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Diversifying crop rotations for sustainable production and climate change adaptation

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Abstract

Industrial cropping systems are increasingly simplified, with fewer crops being grown, requiring extensive use of inputs, and contributing to environmental pollution and climate change. Solutions are needed to reduce the negative effects, while retaining productivity in the face of increasingly frequent detrimental climatic conditions. Crop rotational diversity has shown promises to increase staple cereal yields, especially under low fertilisation regimes and years of low productivity. However, it is unclear how different levels and types of crop rotational diversity mediate the interaction between productivity, contrasting levels of fertilisation, and explicit climatic conditions. Moreover, crop diversification substitutes staple cereals with alternative crop types, e.g., legumes, broadleaves, and perennial mixtures of grasses and legumes, raising the question whether sufficient and sufficiently nutritious food can be produced with less nitrogen inputs in diverse rotations. Using data from 32 long-term field experiments (10-64 years) from Europe and North America, I show that crop rotational diversity, expressed as species diversity or functional richness, enhances cereal yields over time, particularly under regimes of low fertilisation. I also show that crop rotational diversity can reduce cereal yield losses caused by detrimental climatic conditions. Using a sub-set of the European data, I also show that increasing functional richness can increase the outputs of human-available calories and macronutrients, i.e., carbohydrates, proteins, and fats, with increasing benefits over time, and requiring less nitrogen than cereal-only rotations. In summary, crop rotational diversity can benefit food security and sustainability, and provide climate adaptation to cropping systems.

Keywords: Sustainable crop production, climate change adaptation, nitrogen use efficiency, crop diversification, crop rotation, long-term experiments

Diversifiering av växtföljder för hållbar produktion av kalorier och makronäringsämnen och anpassning till klimatförändringar

Sammanfattning

Industriella odlingssystem blir alltmer förenklade, med färre grödor som odlas, kräver omfattande användning av insatsvaror och bidrar till miljöföroreningar och klimatförändringar. Lösningar behövs för att minska de negativa effekterna och samtidigt bibehålla produktiviteten trots allt oftare förekommande ogynnsamma klimatförhållanden. Diversitet i växtföljden har visat sig kunna öka avkastningen på spannmål, särskilt vid låga gödslingsnivåer och under år med låg produktivitet. Det är dock oklart hur olika nivåer och typer av diversitet i växtföljden påverkar samspelet mellan produktivitet, gödslingsnivåer och tydliga klimatförhållanden. Dessutom ersätter diversifiering av grödor spannmål med alternativa grödtyper, t.ex. baljväxter, oljeväxter, rotfrukter och fleråriga vallar, vilket väcker frågan om tillräcklig och tillräckligt näringsrik mat kan produceras med mindre kvävegödsel i olika växtföljder. Med hjälp av data från 32 långliggande fältförsök (10-64 år) från Europa och Nordamerika visar jag att diversifierade växtföljder, uttryckt som artdiversitet eller funktionell rikedom, ökar spannmålsskörden över tid, speciellt om gödselgivan var låg. Jag visar också att diversifiering av växtföljden kan minska skördeförlusten av spannmål under ogynnsamma klimatförhållanden. Från en del av den Europeiska data visar jag också att ökad funktionell rikedom kan öka produktionen av kalorier och makronäringsämnen tillgängliga för humankonsumtion, dvs. kolhydrater, proteiner och fetter, med ökande fördelar över tid, och kräver mindre kvävegödsel än växtföljder med endast spannmål. Sammanfattningsvis kan diversitet i växtföljden gynna livsmedelssäkerhet och hållbarhet och göra odlingssystemet mer klimatanpassat.

Nyckelord: Hållbar växtproduktion, anpassning till klimatförändringar, kväveanvändningseffektivitet, diversifiering av grödor, växtföljd, långsiktiga experiment

Dedication

To Afifa

Contents

List of publications

This thesis is based on the work contained in the following papers, referred to by Roman numerals in the text:

I. Smith M, Vico G, Costa A, Bowles T, Gaudin ACM, Hallin S, Watson CA, Alarcòn R, Berti A, Blecharczyk A, Calderon FJ, Culman S, Deen W, Drury CF, Garcia y Garcia A, García-Díaz A, Hernández Plaza E, Jonczyk K, Jäck O, Lehman RM, Montemurro F, Morari F, Onofri A, Osborne SL, Tenorio Pasamón JL, Sandström B, Santín-Montanyá I, Sawinska Z, Schmer MR, Stalenga J, Strock J, Tei F, Topp CFE, Ventrella D, Walker RL, Bommarco R (2023). Increasing crop rotational diversity can enhance cereal yields. Communications Earth and Environment, 4 (1), pp. 4-9. https://doi.org/10.1038/s43247-023-00746-0

