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Increasing crop rotational diversity can enhance cereal yields

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Increasing crop rotational diversity can enhance cereal yields

Monique E. Smith¹, Giulia Vico ², Alessio Costa ², Timothy Bowles ³, Amélie C. M. Gaudin⁴, Sara Hallin ⁵, Christine A. Watson^{2,6}, Remedios Alarcón ⁷, Antonio Berti⁸, Andrzej Blecharczyk ⁹, Francisco J. Calderon¹⁰, Steve Culman¹¹, William Deen¹², Craig F. Drury¹³, Axel Garcia y. Garcia ¹⁴, Andrés García-Díaz¹⁵, Eva Hernández Plaza¹⁶, Krzysztof Jonczyk¹⁷, Ortrud Jäck², R. Michael Lehman¹⁸, Francesco Montemurro¹⁹, Francesco Morari⁸, Andrea Onofri²⁰, Shannon L. Osborne¹⁸, José Luis Tenorio Pasamón²¹, Boël Sandström²², Inés Santín-Montanyá ²¹, Zuzanna Sawinska ⁹, Marty R. Schmer²³, Jaroslaw Stalenga¹⁷, Jeffrey Strock²⁴, Francesco Tei²⁰, Cairistiona F. E. Topp ²⁵, Domenico Ventrella¹⁹, Robin L. Walker⁶ & Riccardo Bommarco ¹✉

Diversifying agriculture by rotating a greater number of crop species in sequence is a promising practice to reduce negative impacts of crop production on the environment and maintain yields. However, it is unclear to what extent cereal yields change with crop rotation diversity and external nitrogen fertilization level over time, and which functional groups of crops provide the most yield benefit. Here, using grain yield data of small grain cereals and maize from 32 long-term (10–63 years) experiments across Europe and North America, we show that crop rotational diversity, measured as crop species diversity and functional richness, enhanced grain yields. This yield benefit increased over time. Only the yields of winter-sown small grain cereals showed a decline at the highest level of species diversity. Diversification was beneficial to all cereals with a low external nitrogen input, particularly maize, enabling a lower dependence on nitrogen fertilisers and ultimately reducing greenhouse gas emissions and nitrogen pollution. The results suggest that increasing crop functional richness rather than species diversity can be a strategy for supporting grain yields across many environments.

Commodity cropping has generally focussed on increasing yields while overlooking its contribution to environmental degradation, climate change and biodiversity loss. As such, global food production threatens people, the planet and agriculture itself¹. Expanded use of agroecological solutions that maintain crop yields while minimizing the use of external inputs has been proposed as a way forward^{2,3}. A promising practice is to diversify agriculture by rotating a greater number of crop species in sequence in the same field^{4,5}. Nevertheless, production of global staple commodity crops is often dominated by short rotations of two crop species⁶, sometimes even with only one crop grown season after season (monoculture)^{6–8}, resulting in loss of crop diversity on the regional scale^{9–12}.

Farmers around the world^{16,13} have known for a long time that grain yields decline with monoculture or short crop rotations and that high external inputs are required to support production in simplified systems^{14,15}. Soil fertility and nutrient use efficiency can be improved or maintained in the long term when more species and functional groups are included, as soil microbial biomass, water holding capacity, soil carbon, and nitrogen (N) availability and plant uptake are enhanced^{6,16,17}. Increased diversity of crop species and functional groups in the rotation also decreases weed, pest and disease pressure⁶. A diverse rotation can thereby require less fertilisation and crop protection inputs, which is central to easing the pressure of agriculture on climate, soil, and biodiversity. Supporting this, a recent meta-analysis found crop rotational diversity (CRD) resulted in higher yields when N input was low at least when legumes were present¹⁸. But to what extent diverse rotations can maintain grain yields and compensate for reduced fertiliser inputs over time and for different grain crop species is not clear. Most studies on CRD only compare monoculture to diverse rotations¹⁹, focus on a single site^{20,21}, are short term^{22,23}, or only include a single indicator crop²⁴ or a few species in their diverse rotation^{25,26}. Therefore, it remains unclear how crop production benefits and fertiliser dependency develop when gradually moving from monoculture to high CRD, whether increased diversity of crop species, crop functional groups, or inclusion of specific crops renders the greatest grain yield benefits, and how these effects change over time, under contrasting fertilisation and for different cereals.

Much of our understanding on diversity-productivity relationships comes from long-term experiments in grasslands. They demonstrate that plant biomass production increases with the number of plant species or functional groups in the community²⁷. The increase is explained by a combination of species selection effects and niche differentiation and facilitation between species, i.e., niche complementarity^{28–31}. It is hypothesised that these outcomes would be replicated in arable ecosystems, with crop yields increasing with CRD³², but this has not been verified across a wide range of diversity levels. Grasslands are characterized by many species of intermixed annual and perennial plants and comparably low levels of nutrient inputs and soil disturbance, e.g., by tillage. By contrast, global staple commodity crops, such as maize and small grain cereals, are annual plants and typically grown as a single crop in fertilised and frequently tilled soils³³. Furthermore, grassland experiments mainly test outcomes of spatially-intermingled diversification, whereas CRD refers mostly to temporal diversification where crop species are changed from season to season (intercropping and under-sowing being some exceptions). Species interactions are thus more indirect in cropping systems than in grasslands and, for instance, mediated by changes in soil properties³⁴ that may take longer to accrue³⁵. Lastly, in grassland experiments examining relationships between biodiversity and ecosystem functioning, species mixes have been selected such that species diversity is not confounded by functional traits. In CRD experiments, species are chosen based on

their agronomic traits and market value and often are confounded by functional traits²¹. These differences impede a direct translation of diversification outcomes from grassland experiments to arable ecosystems and highlight the need to test relationships between diversity and productivity in agricultural experiments that cover a range of crop species diversities and functional traits.

