."

co-occurring species by altering its environment in some way (Chu et al., 2008; Hooper et al., 2005); and (3) due to *the sampling effect*, a more species-diverse community is more likely to include a species that is highly productive in that environment (Hector, 1998; Hooper et al., 2005).

In polyculture experiments, the sampling effect comes into play when researchers have to choose a secondary crop, *if* they include the secondary crop in the total yield, which many studies do not. Then, if the sampling effect can be ignored, as Iverson et al. (2014) do for the 26 studies in their meta-analysis, the remaining mechanisms that could explain an increase in plant productivity are the facilitation and the complementarity hypotheses.

It is also important to distinguish between experiments that are testing the effects of *functional diversity* and experiments that are testing the effects of *overall diversity*; whereas the sampling effect would apply to overall diversity, facilitation and complementarity would apply (most) to functional diversity (Hooper et al., 2005), since many differences in plant structure and physiology can be assigned assigned a functional attribute. Since most polycropping experiments are designed with secondary crop functionalities already in mind, the sampling effect will not often contribute to the measure of productivity.

The complementarity hypothesis could explain some of the increases in yield that Iverson et al. (2014) see for the substitutive study designs in their analysis: according to niche theory, competition between functionally similar species

could have an inhibitory effect on productivity (Hooper et al., 2005), but the competition between individuals of the same species can be even fiercer, since these will be functionally almost identical. Even if species belong to the same guild[2], structural differences (e.g. root depth) could reduce intraspecific competition in favor of less severe interspecific competition.

Of more relevance to the remainder of this thesis is the facilitation hypothesis. The results from Iverson et al. (2014) suggest that adding a secondary crop consistently improves pest resistance (fig. 3. Thus, crop diversity can be said to provide an important ecosystem service. This begs the question how this facilitation occurs. Also, it is unclear to which extent facilitation and complementarity are contributing to the increase in yield in the substitutive designs and in the additive designs with legumes.

2.1 Ecosystem services

Agroecology considers agroecosystem function not just in terms of crop yield, but also in terms of other ecosystem services, many of which are neglected and consequently downgraded by conventional agriculture with its large monocultures (Manning, 2004), so that desiccation and erosion follow, while downstream of agricultural sites, leached nitrate, pesticides and minerals cause large-scale damage to natural ecosystems (Dewar, 2007; Matson et al., 1997).

Hajjar et al. (2008) review how a number of ecosystem services (pollination, pest and disease control, and soil nutrient dynamics) are improved by crop diversity. Although there's an even longer array of other essential ecosystem services that could potentially be enhanced by polyculture (e.g. fresh water retention, wildlife refuge), for the purpose of this present paper, only resistance, resilience and soil nutrient dynamics will be related to biodiversity, as they can be directly related to the stability of more or less diverse agroecosystem soils.

Resistence and resilience. *Resistence* is the ability of (a selected part of) an ecosystem to withstand disturbance or stress[3] without changing state, while a *resilient* system is a system that can easily return to the original state after a (prolonged) disturbance. These are the engineering definitions. The ecological definition of *resilience* is how many stress can be incurred by a system without changing from one stable state to an alternative stable state (ASS). (See fig. 2 for a comparison of ecological and engineering resilience.) In this present paper, the engineering definitions are used, except where ecological resilience and ASS are explicitly mentioned.

In their recent meta-analysis, Iverson et al. (2014) reported a 31% to 36% increase in pest resistance for polycultures compared to monocultures (fig. 3), with sustained yield in additive designs (but only with legumous secondary crops) and improved yield in substitutive designs (fig. 1 3). On average, the polycropping treatments included in Iverson et al.'s (2014) meta-analysis thus increased stability in the face of pest pressure. From these results, it can be argued that plant diversity in agroecosystems increases system stability.

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[2]This follows the ecological definition of a *guild*, as a group of functionally similar plants, not of facilitating/complementary species, as the term is often (mis)used in permaculture circles.

[3]In ecological literature, *stress* is taken to mean "prolonged disturbance".
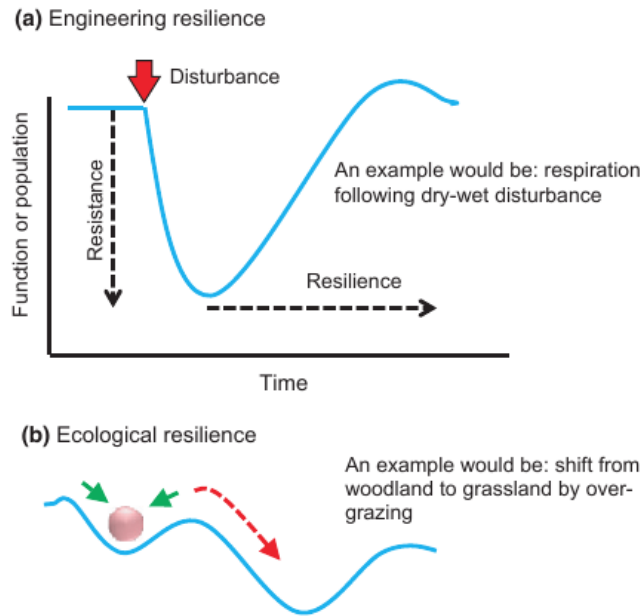
Figure 2. This schematic, reproduced from Griffiths and Philippot (2013), depicts that resilience in the engineering definition is how well the system can recover over time after a disturbance, while resilience in the ecological definition is the ability of the system (ball) to incur stress before it is pushed into an alternative stable state (ASS) (basin). Resilience in the ecological definition does not fully overlap with resistance in the engineering definition, as the former says nothing about the functional loss that can occur before the switch to an ASS, while the latter says nothing about qualitative changes to a system when pushed over the edge from one basin into the other.

2.2 Perennial polyculture

If the resilience, the stability and the yield of agroecosystems can be improved by replacing monocultures with polycultures, then the perennial polycultures propagated by many permaculture evangelists could possibly further boost some of these ecosystem services (Dewar, 2007). Food crops in conventional agriculture are mostly annuals—pioneer species, optimized by selective breeding (and, increasingly, genetic engineering) to cope well with high nutrient loads from artificial fertilizers and regular treatment with pesticides. Although the "Green Revolution" in which these optimizations culminated has enabled the feeding of billions (Khush, 2001; Evenson and Gollin, 2003), it has also had a disastrous impact on the provision by agroecosystem of services other than gross yield (Leach, 1975; Matson et al., 1997).