II. Costa A, Bommarco R, Smith ME, Bowles T, Gaudin ACM, Watson CA, Blecharczyk A, Garcia y Garcia A, Jonczyk K, Mazzon M, Marzadori C, Montemurro F, Onofri A, Tenorio Pasamón JL, Sandström B, Santín-Montanyá I, Sawinska Z, Stalenga J, Tei F, Topp CFE, Ventrella D, Walker RL, Vico G. Functionally rich rotations can produce nitrogen-efficient calories and macronutrients (manuscript)

III. Costa A, Bommarco R, Smith ME, Bowles T, Gaudin ACM, Watson CA, Alarcòn R, Berti A, Blecharczyk A, Calderon FJ, Culman S, Deen W, Drury CF, Garcia y Garcia A, García-Díaz A, Hernández Plaza E, Jonczyk K, Jäck O, Navarrete Martinez L, Montemurro F, Morari F, Onofri A, Osborne SL, Tenorio Pasamón JL, Sandström B, Santín-Montanyá I, Sawinska Z, Schmer MR, Stalenga J, Strock J, Tei F, Topp CFE, Ventrella D, Walker RL,Vico G. Crop rotational diversity can mitigate climate-induced grain yield losses (submitted manuscript)

IV. Costa A, Bommarco R, Smith ME, Bowles T, Gaudin ACM, Watson CA, Berti A, Blecharczyk A, Garcia y Garcia A, Jonczyk K, Mazzon M, Marzadori C, Montemurro F, Morari F, Onofri A, Tenorio Pasamón JT, Sandström B, Santín-Montanyá I, Sawinska Z, Stalenga J, Tei F, Topp CFE, Ventrella D, Walker RL, Vico G. Functionally rich crop rotations increase calorie and macronutrient outputs (manuscript)

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The contribution of Alessio Costa to the papers included in this thesis was as follows:

I. Third author. AC contributed to building the hypotheses together with MES, GV, and RB. AC acquired part of the data together with MES. AC and MES collated the data. AC developed the statistical model and model selection together with MES, GV, and RB. AC contributed to the interpretation of results and writing of the paper. All co-authors contributed to the final version of the manuscript.

II. Main author. AC conceived the idea together with GV. AC and MES acquired and collated the data. AC and GV developed the statistical model and analysed the data. AC interpreted the results together with GV, RB and MES. AC and GV wrote the manuscript supported by RB and MES.

III. Main author. AC conceived the idea together with GV. AC, MES and GV acquired the data. AC and MES collated the data. AC developed the statistical model with GV. AC analysed the data and interpreted the results with GV and RB. AC led the writing supported by GV, RB, and MES. All co-authors contributed to the final version of the manuscript.

IV. Main author. AC conceived the idea together with GV and RB. AC and MES acquired and collated the data. AC developed the statistical model. AC analysed the data together with GV. AC interpreted the results together with GV, RB and MES. AC led the writing supported by GV, supported by RB, and MES. All co-authors contributed to the final version of the manuscript.

Abbreviations

LTE	Long-Term Experiment
N	Nitrogen
N_{tot}	Total Nitrogen inputs

1. Introduction

Cropping systems face multiple challenges that ultimately threaten food production. Loss of biodiversity, soil degradation and climate change can all negatively impact crop production, causing increasingly frequently yield stagnation and declines in major cropping regions (Bennett et al., 2012). At the same time, industrial crop production contributes to these environmental challenges through intensive use of polluting inputs and practices to produce high yields (Campbell et al., 2017). To continue feeding a growing human population and reduce rising hunger and malnutrition in a sustainable way, we need cropping systems that nurture soil health and promote climate-change resilience, while keeping high levels of production and providing nutritious food (Bommarco et al., 2013; Kremen & Merenlender, 2018). Improving human nutrition and promoting sustainable and climate-change adapted agriculture are part of the UN 2030 agenda for Sustainable Development (UN DESA, 2023). It remains to be explored to which extent sustainability and climate change adaptation can be promoted in cropping systems without sacrificing productivity.

1.1 Environmental impacts of industrialized cropping systems

Following the Green revolution, in many regions, cropping systems transitioned from growing diverse crops in rotations, to simplified and industrialized ecosystems where one or few crops are grown, such as monocultures or short rotations. Industrial crop production has mostly specialized on growing few selected species, with a focus on cereals (Bennett et al., 2012; Crossley et al., 2021; Schaak et al., 2023). Cereals like rice and wheat have been staples in human diets for millennia, are easy to store and have long shelf-life (Cassman & Grassini, 2020). Specializing on few cereals reduces costs of production, logistic and storage (de Roest et al., 2018; Magrini et al., 2018). However, to produce high yields, these systems need the support of substantial inputs, such as pesticides, herbicides and fertilisers. These negatively affect the environment by causing biodiversity loss, increasing greenhouse gas emissions, polluting water, and degrading soils (Campbell et al., 2017).