Focusing on small grain cereals and maize as indicator crops, we hypothesise that diversifying rotations raises rainfed grain yields within a few years after implementation and that there is a gradual rise in yield benefits over time from a long-term build-up of supporting ecosystem functions in the agroecosystem. We expect this effect to be stronger with a higher number of functional groups in the rotation, but also that specific functional groups can be more influential than others. Because CRD can increase nutrient use efficiency and N availability^{36,37}, we expect CRD benefits to be higher under lower external N fertilisation rates.

Long-term agricultural experiments provide unique opportunities to explore relationships between CRD and crop yield over time and assess linkages between yield outcome and presence of specific crop functional groups^{20,38,39}. This is because CRD levels are compared under similar ecological, edaphic, climatic and management conditions at each site. The experiments often include treatments with contrasting fertiliser input levels allowing for examination of effects of CRD under high and low N inputs. Furthermore, crop yield data collected over decades allow tracking of yield benefits and potential trade-offs of CRD over time scales comparable to those of the processes involved. We analysed grain yields of spring-sown maize and small grain cereals, and of winter-sown small grain cereals from 32 long-term (≥ 10 years) crop rotation experiments across Europe and North America (Fig. 1), encompassing 957 site-years and 27,460 grain yield observations. This dataset covers wide ranges of pedoclimatic conditions, CRD designs, productivity levels and management practices, including contrasting external inputs of organic and inorganic N fertilisers (Supplementary Tables 1, S2). We used maize and small grain cereals as indicator crops because they dominated all rotation treatments and are global staple crops. We measured CRD based on two metrics: species diversity and functional richness. Species diversity was calculated using a modified version of the inverse of Simpson's index of diversity, whereby species diversity of 1 represents a monoculture and 3 represents a 3-year rotation with three different crop species. Functional richness accounts for presence of agronomically or ecologically diverse species in the rotation (see methods for more details on CRD metrics and Supplementary Table 2 for information about CRD for each rotation per site).

Results and discussion

Grain yields increased with higher species diversity for all indicator crops, and the effect depended on the external N input rate (Fig. 2). Using monoculture with low external N input at year zero as the baseline, the maximum yield gain 35 years after the start of diversification was 0.94 t/ha (95% confidence intervals (CI) [0.74, 1.13]) for spring small grain cereals, occurring at species diversity of 3.9; 1.32 t/ha (95% CI [1.01, 1.62]) for winter small grain cereals at species diversity of 3.91; and 4.19 t/ha (95% CI [3.60, 4.78]) for maize at species diversity of 4.57 (Fig. 2). For winter small grain cereals, CRD gains decreased at species diversity >3.91 at low external N input and >3.86 at high external N input, remaining only slightly above the monoculture rotation yield at the highest species diversity at year 35 (Fig. 2, Supplementary Table 3). Spring small grain cereal yields tended to decline at the highest CRD but at lower rates than winter small

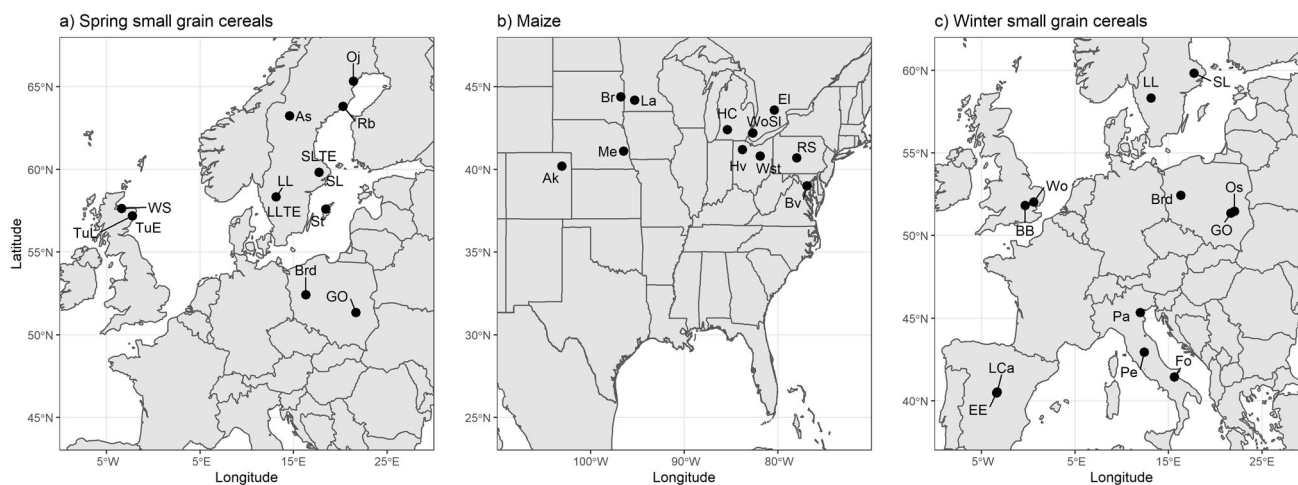


Fig. 1 Location of long-term agricultural experiments of each indicator crop. Spring-sown small grain cereals (**a**) were grown in Europe, maize (**b**) in North America, and winter-sown small grain cereals (**c**) in Europe. See Supplementary Table 1 for the clarification of site codes and additional site information.

grain cereals, mostly after 35 years (Fig. 2, Supplementary Table 3). For maize, there was only a slight decline close to the maximum diversity covered by the experiments, and only present at low external N input and at years 5 and 20, i.e., disappearing by 35 years (Fig. 2, Supplementary Table 3). We note though that the maize experiments tested a lower maximum species diversity compared with the small grain cereals (4.57 and 6.00 respectively; Supplementary Table 2).