The farming of annuals is usually accompanied by tilling, often both in the spring and in the fall; the subsequent wind and water erosion are a problem in a significant fraction of the world's cultivated lands (Dewar, 2007). By substituting (some) annuals with perennials, "erosion could be reduced by as much as
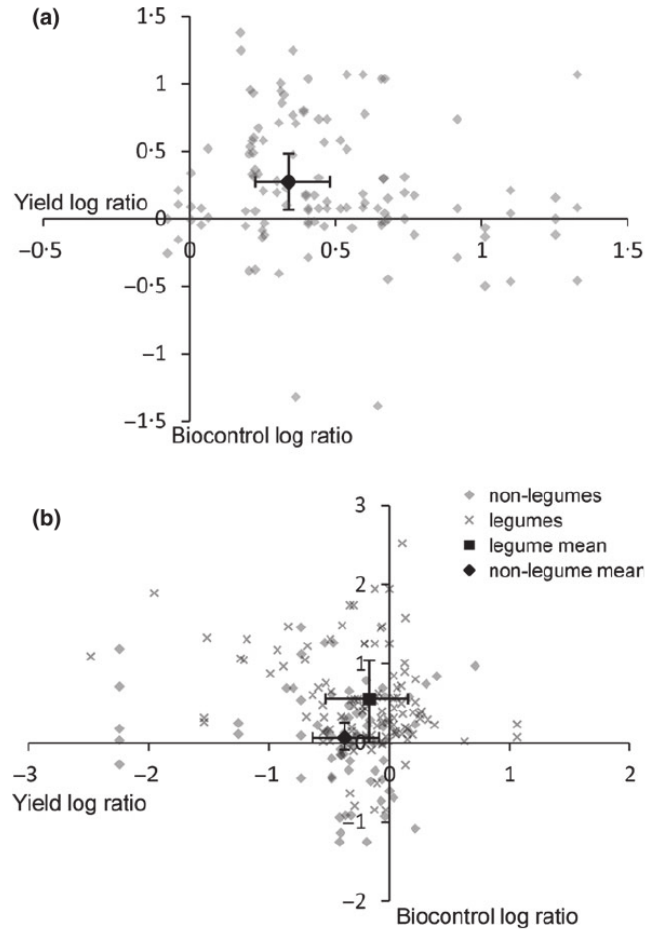
Figure 3. This figure, reproduced from Iverson et al. (2014) their recent meta-analysis of 311 observations from 26 studies on the effect of intercropping on primary crop yield, shows that for substitutive experiments (a), both pest-resistence (biocontrol) and per-plant productivity increase; while for additive experiments (b), pest-resistance increases at the cost of per-plant productivity, unless the secondary crop is a legume in which case there's no significant decrease in productivity. Large diamond in (a) = model mean estimate for all studies $\pm95\%$CI; and in (b) = model mean for observations with a non-legume as secondary crop. Spearman rank correlation (a): $\rho = 0.178(N = 117, P = 0.055)$; (b): $\rho = 0.085(N = 184, P = 0.252)$.

50% because soils are left relatively undisturbed and covered with vegetation" (Pimentel et al., 1997).

Biomass. In terms of total biomass, it is assumed in popular permaculture literature that more mature, late successional ecosystems have more primary production (e.g. Shepard, 2013). Indeed, more complete light harvesting and soil nutrient mobilization could be possible due to the layered, closed canopy, and the deeper, more complex root system (amended with mycorrhiza) of natural forests and forest gardens. But, at the same time perennials invest more of their energy in support structures such as (woody) stems and roots (Ferguson and Lovell, 2014).

There's no definite correlation between diversity and primary productivity. For example, in a recent multi-year experiment, Griffith et al. (2011) found that polycultures of perennial grasses were outperformed by monocultures of the highest performing species (Griffith et al., 2011); in sharp contrast with a extensive meta-analysis by Cardinale et al. (2007), who found proof for both selection effects and species complementarity: the latter increased over time when the species assemblages matured. This same shift—from selection to complementarity—as the main cause of the biodiversity-productivity relationship has also been observed by Fargione et al. (2007). In these cases, the sampling/selection effect improves the primary productivity of maturing ecosystems, until time has allowed the more competitive species to emerge, after which the effects of complementarity starts to outweigh the effects of selection (Cardinale et al., 2007; Fargione et al., 2007).

Crop yield. In popular permaculture literature (e.g. Mollison and Holmgren, 1978; Mollison et al., 1991; de Waard, 1996; Shepard, 2013), it is generally assumed that perennial polycultures can outperform annual poly- and monocultures not just in the broad sense—in the long-run economical and ecological viability of the agroecosystems—but also in terms of harvestable produce.

Although many food stuffs in our contemporary diets (e.g. many herbs, and tree-based products) are derived from perennials, the bulk of our calories originates are derived from annuals, particularly grains such as wheat[4] and maize, to the extend that worldwide in 2012, only 164 million (11%) out of the total 1.6 billion hectares of cropland where planted with "permanent crops" FAO (2015).

Quantitative comparisons between the yield of annual mono- and polycultures versus perennial polycultures are difficult and therefore practically nonexistent so far. Such studies would have to involve some intermediate measure of yield (e.g. nutrient content) to be able to compare different crop species. Nevertheless, figures are available on the productivity of perennial mono- versus polycultures (e.g. Dai et al., 2009, 2013, see also table 3).

3   Microbial indicators of soil health

Soil health, as a subset of soil quality, represents the ecological condition of a soil, rather than an inherent (physico-chemical) property (Karlen et al., 1997).

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[4]Worldwide wheat production alone covers more than 240 million ha; all cereals together, more than 720 million ha (FAO, 2015), almost half of the worlds total cropland area.

A poor quality soil can still be healthy—fertile and resilient—in that all its beneficial properties are optimally expressed in a way that maximally benefits the plant community.

Nutrient cycling in the soil depends in a large part on microbial life: nitrogen from the atmosphere is made available to plants by nitrogen-fixing bacteria, which, in turn, profit from root exudates of their hosts (mostly legumes) (Galloway et al., 1995; Peoples et al., 1995); 80% of plants depend on the extended hyphea of *arbuscular mycorrhizal fungi (AMF)* for optimum nutrient absorption (Jeffries et al., 2003); and recalcitrant dead plant material is broken down back into soluble form by microorganisms during decomposition and mineralization processes (Salamanca et al., 2002; Ndaw et al., 2009).

