DIVERSify: Designing InnoVative plant teams for Ecosystem Resilience and agricultural Sustainability

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Project Co-ordinator:	Dr Alison Karley, JHI
Tel:	+44 (0)1382 568820
Email:	Alison.Karley@hutton.ac.uk

Key mechanisms promoting performance of plant teams (Report, Public)

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Lead Author: Christoph Scherber (WWU)

Contributing Author(s): Alison J. Karley (JHI), Christian Schöb (ETHZ), Anja Schmutz (ETHZ), Martin Weih (SLU), Stefano Tavoletti (UNIVPM), Eveline Adam (SZG), Carlota Vaz Patto (ITQB), Diego Rubiales (CSIC), Ángel Villegas Fernández (CSIC), Lars P. Kiær (UCPH)

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Executive Summary

Growing two or more plant species in close proximity ('plant teams') is a practice that can be adopted to improve crop production in terms of increased productivity, more efficient resource use, reduced reliance on crop protection chemicals, and biodiversity enhancement. Here, we summarise findings from experimental trials conducted across Europe to quantify plant team performance and elucidate the underpinning mechanisms, whether due to facilitation between plants (i.e. one plant benefits the performance of another directly or indirectly) or complementarity in resource use (i.e. maximising space or resource utilisation). We highlight trial findings suggesting that carefully chosen plant teams could provide improved yield stability and greater resilience against environmental stress for future-proofing agriculture in Europe and globally.

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1. Introduction

Growing two or more plant species in close proximity (intercropping or 'plant teams') is a practice that can be adopted to improve crop production. Plant team performance is affected not only by agricultural management, but also by ecological interactions among plant team components (plant-plant interactions) and with other organisms (e.g. plant-insect interactions). Within the DIVERSify project, Work package 2 set up experiments to elucidate key mechanisms of plant team performance using monocultures and mixtures of crop species in different pedoclimatic zones and under different agricultural management intensities.

The "performance" of a plant team can be measured in several ways, depending on why a plant team is being grown, or on the products that are derived from growing plant teams. For example, performance may simply be the grain yield, often given as the difference to the value expected from observations of monocultures in the form of relative yield, land equivalent ratio, or competition indices. But it can also mean providing particular ecosystem services (e.g. crop pollination, soil water retention, crop health) or ensuring particular product properties (e.g. nutrient content).

Thus, when talking about the "performance" of a plant team, it is useful to distinguish between measurable outcomes, and the mechanisms generating these outcomes. Outcomes include:

(i) increased productivity and improved quality;

(ii) Increased resource use efficiency (light, water, nutrients);

(iii) Weed, pest and disease suppression;

(iv) Improved pollination effectiveness;

(v) Biodiversity enhancement.

If these effects occur consistently in plant teams, they can result in improved yield stability and stress resilience. The underpinning mechanisms are usually a mixture of:

(i) facilitation between plants (e.g. by increasing soil fertility); and/or

(ii) complementarity (e.g. by maximising space/resource utilisation).

2. Hypotheses

Compared with monocultures, plant teams are capable of showing improved agronomic performance (higher productivity, yield stability) and environmental performance (reduced reliance on inputs, supporting greater biodiversity) as a result of mechanisms that lead to increased resource acquisition, weed suppression, attraction of beneficial organisms, or protection against pests or pathogens (Brooker et al. 2015) The two key concepts to explain the mechanisms underpinning these outcomes in plant teams are:

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(1) **Facilitation**. An interaction "in which the presence of one species alters the environment in a way that enhances growth, survival, or reproduction of a second, neighbouring species" (Bronstein 2009);

(2) **Niche Complementarity**. An interaction by which the component species of the plant team utilise resources differently, either spatially and/or temporally, resulting in the mixture filling out niche space more completely than the components in monocultures.