The benefit of CRD increased over time (Fig. 2). After only five years following implementation, there was a short-term grain yield benefit from CRD of 0.36 t/ha (95% CI [0.16, 0.55]) at species diversity of 4.03 in spring small grain cereal; of 0.62 t/ha (95% CI [0.33, 0.91]) at species diversity 3.54 in winter small grain cereal; and 2.26 t/ha (95% CI [1.81, 2.71]) at species diversity of 3.72 in maize compared with a baseline of monoculture, at year zero and low external N input (Fig. 2). This benefit increased over time, for example, the diversity levels from the 5-year estimates provided an additional 0.58 t/ha (95% CI [0.38, 0.77]) in spring small grain cereal, 0.68 t/ha (95% CI [0.37, 0.98]) in winter small grain cereal and 1.70 t/ha (95% CI [1.18, 2.22]) in maize from 5 to 35 years after implementation (Fig. 2, Supplementary Table 3). For comparison, the yield increase over the same period in monocultures was 0.08 t/ha (95% CI [−0.11, 0.28]) for spring small grain cereal; 0.08 t/ha (95% CI [−0.23, 0.39]) for winter small grain cereal; and 0.58 t/ha (95% CI [0.06, 1.09]) for maize (Fig. 2, Supplementary Table 3). Our models account for most of the year-to-year variation in our random structure so that the CRD yield gains were not inflated by technological advances over time. Therefore, these increases are relatively low compared to those including technical advances, e.g., yield benefits between 69 and 126 kg/ha/year in maize⁴⁰.

To test whether functional group richness acts independently of species diversity in crop rotations, as observed in grasslands^{41,42}, we divided the crop species into four agronomic and ecological functional groups: annual cereals, annual legume, annual broadleaf, and ley, i.e., biennial or perennial grass and/or legume in single or mixed plantings (Supplementary Fig. 1). We then analysed grain yields using functional richness (1–4) as a measure of CRD in the same way as for species diversity. Generally, the greatest production benefits occurred in rotations with two to three functional groups (Fig. 2, Supplementary Table 4). For both small grain cereals, the yield benefit from three functional groups increased with time, while for maize two functional

groups led to the highest grain yield in the long term. For spring small grain cereals, the yield benefit declined as a fourth functional group was added to the rotation, at low fertilisation, but compared with monocultures a yield benefit remained with four functional groups after 35 years (Fig. 2, Supplementary Table 4).

We used the same functional group classification to examine the relationship between grain yields and the presence or absence of the remaining functional groups (annual legumes, annual broadleaves, and ley). Benefits from CRD stemmed from the inclusion of specific crop functional groups in the rotation, such as annual leguminous crops that can fix atmospheric N₂, rather than crop diversity per se. This is aligned with species selection effects shown to enhance ecosystem functioning, such as resource capture, biomass production, decomposition, nutrient cycling, at higher diversity²⁸. Certain groups of crops are particularly efficient at breaking a sequence of agronomically and ecologically similar cereal species⁴³. However, the indicator crops reacted differently to the inclusion of crop functional groups. Winter small grain cereal production benefited from the inclusion of annual broadleaf and legume crops (Supplementary Table 5). Including legume and annual broadleaf crops benefited spring small grain cereal yields, whereas including ley had no impact (Supplementary Table 5). Maize yields benefited from including annual legume and ley crops, but not from annual broadleaf crops in the rotation (Supplementary Table 5). Hence, several functional groups contributed to gains in the indicator crop yields and we surmise that ecological complementarity mechanisms over time and space are likely important explanatory factors for CRD benefits. The low R² values in the models indicate that results are part of the many factors that affect grain yields, and possibly a result of merging data from a range of pedoclimatic conditions. However, the robust and increasing effect sizes that we despite this see from CRD and fertiliser management call for serious agronomic consideration.

If nitrogen use efficiency mainly explain rotational benefits then we would expect rotational benefits to decline or disappear at high rates of external N fertilisation¹⁸. When comparing the grain yield benefit from CRD in terms of both crop diversity and functional group richness at high and low external N inputs, responses differed among indicator crops (Fig. 2, Supplementary Tables 3 and 4). This suggests that the biophysical source of production benefits varies among crops. Maize yields increased more strongly with CRD under low rather than high external N