From a monoculturalist's perspective, a healthy soil is a soil that can sustain the greatest possible yield from the smallest possible input. Although the cheap artificial fertilizers and pesticides characteristic of the green revolution have enabled record yields of many crops (Evenson and Gollin, 2003; Khush, 2001), question marks have to be placed around the sustainability of investing 7–8 oil calories for every food calorie (Leach, 1975). Besides that, agricultural soils are degraded by deep-plowing (Dewar, 2007) and the chemical run-off causes environmental problems downstream, so that, for example, eutrophication of estuaries has become a global health and conservation issue (Nixon, 1995; Anderson et al., 2002).

If perennial polyculture can mitigate these problems, than the question is if the effects of polyculture are measurable in the living part of the soil, especially the part that is played by soil microbes. But first, reliable indicators of soil health have to be selected.

As of yet, there's no definite answer on the question which are reliable and universal microbial indicators for soil health (van Bruggen and Semenov, 2000). Van Bruggen and Semenov (2000) "suggest a systematic ecological approach to the search for indicators for soil health and disease suppression, namely, measuring biological responses to various stress factors and the time needed to return to the current state." Subsequently, a number of ex-
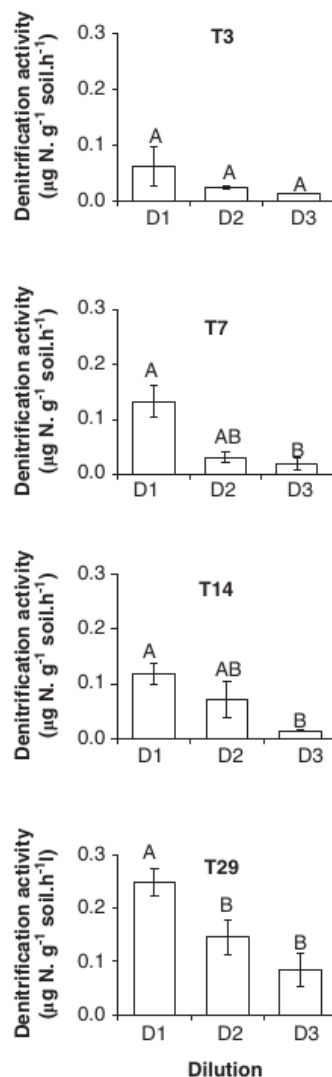
Figure 4. Potential denitrification activity decreases in serially diluted soil microcosms. The same letters above the bars indicate that they're not significantly different ($P < 0.05$). "Measurements were performed 3, 7, 14 and 29 days after addition of the residues [for denitrification] (T3, T7, T14 and T29)." Dilution levels were D1 (undiluted); D2 ($1/10^3$); and D3 ($1/10^5$). Treatments where in triplicate. Reproduced from Philippot et al. (2013).

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amples of this approach have cropped up (e.g.
Girvan et al., 2005; Philippot et al., 2013).
Philippot et al. (2013) showed, by a dilution
approach, that, after the microbial biomass
recovered, the soils that were more diluted and less diverse recovered less of
their denitrification potential during the duration of the study. Girvan et al.
(2005) discovered that soils that were naturally more diverse were more resilient
to toxic perturbations.

These results suggest that microbial diversity can be an important indicator
of soil stability. However, there's no unambiguous relationship between plant
diversity and diversity of soil microbes. It is therefore that stability and self-
recovery from stress have been suggested as additional indicators of soil-health
(van Bruggen and Semenov, 2000). More recently than and congruently with
van Bruggen and Semenov (2000), Griffiths and Philippot (2013) conclude, after
reviewing 50 studies on the resistance and resilience of microbial functioning in
soils, that "biological stability is not solely owing to the structure or diversity of
the microbial community", but "results from a combination of biotic and abiotic
soil characteristics and so could [itself] provide a quantitative measure of soil
health[.]"

3.1   Microbial diversity and soil function

In section 2, the effect of plant diversity on ecosystem productivity was already
discussed, in terms of various ecosystem services, including total primary pro-
ductivity and crop yield, both of which were not reliably increased by plant
species diversity (Hooper, 1998; Iverson et al., 2014), although community com-
position definitely made a difference in various studies (Hooper and Vitousek,
1997). It is unlikely that from these results, inferences can be made for mi-
crobial diversity, first and foremost because microbial diversity—even in mono-
cultures—is orders of magnitude greater than diversity of macrobiota[5]. Also,
the microbial environment is always very heterogeneous in spatial, chemical and
temporal dimensions. Finally, microbes play different (and more varied) roles
in ecosystems than do plants; their functional diversity operates on a different
scale and in different niches.

When discussing microbial diversity and soil health, a number of functions
can be distinguished, all of which contribute to soil health in some way: (1)
the decomposition/mineralization of dead organic matter; (2) the nitrification
of atmospheric nitrogen; (3) the provision of resistance against plant pathogens;
and (4) the optimization of nutrient availability in the rhizosphere. If and how
microbial diversity can enhance and or stabilize some of these functions is of
great concern to research scientists, because this knowledge allows for more
effective soil management.

Microbial diversity and functional stability.   Because soil microbe count
and diversity is huge in comparison to plant (species) number, one hypothesis
regarding the effect of microbial diversity on the stability of soil functioning can
be inferred from the observation (in section 2) that it is plant *functional* diversity

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[5]There are "about one million species of archaea and bacteria per gram of soil " (Lemanceau
et al., 2015)

and not diversity *per se* which has a positive impact on productivity: compared to plant communities, functional stability of soil microbial communities will be relatively strong because of a large amount of functional redundancy. This hypothesis can only be valid if there is such a thing as functional redundancy. For microbial species, with their horizontal mobility of functional genes, the assumption of redundancy is an especially well-justified starting point, but it is important to remember that it is only one out of "two extreme viewpoints on redundancy: (1) Each species in an ecosystem plays a fundamental role, such that removal of each species incrementally weakens the integrity of the system, just as removal of rivets from an airplane weakens its structure (the rivet hypothesis). Alternatively, (2) a community is composed of a few functional groups, each with several ecologically equivalent species, such that species can be lost from the community with little effect on ecosystem processes, as long as each functional group is represented (the redundancy hypothesis)" (Chapin et al., 1992).