In the DIVERSify project, research has focused on providing evidence for the processes and outcomes that optimize the performance of plant teams compared with monocultures and identifying the underpinning mechanisms of facilitation and complementarity. Where possible, we highlight the morphological, phenological, and physiological plant traits that are likely to underpin niche complementarity or facilitation in plant teams. The DIVERSify field trials have been conducted at locations spanning the pedoclimatic zones of Europe, and were designed to test several hypotheses based on theoretical assumptions about the benefits of increased diversity (e.g. Letourneau et al., 2011) as follows:

- Plant teams show improved productivity compared with monocultures;
- Plant teams show greater resource uptake, increasing resource use efficiency compared with monocultures;
- Plant teams show better weed suppression than monocultures;
- Plant teams show lower incidence of pest organisms and reduced disease incidence than monocultures;
- Plant teams support greater biodiversity, particularly of beneficial organisms, than monocultures;
- Plant teams provide greater yield stability and resilience to weather extremes.

3. Productivity and resource use

Productivity (biomass production per unit of time and space) is one of the most frequently measured outcome variables (often only measured once per season as biomass per unit of space) that can increase in mixtures when compared to the productivity expected from monocultures of the component crops. This is an important aspect of intercropping and growing plants in mixtures. Throughout the DIVERSify trials, we have frequently found - when using yield as a final measurement - plant teams whose mixture performance is increased relative to monocultures. Here we summarise what we have learned about the mechanisms of resource capture that help to underpin such enhanced plant team performance.

3.1. Light use

Complementarity between plant species for maximising light capture is one of the core mechanisms analysed within the DIVERSify trials, as many of the measured aboveground plant characteristics correlate directly or indirectly with light acquisition traits. These include, for example, aboveground plant height (as an individual plant trait), but also plant community measures such as canopy

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reflectance or leaf area index. Complementarity in light acquisition traits is among the key mechanisms promoting plant team performance, as plant species with complementary architecture show greater space filling (**Figure 1**). Further, plasticity in leaf traits such as specific leaf area can aid light capture if a species is partially shaded in the plant team canopy.

Figure 1. Complementarity in light acquisition traits (growth form) in (a) a wheat-faba bean intercrop and (b) a wheat-linseed intercrop. Photographs by C. Scherber.

3.2. Nitrogen use

Nitrogen use efficiency in mixtures and monocultures has been intensively studied in Sweden (by SLU), Denmark (UCPH) and Scotland (JHI). In multi-year cereal-legume field trials conducted by SLU, involving both pea-barley and faba bean-wheat mixtures, cereals had higher nitrogen uptake efficiency than legumes. This pattern is similar to the findings in Denmark (UCPH), but different from those seen in field trials of pea-barley mixtures in Scotland (JHI), in which both the legume and the cereal showed improved grain nitrogen concentrations in plant teams compared with monocultures.

In the Swedish trial (SLU), the cereal partners strongly benefitted from growing in mixtures at the cost of reduced performance of the legume partners; differences in yield between the plant teams were accomplished more by differences in crop net nitrogen (N) uptake, and less by differences in yield production per unit of nitrogen in the crop. Thus, the cereals increased their efficiency for N uptake and therefore benefitted more than the legumes in the mixtures, in terms of accumulated N and grain yields. In general, the N economy of the mixtures was greatly influenced by the cultivar identity in the mixture, and the cultivar identity effect on mixture performance often appeared to be more important than the diversity level (i.e. single species *vs*. two-species mixtures) in which each species was grown. For faba bean cultivars differing in nitrogen accumulation and grain yield (varieties “Diskett” and “Alderon”), additional growth container experiments showed that cultivars differed in nitrogen allocation to aboveground *vs.* belowground plant parts in monocultures compared with mixtures. By contrast in the pea-barley trials in Scotland (JHI), increased grain productivity in plant teams was driven by increased pea yield rather than barley yield. The differential responses between these trials

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of the legume and cereal crop species might relate to site-specific conditions (temperature, water/nutrient availability) affecting the degree to which intraspecific competition for resources was reduced in plant teams.