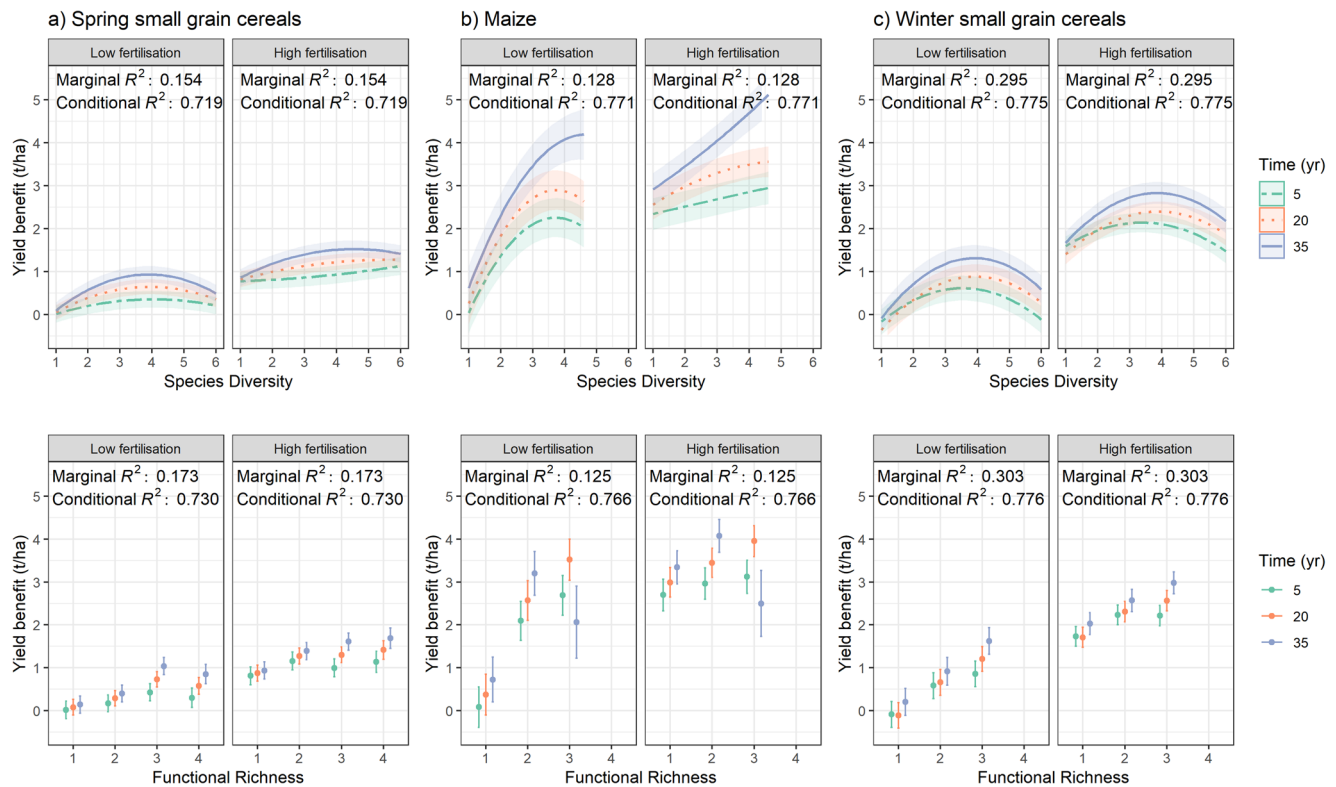


Fig. 2 Yield benefit for each indicator crop from crop rotational diversity (CRD) and external nitrogen (N) fertilisation. The y-axis presents model-predicted yield benefit compared to monocultures at year = 0, based on mean-centred observations, i.e., the difference from to the long-term within-site average across all CRD treatments for each indicator crop (a–c). Yield-benefit of 0 indicates model predictions remained the same, negative values are yield losses and positive values are yield benefits as yields in monocultures at year = 0. CRD is characterized by species diversity (based on the inverse Simpson’s diversity index; top row) and functional richness (the number of functional groups included in the rotation; bottom row), and external N fertilisation is classified as low or high (left and right panel in each pair). The curves (top row) and symbols (bottom row) represent model predictions after 5, 20, and 35 years since the beginning of the experiment (dotted green lines, dotted orange lines, and solid blue lines, respectively). We chose these three time periods to represent the early-, middle- and long-term effects of time within our range of data. The 95% CIs are indicated by the shaded areas surrounding the lines (top row) and error bars (bottom row).

inputs. For example, at year 35 there was an average positive yield slope of 1 t/ha per species diversity unit (95% CI [0.74, 1.26]) under low external N input, compared with 0.61 t/ha per species diversity unit (95% CI [0.40, 0.82]) at high external N inputs (Fig. 2, Supplementary Tables 3 and 4). This indicates that CRD enhances nutrient-mediated benefits particularly well in maize. For winter and spring small grain cereals, yield benefits from low to medium CRD were also steeper at low external N input but to a lesser extent than in maize (Fig. 2, Supplementary Tables 3 and 4). Again, in year 35, the average increase in grain yield with diversity for winter small grain cereals was 0.48 t/ha per species diversity unit (95% CI [0.40, 0.56]) at low external N input and 0.41 t/ha per species diversity unit (95% CI [0.34, 0.48]) at high external N input. For spring small grain cereals, the benefit was 0.29 t/ha per species diversity unit (95% CI [0.24, 0.34]) at low external N input and 0.19 t/ha per species diversity unit (95% CI [0.14, 0.23]) at high external N input. Thus overall, increasing CRD had a greater positive effect on grain yield at low external N fertilisation rates (Fig. 2), suggesting other rotation effects, such as enhanced pest regulation or soil water holding capacity, have secondary roles compared with complementary use of resources, particularly for maize.

To further explore the interaction of fertilization rate and CRD, we compared yield benefits from diversified rotations, managed to yield-maximising species diversity or functional richness, with low external N fertilisation, against yield benefits from adding high amounts of N fertiliser to monocultures (Fig. 3). For small

grain cereals in the early years of the experiments, increasing fertiliser input to monocultures produces greater yield benefits compared with diversified rotations with low external N fertilisation (Fig. 3a). However, the high N input monoculture yields remain stable over time and the low N input diversified rotations eventually produce comparable yield benefits (Fig. 3b, c). The combination of diversified rotations and N inputs results in the greatest yield benefits, which indicates a gap in N use when only diversifying, or only increasing external N input. In maize, the difference is small between these management techniques or their combination already after the first five years (Fig. 3). Nevertheless, as with small grain cereals, yields in the monoculture with high external N fertilisation are stable over time and the diversified treatments begin to produce higher yields by 20 years (high N) or 35 years (yield-maximising species diversity with low N) (Fig. 3). This perceived N effect that builds up overtime in diverse rotations could be from increased N supply from soil organic matter, or from crops with different root systems that fill different niches, more effectively scavenge, retain and recycle N, and also by inclusion of nitrogen fixing legumes in the rotation^{18,44,45}. Therefore, if reducing external N fertilisation is a target, e.g., as in the European Union Farm to Fork strategy⁴⁶, a switch from monoculture to carefully managed diverse rotations will reduce the fertiliser use over time as the yield enhancing rotation effect gradually increases. This would reduce yield loss from low soil fertility in the early years of a new rotation. However, if producing the highest yields is the main target then diverse rotations