If species can be redundant and redundancy has a stabilizing effect, redundancy can by hypothesized to act on the stability of the aforementioned ecosystem services through a few distinct stabilizing mechanisms:

1. the *averaging effect*, when functionally similar species react differently to natural or man-made environmental changes so that, on average, ecosystem function remains the same (Doak et al., 1998);

2. the *negative covariance effect*, when highly competitive species negatively covary, causing lower overall variance (Tilman et al., 1998);

3. the *insurance effect*, when multiple redundant species can fill in the gaps left by functionally similar species (Naeem and Li, 1997); and/or

4. competition with invading species and suppression of pathogens.

In line with the insurance effect, Philippot et al. (2013) have found that the denitrification potential of serially diluted microcosms decreased significantly in the less diverse microcoms. They "inoculated sterile soil [. . .] with serial dilutions of a soil microbial suspension." Dilution levels were D1 (undiluted); D2 ($1/10^3$); and D3 ($1/10^5$). D2 and D3 led to a 25 and 75% decrease in OTUs respectively. The treatments where in triplicate. "Removal of 75% of the total OTUs in the $1/10^5$ dilution treatment led to a decrease in potential activity of about 48 to 88%" (fig. 4).

Also in line with the insurance hypothesis, Tardy et al. (2014) recently demonstrated that functional and structural stability of microbial diversity decrease along a gradient of decreasing microbial diversity (created using a dilution to extinction approach). Similarly, in a study by Girvan et al. (2005), "the genetic diversity of the naturally more diverse soil was more resistant to benzene perturbation than the less diverse soil," although their results may partially be explained by the source of these soils: their more and less diverse soils originated from organo-mineral/improved pasture-land and mineral/arable field, respectively. In an experiment with "replicated microbial microcosms with varying number of species per functional group," Naeem and Li (1997) "found that as the number of species per functional group increased, replicate communities were more consistent in biomass and density measures" (fig. 5).
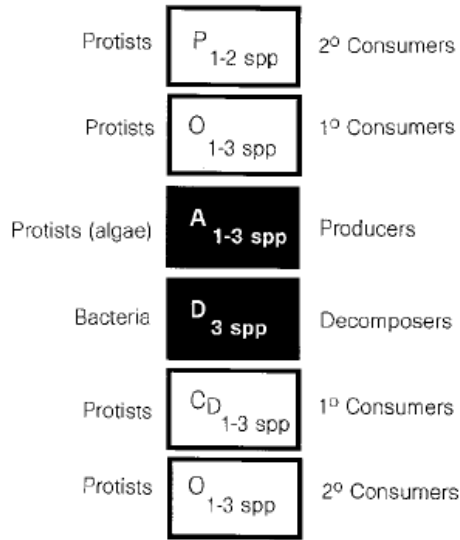
Figure 5. Experimental design of Naeem and Li (1997). Core functional groups consisted of autotrophs (A) and decomposers (D). Additionally functional groups are primary (1°) and secondary (2°) consumers of either A or D. All consumers were protists. Primary consumers of A and D were omnivores (O) and consumers of detrivores (C_D), respectively. Secondary consumers were predators (P) for A and omnivores for D. Species were randomly chosen from a pool of selected species for each functional group. Reproduced from Naeem and Li (1997).

In section 2, the sampling effect was discussed as one of the possible mechanisms by which plant diversity can affect plant productivity. That the sampling effect also applies to microbial diversity is demonstrated by Philippot et al. (2013), who "found that the denitrifier community composition between replicates was more similar for the low than for the high dilution treatments." The sampling effect works much the same way as the insurance effect: higher species diversity increases that change that a highly productive species will be sampled, but also that no functional gaps will be left after disturbance.

3.2 Microbial biomass and soil function

Although "microbial biomass is a well-known marker of soil biological functioning" (Lemanceau et al., 2015), it is most useful as "a sensitive and early indicator of changes in soil management" (Lemanceau et al., 2015), because microbial biomass depends on soil type and carbon content (Lemanceau et al., 2015), making it less suitable for comparison between different soil types and fertilization regimes. However, measuring the recovery-rate of microbial biomass after disturbance is a decent measure of soil stability and thus a good choice when studying the effect of polyculture on microbial soil health.

With quantitative polymerase chain reactions (qPCR) techniques, it is even possible to quantify functional communities, identified by particular functional genes (Lemanceau et al., 2015).

4 Polyculture and microbial soil health

Section 2 evidenced that polycultures can be as productive, or, under certain conditions, more productive than monocultures, while simultaneously improving essential ecosystem services (Iverson et al., 2014). Among the mechanisms which could explain these productivity improvements under polyculture are those that involve microbial actors—actors which can enhance resistance against pathogens

([Lemanceau et al., 2015](#)), as well as actors that enhance soil enzymatic activity ([Lemanceau et al., 2015](#)). Section [3](#) summarized some possible indicators of a microbially healthy soil, to determine which of these could best be used to test the hypothesis that the agroecological improvements of polycultures are reflected and/or mediated by soil microorganisms. To this end, the available literature was reviewed to find studies that investigate the relationship of polyculture with soil health in terms of diversity and resilience of the soil microbial community.

4.1 Search methodology

Relevant studies were identified by first using the keywords listed in table [1](#) to perform a series of searches in Thomson Reuters' Web of Science literature search engine. Many of these hits were duplicates, and were consequently discarded. Among the unique hits, the majority was disqualified upon reviewing the title or the abstract of the papers. Papers for which no full text version was available (for free) for Rijksuniversiteit Groningen (RuG) students were also disqualified. Studies which were obviously not about polyculture or which didn't measure the effect on soil microbiological measures were also excluded. (Where this was less immediately obvious, the papers were eliminated in the next disqualification round.)