Increased nutrient availability through nitrogen fertiliser addition has the potential to modify plant-plant interactions and plant acquisition of nutrients. Trials in Scotland (JHI) showed that adding nitrogen fertiliser had only minor effects on plant team performance: some barley cultivars showed differential responses to pea when grown under low *vs.* standard nitrogen input levels. In trials conducted in Italy (UNIVPM), durum wheat-faba bean and bread wheat-faba bean mixtures had more efficient nitrogen use, reducing the fertiliser requirements, and a higher grain protein content was obtained from wheat in mixtures than in wheat monoculture. Moreover, different faba bean varieties showed a different competitive ability with wheat, suggesting that an optimal choice of wheat-faba bean variety combinations should be selected to optimise plant-plant interactions and performance of the plant teams. In general, analyses across all partner sites show a significant interaction between management intensity and diversity (monoculture vs species mixtures), indicating that high- and low-input farming systems differ in their yield responses to intercropping. This effect was due to more consistent yields in mixtures under low and high input management, whereas monocultures (particularly cereals) showed reduced yield under low input management (**Figure 2**).

Figure 2. High (left) vs. low-input mixtures in barley-pea intercropping, leading to massively different crop yields depending on nutrient addition and weed cover (mainly *Chenopodium album*, right-hand side of the image). Photograph by C. Scherber.

In summary, the findings show that crop species differ in their nutrient use efficiencies and that growing cereals in mixtures with legumes can reduce the need for nitrogen fertiliser inputs. It is important to consider, however, the cultivar identity of each species in the mixture; this is an

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important aspect to address with respect to future farming systems where fertiliser inputs may be increasingly regulated. The mechanisms underpinning improved nutrient use appear to include increased nutrient availability (e.g. due to mobilisation of soil phosphorus resources by legume root exudates: Li et al., 2016), decreased competition for nitrogen uptake by cereals (due to biological nitrogen fixation by legumes) and more efficient use of acquired nutrients by one or both plant species.

3.3. Water use

Water use efficiency in crop mixtures and monocultures has been intensively studied in Switzerland (by ETHZ) and Portugal (by ITQB). In Switzerland, six crop species were studied in monoculture and species mixture combinations and received either natural rainwater or water enriched with stable isotopes of hydrogen to monitor water uptake from different depths in the soil substrate. Initial findings indicate that crop species differed in the depth of soil from which they extracted water, with oilseed rape having deepest roots and tending to take up water from greater depths in mixtures compared with monocultures. These findings indicate that root length plasticity could enable partitioning between species of water use at different soil depths, leading to greater water use efficiency in species mixtures.

In Portugal (ITQB), trials were set up to manipulate water availability (irrigated or not) in pot experiments using grassland mixtures comprising nine cereal and legume species. The water-deficit treatment corresponded to 50% of field capacity. Leaf gas exchange parameters, chlorophyll *a* fluorescence, photosynthetic pigments, and relative water content were measured under well-watered and water-deficit conditions. There were species-specific differences for all the traits. Some species, however, showed smaller trait variation than others when comparing the two water treatments. For example, *Trifolium vesiculosum*, *T. incarnatum*, *T. suaveolens,* and *Vicia villosa* appeared to be particularly resilient under water scarcity, as their photosynthetic performance was stable under both water regimes studied. In contrast, *Lolium multiflorum* (diploid and tetraploid cultivars) suffered more from water shortage with a negative effect on photosynthesis. These results provide clues for the development of innovative plant teams combinations more adapted to climate change conditions aiming at a more productive and sustainable grassland agriculture.

Overall, especially in semiarid and arid areas, water use efficiency can be an important driver of plant team performance and an important point to consider when adapting cropping systems to the changing climate (**Figure 3**). For example, in Austria (SZG), in the dry year, grass pea had higher yields in a faba bean/grass pea mixture whereas in the wet summer conditions, faba bean was the dominating crop. If species in mixtures can occupy different niches belowground leading to complementary use of the available soil water, mixtures may ultimately be more resilient against drought events than monocultures.

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Figure 3. Severe summer drought at WWU in 2018. Photograph by C. Scherber.

4. Antagonist suppression

4.1. Weeds

Suppression of antagonists, such as weeds or plant pathogens, in mixtures compared with monocultures has been investigated in Germany (WWU), Scotland (JHI), Austria (SZG), and Italy (UNIVPM). Management treatment (pre-emergence herbicide application) had the strongest effect on weed cover and biomass in monocultures and mixtures, but mixtures additionally reduced weed cover (**Figure 4**), indicating that plant teams can contribute to weed suppression through improved light capture and efficient nutrient and water uptake, therefore outcompeting the weed understorey. As weed infestation is among the most important causes of poor crop productivity globally and leads to seed contamination, reduced weed infestation in mixtures can be considered one of the most important outcomes governing plant team performance. Employing plant mixtures can thus be an important strategy to reduce the amount of herbicide use in cropping systems worldwide.