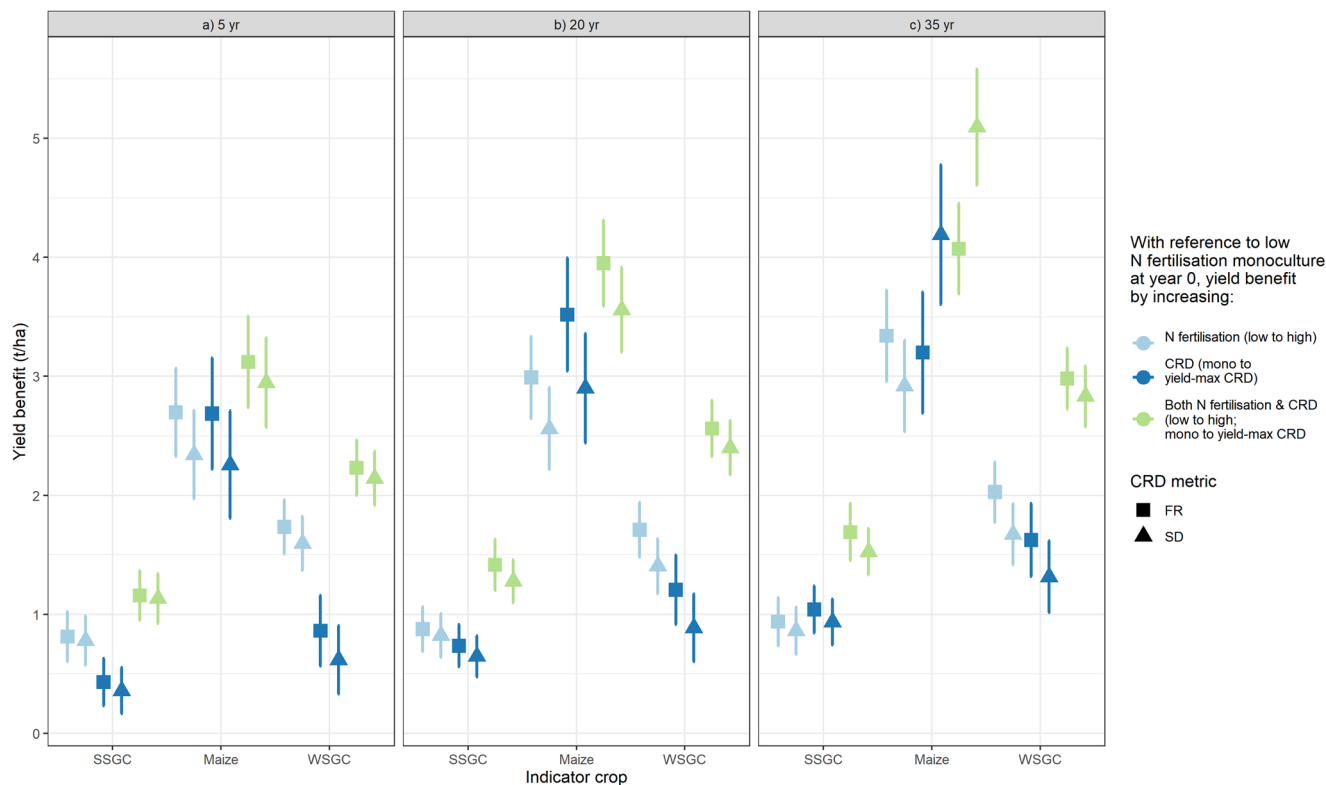


Fig. 3 Comparison of yield benefits from increasing either crop rotational diversity (CRD), external nitrogen (N) fertilisation or both over time. Mean ($\pm 95\%$ CI) yield benefits, with reference to monocultures at year = 0 receiving low external N inputs, from only increasing N fertilisation to high (maintaining monocultures; light blue), only increasing CRD to yield-maximising CRD (maintaining low N fertilisation; dark blue) and increasing both N fertilisation (from low to high) and CRD (from monoculture to yield-maximising CRD; green). The three time periods represent the **a** early-, **b** middle- and **c** long-term effects of time within our range of data. The yield benefit estimates were derived from the fitted models for both CRD metrics, i.e., functional richness (FR; the number of functional groups included in the rotation; squares) and species diversity (SD; based on the inverse Simpson's diversity index; triangles), for each group of indicator crops, i.e., spring small grain cereals (SSGC), maize and winter small grain cereals (WSGC). Predictions were derived from mean-centred observations i.e., the difference from to the long-term within-site average across all CRD treatments, after 5, 20, and 35 years since the beginning of the experiment. Yield-benefit of 0 indicates model predictions remained the same, negative values are yield losses and positive values are yield benefits compared with yields in monocultures at year = 0.

with high external N are superior. This would also reduce the need of land used for crop production by producing a given yield in a smaller area¹⁸, particularly when the other crops in the rotation support a diverse plant-based diet⁴⁷.

Niche complementarity among crop species likely explain part of the yield improvement with increasing CRD given the individual responses of each indicator crop to the different functional groups in the rotations^{28–31}. Several niches and ecological processes below- and above-ground can mediate this effect. For example, differences in functional traits and environmental niches among crops lead to contrasting communities of weeds, herbivorous insects and pathogens, and growing diverse crops in rotation often shifts resource partitioning to support natural enemies to herbivores^{48,49} and breaks life cycles of weeds and pests^{6,7}. Soil microbial activity is often enhanced in diverse rotations, which can also improve suppression of crop pests^{17,50–52}. Roughly half of our experiments are managed similarly across CRD treatments for crop protection, e.g., against weeds and pests (Supplementary Table 1). This probably leads to an underestimation of the positive effects of CRD on crop protection, given that the need for chemical weed control is often reduced with increasing CRD^{14,53}. Crop protection measures and other management practices would need to be treatment-specific in long-term experiments to assess the full benefit of CRD on crop protection and yield. Diversity in root depth and architecture and resource needs among crops is probably also relevant

because nutrient and water uptake become complementary. Soil organic matter accumulates and changes in quality⁵⁴, which affects soil biota throughout the rooting profile^{55,56} with cascading effects on multiple additional processes, such as microbiome interactions in the rhizosphere, decomposition and soil aggregation. Enhanced soil organic carbon content improves soil structure, nutrient stocks, water retention, and ultimately yields^{51,57}. Crop rotational diversity can also increase N availability and use efficiency for all crops in the rotation because crops with different root systems, N needs and uptake dynamics more efficiently scavenge, retain and recycle N over time. For example, the deeper roots of winter wheat are better at reducing N leaching and providing yield benefits to subsequent crops compared with shallower rooted spring wheat⁵⁸. Inclusion of nitrogen fixing legumes in the rotation is also an important factor^{18,44}, at least for maize and winter small grain cereals, along with increased N supply from soil organic matter^{59,60}. However, the relative importance among these different mechanisms is still poorly understood, especially over time, and requires further research^{6,61}.