Keywords	Hits minus overlapping hits minus disqualified hits		
"polyculture/polycropping/intercropping soil microbial diversity"	$4 + 0 + 58$	$- \ 9 - 31$	$= 23$
"polyculture/polycropping/intercropping soil microbial community stability"	$1 + 0 + 5$	$- \ 0 - \ 3$	$= \ 3$
"polyculture/polycropping/intercropping soil functional stability/resilience"	$(1 + 0 + 1) + (1 + 0 + 0)$	$- \ 2 - \ 1$	$= \ 0$
"polyculture/polycropping/intercropping AMF diversity"	0	$- \ 0 - \ 0$	$= \ 0$
"polyculture/polycropping/intercropping bacterial diversity"	$(3 + 0 + 34)$	$- 19 - 25$	$= \ 0$
"polycropping"	7	$- \ 0 - \ 7$	$= \ 0$

Table 1. Search results when probing Thomson Reuters'™ Web Of Science™ (on June 25, 2015) for different search terms related to the effect of intercropping on microbial measures of soil health. On first inspection of the titles and the abstract, many articles were disqualified if no control treatment was present, if the study didn't involve polyculture, or if the effect on microbial indicators of soil health was not measured. Articles were also disqualified if no 'free' full text version was available to Rijksuniversiteit Groningen (RuG) students. Finally, reviews were also excluded.

After assessing the methodology of the remaining papers, a second disqualification round followed, during which purely descriptive publications were excluded which did not follow an experimental design with a control treatment. All the publications passing those criteria are listed in table [3](#), including a brief, qualitative summary of the results found in these studies.

Polyculture practice	Yield	Microbial indicators of soil health				Reference
		Biomass (m)	Diversity (H')	Activity (r)	Stability	
Grass-legume	-	$_F\uparrow$	-	-	-	Zhao et al. (2015)
Maize-faba bean	-	$_{B,A}{}^{AO}_{B,A}$	${}^{AO}_{B,A}$	-	-	Zhang et al. (2015)
Maize rhizosphere	-	$_A\downarrow {}^{AO}_A\uparrow$	$_B\downarrow {}^{AO}_B\downarrow {}^{AO}_A\uparrow$	-	-	
Faba bean rhizosphere	-	$_B\downarrow {}_A\downarrow\uparrow {}^{AO}_{B,A}\downarrow$	$_B\downarrow {}^{AO}_B\uparrow {}^{AO}_A\downarrow$	-	-	
Soybean-maize	-	-	$\lambda, H'_B\uparrow$	-	-	Zhang et al. (2011)
Faba bean-maize	$1^{ary}\uparrow$	$_B$-	S_B-	-	-	Zhang et al. (2010)
Maize-peanut	-	$_F\downarrow {}_B\uparrow$	-	${}^{U,S,Pe}_E\uparrow {}^{PO}_E$-	-	Khan et al. (2014)
Intercropping with aromatic plants	-	$_B\uparrow {}_F\uparrow {}^{N,DN,Azo}_B\uparrow$	-	${}^{P,U}_E\uparrow$	-	Chen et al. (2014)
Soybean-maize	1^{ary}-	-	H'_B-	-	-	Herrmann et al. (2014)
Red clover-apple orchards	-	-	$H', S^F_M\uparrow$	-	-	Jiao et al. (2013)
Sedum-Apium graceolens	-	$_B\uparrow {}_F\uparrow$	${}^F_M\uparrow$	${}^{U,C}_E$-	-	Nai et al. (2013)
Pepper-garlic	-	$_B {}^{Ac}_B {}_F$	-	${}^{I,AP,U,C}_E$	-	Ahmad et al. (2013)
Pepper-normal garlic	-	$_B\uparrow$	-	${}^U_E\uparrow$	-	
Pepper-green garlic	-	${}^{Ac}_B\downarrow {}_F\downarrow$	-	${}^{I,P,C}_E\uparrow$	-	
Peanut–*Atractylodes lancea*	$1^{ary}\uparrow$	$_{G-}\uparrow$	-	${}^{U,I}_E\uparrow$	-	Dai et al. (2013)
Peanut-medicinal plants	$1^{ary}\uparrow$	-	$_F\uparrow {}_{PF}\downarrow$	-	-	Dai et al. (2009)
Barley-legume	1^{ary}-	$_M$	-	-	-	Tortorella et al. (2013)
Barley-pea	$2^{ary}\downarrow {}_m\uparrow$	-	-	-	-	
Barley-faba bean	$2^{ary}\downarrow$	-	-	-	-	
Soybean-maize	-	-	$H'_{B,F}$	-	-	Kihara et al. (2012)
Nyabeda, Kenya	-	-	$H'_{B,F}\uparrow$	-	-	
Matayos, Kenya	-	-	-	-		
Alfalfa-*Elsholtzia-Sedum*	-	$_M\uparrow$	$H'^F_M\uparrow$	-	-	Ma et al. (2012)
Oat-common vetch	-	-	$S, H'_B\uparrow$	-	-	Qiao et al. (2012)
Cucumber intercropping*						Zhou et al. (2011)
Cucumber-onion	$1^{ary}\uparrow$	-	$S, H'_B\uparrow S, H'_F$-	${}^{U,PO}_E\uparrow$	-	
Cucumber-garlic	$1^{ary}\uparrow$	-	$S, H'_B\uparrow S, H'_F$-	${}^{U,PO,C}_E\uparrow$	-	
Coffee-legume						Balota and Chaves (2010)
Coffee-*Leucaena leucocephala*	-	-	${}^{AS,P,U}_E\uparrow$	-		
Coffee-*Crotalaria breviflora*	-	-	${}^U_E\uparrow$	-		
Alfalfa–Siberian wild rye*	$1,2^{ary}\uparrow$					Sun et al. (2009)
Alfalfa rhizosphere		$_M\uparrow$	-	${}^{U,I,P}_E\uparrow$	-	
Wild rye rhizosphere		$_M\downarrow$	-	-	-	
Wheat-canola	1^{ary}-	$_M$-	H'_M-	-	-	Hummel et al. (2009)
Soy-Mixed trees*	-	${}^{AM}_F\uparrow {}_{G+}/{}_{G-}\uparrow$	$\beta_M\uparrow$	-	$m, \beta_B\uparrow$	Lacombe et al. (2009)
Wheat-maize-faba bean (2005)						Song et al. (2007)
1^{ary} = Wheat-maize	$1,2^{ary}\uparrow$	$_M\uparrow$	$H'_B\uparrow$	-	-	
1^{ary} = Maize-faba bean	$1,2^{ary}\uparrow$	$_M$-	$H'_B\uparrow$	-	-	
1^{ary} = Wheat-Faba-bean	$1^{ary}\uparrow$	$_M\uparrow$	$H'_B\uparrow$	-	-	
Maize-pigeonpea	-	$_F/{}_B$-	-	-	-	Fernandes et al. (2011)