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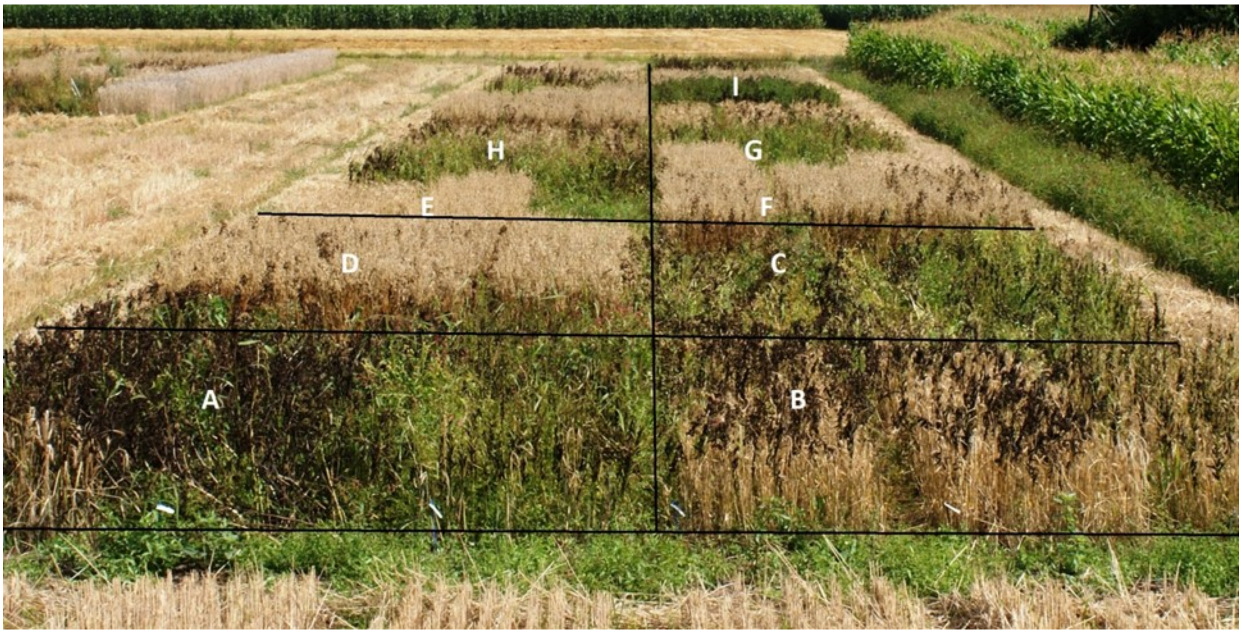

Figure 4. Weed suppression potential of mixed cultures: A, H and I) faba bean monocultures with high amounts of weed biomass (weed leaves green); B) faba bean-wheat mixtures (no weeds); C) faba bean-grass pea mixtures (grass pea still green, no weeds); D and F) faba bean-oat mixtures (no weeds); E and G) wheat, oat and grass pea monocultures (grass pea still green). Photograph by E. Adam.

4.2. Pest and disease suppression

Increased diversity of crop vegetation has been shown in multiple studies to improve pest and disease regulation (e.g. Letourneau et al., 2011). In Scotland (JHI), pest damage and aphid infestation (**Figure 5**) was monitored on pea and barley mixtures and the corresponding monoculture crops under two management conditions – standard inputs (conventional practice) and low inputs (integrated practice). Aphid infestation was highest under conventional management and was suppressed on pea in mixtures compared with monocultures under both management regimes; however, cereal aphid abundance on barley was higher in mixtures, indicating that diversity benefits were not achieved for both crop components in the plant team.

The effects of plant team cropping on disease incidence have been variable, partly due to variable disease pressures across trials and between years. In Spain (CSIC), for example, the severity of two diseases of cereals (*Septoria* spp. on wheat and *Helminthosporium* spp. on barley) was not affected by growing in pea-barley or wheat-faba bean mixtures, while legume diseases such as powdery mildew (on pea) and rust (on bean) showed lower incidence in mixtures.