The effect of niche complementarity on grain yields in diversified rotations could be impacted by the order in which the different crops appear, particularly the crop preceding the gain crop^{62,63}. In our dataset, maize grain yields were mainly preceded by legumes (49% of yield observations) while winter and spring small grain cereals were mostly preceded by other cereals (61%

and 47%, respectively). This could explain why maize has such a strong CRD response, particularly at low external N input¹⁸ and why the CRD effect is slower to build in the small grain cereals (Fig. 3). However, the unbalanced design prevented us from formally testing pre-crop effects.

Intermediate CRD, based on species diversity, leads to the highest yields in small grain cereals. This hump-shaped relation between grain yield and diversity contrasts with results from long-term grassland experiments where biomass yields increased monotonically with diversity²⁷. Most grain farmers have a limited set of crops to choose from, and crop species included at high diversity can be functionally similar to those included at intermediate diversity, thereby reducing positive complementary effects²¹ or possibly causing negative effects, i.e., when pests impact multiple crops from the same functional group^{64,65}. In our experiments where rotations were designed based on local agronomic practices, the highest species diversity mostly had a functional richness of two, few with four, whereas all rotations with the highest functional richness had medium levels of species diversity (Supplementary Fig. 2). A fully crossed design would be needed to bring out the explicit difference between functional and species diversity effects, and define which combination of species and functional groups would lead the maximum possible yield benefit for the indicator crop. Another potential explanation for the hump-shaped pattern is that soil microbial diversity, which underpins many soil functions, have been shown to exhibit a similar pattern with increasing crop diversity³⁴, pointing to the importance of soil functions driving the relationship between crop diversity and yield.

We find high grain yield benefits from CRD despite large variation, e.g., in growing conditions and management across experiments, thereby demonstrating a general trend. Our goal was to test the importance of CRD and not to predict or explain yields in models that account for most of the yield variability. However, the low R^2 values and large confidence intervals from our models indicate that the effects of CRD should be evaluated on a case-by-case basis. In addition, individual farmers would need to assess this yield benefit against other aspects such as market value of the crops included in the more diverse rotation, any additional land required, skills and infrastructure, logistics, soil properties^{44,66}. Crop choice is also important since the investment in agroecosystem fertility, e.g., with fallow or ley, occurs at the cost of grain production. Similarly, here we focused on CRD over time providing indirect benefits for grain yields; however, farmers could increase CRD in space with intercropping to provide more direct benefits, e.g., pest control via push-pull systems^{67,68}. Finally, the relevance of the decline of yield benefits at high CRD for small grain cereals depends on the purpose of optimizing the CRD. The indicator crops we analysed are mainly grown for acquiring carbohydrates. If this is the main aim, it can be argued that low-diversity rotations lead to higher total production than more diverse rotations, despite their lower yield and greater need for curative crop protection, which could also require less land to grow. However, for agriculture to become sustainable it is crucial to consider outcomes from cropping systems beyond cereal yield⁶⁹, a substantial part of which is currently bound for animal feed or biofuels⁷⁰, and alternatively analyse multifunctionality⁷¹ and the total production of energy, proteins and nutrients of the entire rotation matching human dietary needs⁷². If this is done locally, geographically distributed diversification can also overcome food supply chain shortages that arise from climate extremes, pest outbreaks, conflict and global pandemics^{73–76}.

While there will likely be some need to increase total food production with a growing population⁴⁷, the actual production needed depends greatly on the social-ecological context and societal and political decisions regarding energy, diet, plant health

and food waste^{47,69}. Importantly, there is a need to account for externalities associated with heavy use of pesticides and mineral nutrients necessary to maintain yield levels in short rotation cropping^{14,44}. In this context, increasing CRD emerges as a promising practice to support crop yields while reducing societal and environmental costs and easing the pressure of current mono-, bi- or tri-cereal cropping systems on the environment. Beyond these benefits, more diverse crop rotations have been shown to reduce food system vulnerability to stressful weather²⁴ under a changing climate directly, and indirectly by geographically more evenly distributed production of carbohydrates, proteins and nutrients^{19,76}. Given the decadal time scales of reaping the largest benefit from increased CRD, there is an urgency in providing incentives for farmers to adopt CRD practices and supporting them with knowledge and appropriate technologies.

Methods

Long-term experiments. To quantify the relationship between cereal yield and CRD over time, we collected 27,460 rainfed, annual crop yield observations from 32 long-term experiments, located in North America and Europe across a wide geographical and climatic gradient and 957 site-years (Fig. 1, Supplementary Table 1). The selection of experiments was based on two criteria. First, the long-term experiments needed to be designed such that yield information from the same crop species, hereafter indicator crop, was available from at least two rotation treatments with different CRD levels (Supplementary Fig. 3, Supplementary Table 2). As indicator crop, we used maize in North America and winter- and spring-sown small grain cereals in Europe (Fig. 1). Management of the different rotations, e.g., tillage, pesticides and fertilisation, had to be either the same (i.e., not confounded with other management factors) or comparable (e.g., fertiliser or pest control applied as needed) within each site (Supplementary Table 1).

The second selection criteria was that the rotations had to have been in place for a minimum of 10 years. With this duration threshold, all rotation treatments within sites, except Woodside, Scotland, had completed at least two full rotation cycles. In most experiments, indicator crop yield data had been collected each year for periods ranging between 10 and 63 years. Exceptions were El Encin, Spain, and Foggia, Italy, from which yield information was collected every second year. Therefore, the minimum number of indicator crop yield observations from an experiment was seven, even though all experiments had been operating for at least 10 years. We could draw multiple CRD contrasts from some sites, either because the site included multiple experiments or because the experiment included multiple rotations that met our criteria.