Table 3. The effect of plant polyculture on microbial measures of soil health. Each measure is followed by the microbial group in subscript (M=microbes, A=archea, B=bacteria, $G^{+/-}$=gram-positive and -negative bacteria, respectively; prefixed by a P for pathogenics) and the particular subtype/role/activity within that group in superscript (AO=ammonia-oxidization, AS=arylsulfatase, N=nitrifiers, DN=denitrifiers, I=invertase, P=phosphatase, Pe=peroxidase, PO=polyphenol oxidase, C=catalase, S=sucrase, U=urease, Azo=azotobacters, Ac=actinomycetes). M/F in superscript in the diversity column denotes metabolic/function diversity, respectively. Studies marked '*' are explored in more detail in section 4.2.

In the third round, the intention was to select from table 3 only those studies that include a measure of microbial biomass and/or diversity as well as yield figures and, very importantly, stability measurements, i.e., how does (functionality of) the soil microbiome recover after major disturbances. However, only one study was found which included stability measurements (Lacombe et al., 2009). Lacombe et al. (2009) measured the recovery of both microbial biomass and bacterial beta-diversity, but they did not include yield figures. Because microbial stability is the most promising indicator, this study was picked, despite its shortcomings. In addition, a study was selected that compared yield as well as both microbial diversity and enzymatic activity (Zhou et al., 2011); and one that, besides yield compared both microbial biomass and enzymatic activity between intercropped treatments and monocropped controls (Sun et al., 2009).

4.2 Cases

Most of the studies in table 3 that show an increase in yield for polycropping treatments, see an accompanying increase in microbial biomass (Dai et al., 2013; Tortorella et al., 2013; Sun et al., 2009; Song et al., 2007), microbial diversity (Dai et al., 2009; Zhou et al., 2011; Song et al., 2007), and/or soil enzymes (Dai et al., 2013; Zhou et al., 2011; Sun et al., 2009), with only few exceptions where changes were absent, in microbial biomass (Zhang et al., 2010; Tortorella et al., 2013; Hummel et al., 2009), and/or microbial diversity (Zhang et al., 2010; Herrmann et al., 2014; Hummel et al., 2009), or where changes were ambiguous (Sun et al., 2009, for microbial diversity); when measured, soil enzymatic activity was consistently increased for studies that reported improved yield. It bears mention, though, that most of these experiments were undertaken after prior experiments had already found improved yield for the tested polycropping practices.

Perhaps more interestingly, the studies that fail to find improved yield under polycropping also fail to find a change in any of the investigated indicators of microbial soil health (Herrmann et al., 2014; Hummel et al., 2009).

Since none of the listed studies (table 3) met all the ideal criteria for fully testing the hypothesis that the positive effect of some polyculture practices improves microbial soil health (section 4.1), a few studies which, when combined, touch all these different criteria, will now be discussed in more detail.

Cucumber/union/garlic intercropping. Compared with cucumber (*Cucumis sativus*) monocultures, cucumber production is significantly increased when cucumber is intercropped with garlic (*Allium sativum*) or onion (*Allium cepa*) (fig. 6, Zhou et al., 2011). Together with yield, bacterial species richness, diversity, and polyphenol oxidase activity are also increased in both intercropping systems (table 4, Zhou et al., 2011). This was found by Zhou et al. (2011) in a greenhouse experiment at Northeast Agricultural University in Harbin, China, in which they set up the control and both treatments in triplicate, while they tracked yield and various measures of soil health for at least three continuous growing seasons.

Cucumber yield decreased with each consecutive growing season; the smallest decrease was seen for the intercropped treatments, which suggests that intercropping increases soil stability. However, if the intercropped soils retained more of their fertility over time, this was not reflected in the potential of all of

Treatment	Bacterial community			Fungal community		
	Numbers of visible bands (S)	Shannon-Wiener index (H')	Evenness index (E)	Numbers of visible bands (S)	Shannon-Wiener index (H')	Evenness index (E)
OC1	43	3.33	0.79	24	2.64	0.71
GC1	45	3.38	0.81	25	2.69	0.72
CC1	40	3.26	0.78	18	2.47	0.67
OC2	38	3.17	0.76	27	2.76	0.74
GC2	44	3.46	0.83	27	2.82	0.76
CC2	36	2.97	0.71	22	2.57	0.69
OC3	42	3.40	0.81	19	2.31	0.62
GC3	42	3.32	0.79	23	2.72	0.73
CC3	36	2.87	0.68	18	2.32	0.63

Table 4. Microbial diversity as deduced from DGGE analysis of soil samples in 3 successive growing seasons of 3 different growing systems: onion-cucumber (OC), garlic-cucumber (GC), and cucumber-cucumber (CC). Reproduced from Zhou et al. (2011).

the enzymatic processes: although urease activity was consistently increased for intercropped soils throughout all 3 growing seasons, polyphenol oxidase activity was increased only in the first and second season (Zhou et al., 2011). Overall, soil health, by measure of microbial diversity and enzymatic activity, was improved by intercropping; but it's difficult to say clearly if the increases in crop yield were mediated (in part) by changes in the soil microbiome. Temporal and environmental dynamics throughout each growing system may have influenced the outcomes; indeed, Zhou et al. also tracked enzymatic activity throughout the third growing season and found very erratic patterns for all enzymes, except for urease activity (Zhou et al., 2011, fig. 3). The patterns for bacterial and fungal diversity were less erratic (Zhou et al., 2011, table 2).

Wheat/maize/faba been intercropping.   At the Institute of Soils and Fertilizers at Gansu Academy of Agricultural Sciences, at Baiyun (38° 37'N, 102° 40'E), in Gansu province, northwestern China, in 2003, Song et al. (2007) devised a field experiment in which they planted wheat, maize and faba bean in monocoltures (controls) and intercropped: wheat-maize, wheat-faba bean, and maize-faba bean. All four cropping systems were setup in triplicate. In the second and third year (2004 and 2005), the yield and soil parameters were sampled.