In summary, pest and disease suppression has been observed in mixtures compared with monocultures, but this benefit does not always apply equally to each plant species in the mixture. Reduced pest infestation and pathogen infection is thought to be due to facilitation and

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complementarity mechanisms arising from increased heterogeneity of the canopy, such as dilution of the target plant population, physical barriers to pest and pathogen movement and insect repellence by plant volatile compounds (see Brooker et al., 2015).

Figure 5. Antagonistic interactions in intercropping trials. (a) English grain aphids (*Sitobion avenae* FABRICIUS) infesting a barley plant in monoculture and (b) cabbage-stem flea beetles (*Psylliodes chrysocephalus* L.) infesting oilseed rape seedlings in monoculture. Photographs by C. Scherber.

5. Biodiversity enhancement

Increased canopy heterogeneity in species mixtures leads to greater diversity of resources for other organisms in the crop, supporting greater biodiversity through indirect effects of complementary resource use (e.g. larger area of plant canopy due to more efficient use of light and nutrients) and direct effects of facilitation (e.g. insect attraction to flower volatiles). Where these organisms carry out beneficial ecosystem services, such as pollination and pest suppression, there can be further benefits for mixture performance. At a larger scale, plant teams may be an important strategy to provide food (nectar, pollen, honeydew) for pollinators and natural enemies, thus supporting larger populations in the agricultural landscape. Thus, plant team cropping could be an important strategy to counteract recent worldwide insect declines (van Klink et al. 2020).

5.1. Natural enemies of crop pests

Effects of crop diversity and management on natural enemies of crop pests was studied in Germany (by WWU) and Scotland (by JHI). Natural enemies of chewing and sap-feeding plant pests, including hymenopteran parasitoids and arthropod predators (**Figure 6**), were present in greater abundance in crop mixtures compared with monocultures. As their effects on crop yield are indirect (acting via reduced herbivore infestation levels), natural enemy enhancement in mixtures might be considered less influential for plant team performance than other outcomes (such as increased productivity or weed suppression).

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Figure 6. (a) Adult ladybeetle (*Coccinella septempunctata* L.) in a faba bean monoculture**,** feeding on pea aphids (*Acyrthosiphon pisum* HARRIS); (b) a predatory bug (Heteroptera: Anthocoridae) searching for prey on an oilseed rape plant in a three-species crop mixture. Photographs by C. Scherber.

5.2. Pollinator attraction

Pollinator presence has been investigated in field trials conducted in Germany (by WWU) and Scotland (JHI). While high-input legume monocultures (i.e. fertilized and treated with pre-emergence herbicide) supported the highest abundances of pollinating or flower-visiting insects, the species richness of flower-visiting insects (**Figure 7**) was highest in low-intensity crop mixtures (in Germany), and the abundance of hoverfly pollinators was increased in pea-barley mixtures compared with barley monocultures (in Scotland).

Figure 7. (a) A common carder bee (*Bombus pascuorum* SCOP) visiting a high-intensity wheat-faba bean mixture and (b) red-tailed bumblebee (*Bombus lapidarius* L.) visiting oilseed rape in a low-intensity mixture at WWU © Christoph Scherber

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If plant team performance was measured in terms of how “pollinator-friendly” it was, then clearly growing insect-pollinated plants in mixtures with crops that do not rely on insect pollination (like cereals) would be an optimal scenario to enhance the local abundance and diversity of insect pollinators in agricultural crops.

6. Conclusions

A wide spectrum of outcomes affecting plant team performance arising from mechanisms of complementarity and facilitation has been investigated within the DIVERSify experimental trials. Amongst these, crop species mixtures have been shown to enhance complementary resource use through improved light acquisition, water use efficiency and nitrogen use efficiency. Other potential beneficial outcomes from plant team cropping, such as enhanced biodiversity and better weed, pest and disease control, are affected indirectly by complementarity in space occupation and resource use; facilitation between plant team components also plays a role in promoting these benefits. Trial findings also suggest that carefully chosen plant teams could provide greater resilience against environmental stress, making plant teams a potential strategy for climate-smart cropping as the climate changes.

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