The experiments included combinations of fertilisation rates and mineral and/or organic (e.g., slurry, manure) fertilisers in each rotation. To simplify comparisons among sites we defined external N input rates of inorganic and organic fertiliser as “low” for N rates given to the indicator crop that were lower than the local recommendation, and as “high” for N rates equal to or higher than local recommendations based on information provided in site-specific literature (Supplementary Table 1). This resulted in 12 high and 6 low N input sites, and 14 sites with both high and low fertilisation rates. Inorganic fertilisation was included in 15 sites, organic fertilisation in 9 sites, and 8 sites included comparisons of both organic and inorganic fertilisation (Supplementary Table 1).

Crop rotational diversity metrics. We quantified CRD using two metrics, species diversity and functional richness. We calculated species diversity using the inverse of Simpson's diversity index⁷⁷ defined as $SD = 1/\sum p_i^2$, where p_i is the proportion of individuals of each species. The Simpson index is generally used for measuring spatial diversity based on the proportional abundance of species over a unit area. Here we considered species diversity in time and calculated the proportion of years when a given crop species was grown accounting for the temporal rotational species richness and abundance evenness. For example, in a 4-year rotation composed by a maize–maize–soy–winter wheat succession, maize occupies 2/4 of the rotation length, and winter wheat and soy 1/4 each. The species diversity for this site was $1/(p_{maize}^2 + p_{soy}^2 + p_{w.wheat}^2) = 1/((2/4)^2 + (1/4)^2 + (1/4)^2) = 2.7$. The species diversity was 1 for a monoculture and 3 for a three-year rotation with three different crop species making it comparable with other studies using different CRD indices²⁴. We preferred this index over species richness because it encompasses richness and evenness thus penalising rotations that have multiple years of one species grown in a sequence that result in yields being similar to monoculture by the third year (e.g., maize–maize–maize–alfalfa–alfalfa²¹).

For our second metric of CRD, functional richness, FR , we binned each crop species into four functional groups: annual cereal, annual legume, annual broadleaf, and ley, i.e., biennial or perennial grass and/or legume in single or mixed plantings. In this last group we also included the case of two or more years of alfalfa (Supplementary Fig. 1). Therefore, each rotation was given a value for functional richness between one and four discrete classes.

Statistical methods. To account for differences in crop species and growing conditions among sites, we calculated mean-centred yields for each indicator crop species in each site, i.e., taking away the long-term within-site average across all CRD treatments and external N input levels for an indicator crop species for each yield observation (see Supplementary Fig. 3 for distribution of data). All North American sites had maize and all European sites had a winter- and/or spring-sown small grain cereal as indicator crop. We analysed the mean-centred yields from these three indicator crop groups separately given the geographical divide and differences in responses previously found¹⁹.

To explore the relationship between mean-centred yield and CRD over time under contrasting fertilisation, we built Gaussian mixed-effects models (Eqs. 1–5) in the lme4 package version 1.1-2678 in R version 4.1.079. The three fixed terms in the models were: CRD, either as continuous species diversity (Eqs. 1–3) or categorical functional richness (Eqs. 4–5) in separate models, time in years since the start of the experiment and fertilisation level as a categorical variable (high vs. low input). Several model variants were developed with the most complex model including interaction terms CRD x time and CRD x fertilisation and second-order polynomials of species diversity and year. We carried out model selection by dropping each factor or interaction one by one and the model rendering the lowest Akaike Information Criterion⁸⁰ was selected and used to estimate mean-centred yields (see Supplementary Tables 6 and 7 for model selection results). The model selection process thereby resulted in slightly different final models for the indicator crops (Eqs. 1 and 4 for spring small grain cereals, and Eqs. 2 and 5 for maize and Eqs. 3 and 5 for winter small grain cereals) whereby mean-centred yields were modelled as follows:

$$\text{Yield} = \beta_0 + \beta_{SD}SD + \beta_t t + \beta_{SD^2}SD^2 + \beta_{SDt}SDt + \beta_{SD^2t}SD^2t + \beta_{fert_H}fert_H + \beta_{fert_HSD}fert_HSD + \beta_{fert_HSD^2}fert_HSD^2 \quad (1)$$

$$\text{Yield} = \beta_0 + \beta_{SD}SD + \beta_t t + \beta_{SD^2}SD^2 + \beta_{t^2}t^2 + \beta_{SDt}SDt + \beta_{SD^2t}SD^2t + \beta_{SD^2t^2}SD^2t^2 + \beta_{fert_H}fert_H + \beta_{fert_HSD}fert_HSD + \beta_{fert_HSD^2}fert_HSD^2 \quad (2)$$

$$\text{Yield} = \beta_0 + \beta_{SD}SD + \beta_t t + \beta_{SD^2}SD^2 + \beta_{t^2}t^2 + \beta_{SDt}SDt + \beta_{SD^2t}SD^2t + \beta_{SD^2t^2}SD^2t^2 + \beta_{fert_H}fert_H + \beta_{fert_HSD}fert_HSD + \beta_{fert_HSD^2}fert_HSD^2 \quad (3)$$

$$\text{Yield} = \beta_0 + \sum_{i=2}^4 \beta_{FR_i}FR_i + \beta_t t + \sum_{i=2}^4 \beta_{FR_i t}FR_i t + \beta_{fert_H}fert_H + \sum_{i=2}^4 \beta_{fert_H FR_i}fert_H FR_i \quad (4)$$

$$\text{Yield} = \beta_0 + \sum_{i=2}^3 \beta_{FR_i}FR_i + \beta_t t + \sum_{i=2}^3 \beta_{FR_i t}FR_i t + \beta_{t^2}t^2 + \sum_{i=2}^3 \beta_{FR_i t^2}FR_i t^2 + \beta_{fert_H}fert_H + \sum_{i=2}^3 \beta_{fert_H FR_i}fert_H FR_i \quad (5)$$

where t is the time in years from the beginning of the experiments, $fert_H$ = high fertilisation, SD is species diversity (Eqs. 1–3) and FR_i is functional richness (Eqs. 4–5).