Intercropping with either maize or faba bean increased the availability of phosphorus and nitrogen availability in the rhizosphere of wheat, while also increasing microbial biomass and diversity (Song et al., 2007).

Perennial polyculture.   If reduced tilling can increase the sustainability of soil management, then replacing perennials with annuals may be worthwhile. Incidentally, the only study in table 3 that investigated soil stability in response to polyculture did so in a tree-based perennial cropping system. Lacombe et al. (2009) established tree-based intercropping systems (in duplicate, at two different sites: Saint-Rémi and Guelph, Canada) with soybean planted between alternating tree rows of 4 different tree species, adjacent to fields with soybean
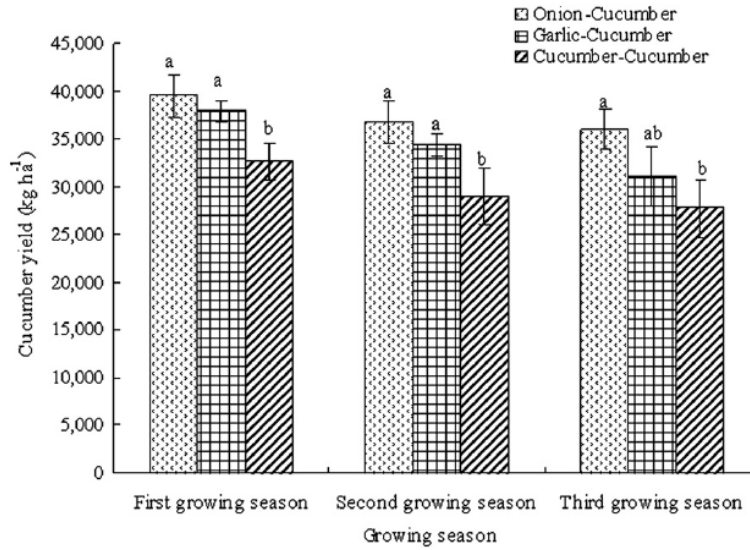
Figure 6. Cucumber yield in different cropping systems. Values that did not differ significant at $p < 0.05$ have identical letters above the error bars. Adapted from Zhou et al. (2011).

monocultures. They did not track soybean yield.

In the fields where soybean was planted between tree rows, Lacombe et al. found a significant increase in AMF biomass compared to monocropped soybean fields. They also found an increase in microbial beta-diversity (which they "measured as the spatial heterogeneity of microbial communities within a given area"), but only on one of the two sites. More importantly, though, they stress-tested five bulked soil samples (N=20) from each field with copper (Cu). "Each bulked sample was comprised of five randomly chosen soil samples within each sampling grid, so as to encompass microbial beta-diversity of each field." After dividing each bulk sample into 24 subsamples, paired subsamples were subjected to 12 different Cu concentrations (Lacombe et al., 2009). As with beta-diversity, a significant increase in stability was only seen for the Saint-Rémi site.

5 Discussion

Over-anthropization of arable lands has led to an ecological crisis. With 40% of earth's land surface dedicated to agriculture (FAO, 2015), it is of considerable importance that this land is used wisely. Periodical ecological upheaval to clear the way for the pioneer species on which most of humanity now subsists may not be the wisest of strategies to preserve the fertility of these fields for future generations, not if we don't want to see repeated everywhere what Plato already described in ancient Greece: "What now remains of the formerly rich land is like the skeleton of a sick man, with all the fat and soft earth having wasted away and only the bare framework remaining" (Jackson, 2002). Erosion, pollution and falling groundwater tables are urgent problems (Dewar, 2007; FAO, 2015), and if perennial polyculture can contribute to the

solution, then vigorous research efforts are more than justified.

Despite the rising popularity of the permaculture philosophy, some of its principles have only recently started to receive much-needed scientific scrutiny from agroecologists (Ferguson and Lovell, 2014). Polyculture is a particularly well-researched practice from within the permaculture philosophy, because it has broadly been applied in many conventional (and often traditional, e.g. Jacob and Alles, 1987; Menzies, 1988) agricultural practices [citation needed]. Therefore, polyculture is a particularly attractive target for investigating the role of the soil microbiome in the productivity of permaculture.

Besides a philosophy, permaculture is also a movement—a movement with momentum (Ferguson and Lovell, 2014). Therefore, it may be advantageous for agroecologists to hook onto the momentum of this growing group of practitioners who genuinely want to make a difference in the current worldwide ecological crisis. This crisis is increasingly well-understood by science, but solutions will likely emerge not from science, but in the field—a field which could definitely profit from the application of egroecology. Any potential solution is worth aggressive investigation given that currently "we humans, a single species among millions, consume about 40 percent of Earth's primary productivity, 40 percent of all there is[, which] may explain why the current extinction rate is 1000 times that which existed before human domination of the planet" (Manning, 2004). There's also urgency in decreasing the rate of consumption of all the energy that has been laid down by ancient life in the form of petroleum; the agricultural dependency on petroleum-based synthetic inputs cannot continue indefinitely into the future, with peak-oil approaching (Hall and Day, 2009), while 7–8 oil calories are spent in the production of every food calorie (Leach, 1975).

With over 7 billion of people to feed—a number that is still growing, while millions are suffering the effects of malnutrition (Black et al., 2008)—intensive agriculture can only be humanely tuned down if this doesn't decrease the amount of food produced.

Permaculture practices promise solutions. For permaculture as a practice to be a viable alternative to conventional agriculture, its claims must be investigated. Since many of these claims are readily testable as scientific hypotheses, much progress could be made by in agroecology by systematically evaluation permaculture claims. One of the cornerstone claims of permaculture is that the substitution of monocultures with polycultures, usually with the inclusion of perennials, will lead to improvements in yield and other ecosystem services (Mollison et al., 1991; Shepard, 2013).

Polyculture productivity relates to changes in the soil microbiome. That indeed intercropping can increase primary (crop) productivity has repeatedly been demonstrated, as well as that, at the same time, important ecosystem services, such as pest resistance, can be improved (e.g. Iverson et al., 2014, see also section 2). What is less clear is if and how these improvements relate to the soil microbiome; if a intercropped soil bolsters improved plant-growth, are some of these positive plant-plant interactions mediated by microbes? In other words: are the agroecosystem improvements reflected by measurable changes in the soil microbial community? This present paper aimed to progress the understanding of the microbial mechanisms by which the productivity and stability increase in

some polycultures could be enacted.