To assess the effect of including a specific crop functional group in the rotation, we ran a separate model using binomial variables indicating the presence of ley, annual legume, and annual broadleaf as fixed terms, excluding interactions with experiment duration. The same model (Eq. 6) was used for all indicator crops where mean-centred yields were modelled as follows:

$$\text{Yield} = \beta_0 + \beta_{leg_Y}leg_Y + \beta_{ley_Y}ley_Y + \beta_{bl_Y}bl_Y \quad (6)$$

where leg_Y is the presence of legumes, ley_Y is the presence of ley and bl_Y is the presence of broadleaf in the rotation.

Several experiments had a factorial design with CRD crossed with management. In these experiments, yield data were available for at least two CRD levels for each management treatment, e.g., tillage. To make best use of the data, we grouped yield information from each site-specific CRD level that was managed in similar ways, e.g., subject to the same tillage, with a dummy variable indicating management group. We included this dummy variable as random effect nested within site in all statistical models listed above. We also included calendar year as a categorical random effect in our models to account for variation due to technological advances over time. We tested model performance for each model by checking residuals and diagnostics with the DHARMA package in R⁸¹, which tests for over- and under-dispersion of residuals, heteroscedasticity and general model fit. We also plotted the observations grouped by site and CRD level over time and external N input to test whether our models were robust to the differences in precision (Supplementary Fig. 3). All models presented here passed these checks. The relatively low explanatory power of our models (low R^2 values, Supplementary Tables 3, 4 and 5) were not unexpected given the large range in geography, management and CRD used in our study. Yield estimates were calculated using the emmeans package (version 1.6.2-1⁸²) and ggeffects package (version 1.1.1) for plots⁸³. We used map data associated with the package rnaturlaerth (version 0.1)⁸⁴ to produce Fig. 1 and all plots were created using ggplot2 package⁸⁵.

Data availability

We have submitted all mean-centred yields, metadata and crop rotation information to the Swedish National Data service (<https://doi.org/10.5878/8af1-0q60>).

Code availability

We published the R code along with the data under ‘associated documentation’ at the Swedish National Data service (<https://doi.org/10.5878/8af1-0q60>).

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Author contributions

R.B. and G.V. discussed the conceptualisation of the study. M.E.S. and A.C. collated and analysed data and produced figures. R.B., G.V., M.E.S., A.C., T.B., A.C.M.G., S.H. and C.A.W. discussed and interpreted results. All remaining authors, R.A., A. Berti, A. Blecharczyk, F.J.C., S.C., W.D., C.F.D., A.G.G., A.G.D., E.H.P., K.J., O.J., R.M.L., F. Montemurro, F. Morari, A.O., S.L.O., J.L.T.P., B.S., I.S.M., Z.S., M.R.S., J. Stalenga, J. Strook, F.T., C.F.E.T, D.V. and R.L.W., managed the long-term experiments and contributed data. M.E.S. and R.B. wrote the manuscript. All authors contributed to revisions and approved this submission.

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Competing interests

The authors declare no competing interests.

Additional information

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Correspondence and requests for materials should be addressed to Riccardo Bommarco.

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¹Swedish University of Agricultural Sciences, Department of Ecology, SE 75007 Uppsala, Sweden. ²Swedish University of Agricultural Sciences, Department of Crop Production Ecology, SE 75007 Uppsala, Sweden. ³University of California Berkeley, Department of Environmental Science, Policy, and Management, Berkeley, CA 94720, USA. ⁴University of California Davis, Department of Plant Sciences, Davis, CA 95616, USA. ⁵Swedish University of Agricultural Sciences, Department of Forest Mycology and Plant Pathology, SE 75007 Uppsala, Sweden. ⁶Scotland's Rural College, Aberdeen UK AB21 9YA, UK. ⁷Madrid Institute for Rural, Agricultural and Food Research and Development, Agro-environmental Department, ES 28800 Alcalá de Henares, Spain. ⁸University of Padova, Department of Agronomy, Food, Natural resources, Animals and Environment, IT 35020 Padova, Italy. ⁹Poznań University of Life Sciences, Department of Agronomy, PL 60-632 Poznań, Poland. ¹⁰Oregon State University, Columbia Basin Agricultural Research Center, Adams, OR 97801, USA. ¹¹Ohio State University, School of Environment and Natural Resources, Wooster, OH 44691, USA. ¹²University of Guelph, Department of Plant Agriculture, Guelph, ON N1G 2W1, Canada. ¹³Agriculture & Agri-Food Canada, Harrow Research and Development Centre, Harrow, ON N0R 1G0, Canada. ¹⁴University of Minnesota, Department of Agronomy and Plant Genetics at the Southwest Research and Outreach Center, Lamberton, MN 56152, USA. ¹⁵Madrid Institute for Rural, Agricultural and Food Research and Development, Applied Research Department, ES 28800 Alcalá de Henares, Spain. ¹⁶National Institute for Agricultural and Food Research and Technology, Spanish National Research Council (INIA-CSIC), Department of Plant Protection, ES 28040 Madrid, Spain. ¹⁷Institute of Soil Science and Plant Cultivation - State Research Institute, Department of Systems and Economics of Crop Production, PL 24-100 Puławy, Poland. ¹⁸USDA-ARS, North Central Agricultural Research Laboratory, Brookings, SD 57006, USA. ¹⁹Council for Agricultural Research and Agricultural Economy Analysis, Research Centre for Agriculture and Environment (CREA-AA), IT 70125 Bari, Italy. ²⁰University of Perugia, Department of Agricultural, Food and Environmental Sciences, IT 06121 Perugia, Italy. ²¹Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria, INIA-CSIC, Environment and Agronomy Department, ES 28040 Madrid, Spain. ²²Swedish University of Agricultural Sciences, Department of Agricultural Research for Northern Sweden, SE 90432 Umeå, Sweden. ²³USDA-ARS, Agroecosystem Management Research Unit, Lincoln, NE 68583, USA. ²⁴University of Minnesota, Department of Soil, Water, and Climate at the Southwest Research and Outreach Center, Lamberton, MN 56152, USA. ²⁵Scotland's Rural College, Edinburgh UK EH9 3JG, UK.

✉email: Riccardo.Bommarco@slu.se