From the cases in section 4.2, a careful conclusion can be drawn: in experimental polyculture setups, an increase in biomass, diversity and stability (stress resilience) of soil microorganisms accompanies the increase in crop productivity, if, and only if, such an increase is seen in comparison to monoculture controls (Song et al., 2007; Lacombe et al., 2009; Zhou et al., 2011).

None of the cases provides perfect evidence for this preliminary conclusion; Lacombe et al. (2009) provide the only results from experimentally comparing microbiotic resilience between monocropped soybean control fields and tree-based soybean intercropping treatments (by applying heavy metal (Cu) stress), but they only find a significant effect for one of their replicates. Also, their replicates are at different sites with a different soil history.

The other two highlighted cases (Song et al., 2007; Zhou et al., 2011) offer some evidence that microbial biomass, diversity and activity can increase under polycropping regimes, but since there's no unambiguous relationship between plant diversity and these indicators (e.g. see table 3 for some counter-examples), researchers should endeavor to include the recovery of these indicators over time after application of stress in their research efforts (van Bruggen and Semenov, 2000; Lemanceau et al., 2015).

Apart from these select cases (Song et al., 2007; Lacombe et al., 2009; Zhou et al., 2011), a quick glimpse at the results from the rest of the studies listed in table 3 reveals that the studies which report an increase in crop yield in their results never report a decrease in microbial biomass, diversity or activity (Dai et al., 2013, 2009), although some see no increase either (Herrmann et al., 2014; Hummel et al., 2009), and Dai et al. (2009) report a relative decrease of pathogenic fungi under intercropping of peanut with traditional Chinese medicinal plants.
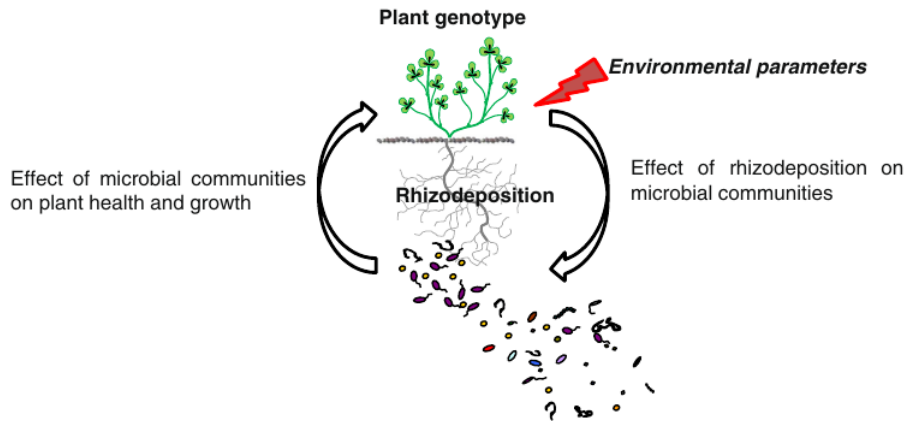

Figure 7. Because "rhizodeposition varies according to plant genotypes and environmental conditions" (Lemanceau et al., 2015), microbial communities in the rhizosphere are differentially affected by different plant species (in terms of abundance, diversity and activity). Since microbial communities in turn impact plant growth and health, it can be hypothesized that plants can affect each other through their microbial interactions. Schematic reproduced from Lemanceau et al. (2015).

Rhizodeposite diversity may improve soil health. Even if we conclude that polyculture can improve microbial indicators of soil health, for now we have to rely on guesswork on if and how microbial actors mediate some of the positive plant-plant interactions. An educated guess *can* be made however, based on the observations that plant species influence the soil microbiome through rhizodeposition and that microbes in turn influence plant growth and health (Lemanceau et al., 2015). Since each plant has its own unique root exudates, this variation in rhizosphere chemistry could increase overall soil microbial diversity and abundance, leading to increase (in stability of) microbial activity in support of essential soil processes.

It's worth recalling that, on the plant level, "diversity begets diversity", when comparing weed diversity between mono- and polycultures (Palmer and Maurer, 1997); And, plant diversity is also increased by below-ground AFM diversity (van der Heijden et al., 1998; Grime et al., 1987), assigned by Grime et al. (1987) to the relative increase in "biomass of the subordinate [weed] species relative to that of the canopy dominant." Extending this to other functional groups in the soil, part of the ambiguous response of primary productivity to increasing crop diversity may potentially be explained: perhaps an increase in yield is only produced if secondary crops improve microbial diversity, biomass, and/or activity beyond the level of monocropping.

A testable hypothesis could thus be: polycropping practices that increase yield do so in part by improving the soil microbial community. The microbial improvements should be measurable as increases in microbial abundance, diversity and activity and, importantly, the stability thereof. To test this hypothesis, the rhizosphere of plants in sterile soil could be inoculated with microorganisms from the corresponding plant species' rhizosphere in polyculture treatments and monoculture controls. Then, if the plants inoculated with microorganisms from the polyculture rhizospheres do no better than the others, the hypothesis is falsified.

Testing how (the stability of) soil microbial diversity, biomass, and activity respond together to different cropping systems is a promising avenue of research that has barely been taken advantage of. Most of the studies found with the search strategy outlined in section 4.1 are no more than a few years old, none older than a decade. The oldest study was Song et al.'s (2007), at 8 years old. Research opportunities await, but future researchers would be well-advised to include stability measurements in their studies. And, ideally, rhizome transplants should be performed to determine the extent to which the soil microbiome mediates the positive plant-plant interactions in productive polycultures.

Permaculture is here to stay. There's a rich body of practices (e.g. no-till planting, livestock integration, mulching) in permaculture that are in dire need of evaluation, based on the urgency of our current ecological predicaments and the strength of the posited claims. Ecological science can benefit from the energy of the parmaculture movement and give back an evidence-based foundation on which science-savvy permaculture practitioners could base more effective agroecosystem interventions. In a sense, many permaculture practitioners are already applied ecologists, since part of the philosophy is to replace hard work with careful observation (Mollison and Holmgren, 1978; Ferguson and Lovell, 2014). Permaculture as a philosophy is here to stay, but science has to provide

the tools for critical evaluations, so that new agroecological disasters can be averted.

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