Among the inputs, fertilisers promote soil nutrients that are used by the crops for their primary processes, such as photosynthesis and respiration. However, a large portion of fertilisers is lost through percolation and surface runoff (Liu et al., 2010). The unexploited fertilisers constitute an unnecessary economic loss to farmers and pollute water sources (EEA, 2022). Further, synthetic nitrogen (N) fertiliser inputs exacerbate climate change, by emitting N_2O in the field, and CO_2 during their production and distribution (Menegat et al., 2022). As N is a fundamental nutrient for most plant processes, such as photosynthesis, efforts have been made to improve the capacity of crop varieties to efficiently acquire and utilize N to produce high yields. Despite such developments, the amount of global harvested plant protein, measured in kg N, per unit total N has diminished from 68% to 45% between 1960 and 2010 (Lassaletta et al., 2014). Aiming to reduce the waste of N and thus its environmental impact, the European Union has proposed to reduce fertilizer use by at least 20% by 2030 (European Commission, 2020). Following a modelling analysis, concerns were raised that such transition could reduce cropping system productivity and increase food insecurity (Beckman et al., 2020). However, the analysis assumed cropping system management to remain constant. It remains to be established whether changes in cropping system management could maintain productivity while lowering fertiliser use.

Intensively managing few crop species favours organisms that benefit from the managed species, such as pests and pathogens, or that benefit from the environmental conditions created, such as weeds (Bennett et al., 2012; Mahaut et al., 2019). At the same time, there is a loss of organisms that can benefit the cropping systems through various ecosystem functions (Cardinale et al., 2012; Kremen & Merenlender, 2018). Examples of beneficial ecosystem functions in cropping systems are breaking down crop residues and recycling soil nutrients, and producing stable soil organic matter, which improve the capacity of soils to store and supply water and nutrients (Kremen

& Miles, 2012). Thus, restoring biodiversity in cropping systems could reduce the need to fertilize (Kremen & Merenlender, 2018). However, arguments are raised that promoting diversified cropping systems might require further agricultural expansion to sustain human population, to the detriment of natural environments (Cassman & Grassini, 2020; Phalan et al., 2011). There is evidence that diversified cropping systems can have higher productivity than industrial ones, although it is context-dependent (Jones et al., 2023; Tamburini et al., 2020). There is a need to empirically determine whether diverse cropping systems can promote sustainability while maintaining productivity at a large scale.

1.2 Growing malnutrition

Despite the global mass-production and distribution of staple crops, millions of people are still malnourished (FAO et al., 2022). The global mass production of cereals enabled high accessibility to a relatively inexpensive source of carbohydrate-rich calories. But many of the cereals we grow are used to produce meat and biofuels, resulting in huge loss of human-available nutrients (Shepon et al., 2016). Further, cereals and meat alone cannot provide all nutrients that are essential for human well-being. Thus, replacing some of the cereals we grow with alternative crops such as legumes and root crops could increase accessibility to more nutrient-balanced diets (Dwivedi et al., 2017). However, alternative crops are less marketable, have higher costs of production and distribution, and are thus less accessible and more expensive than cereals globally (Magrini et al., 2016, 2018). Such constrains could be countered by, e.g., governmental subsidies in favour of diversified cropping systems, but it remains to be empirically established whether pairing cereals with alternative crops provides a higher and more balanced set of human-accessible nutrients.

1.3 Climate change threatens crop production

Combinations of temperature, light, water, and nutrient availability determine photosynthesis, growth, and investment in reproduction organs, and ultimately marketable crop yields. Crop yields are thus substantially impacted by variation in climatic conditions and their combinations (Jägermeyr et al., 2021; Ray et al., 2015). Ongoing changes in the climate results in conditions that threaten crop productivity and food price stability globally (Porter et al., 2014). Warming can lead to temperatures beyond the crop optima, reducing growth and seed production (Eyshi Rezaei et al., 2015; Hatfield & Prueger, 2015). Warming also increases soil evaporation and crop transpiration rates, and hence reduce water availability. Scanter precipitation, and more frequent and longer dry spells also reduce water availability. Even detrimental conditions of short duration can be problematic, but these effects tend to be masked when averaging conditions over long time periods (Troy et al., 2015). Further, combined disturbances such as heat and drought are particularly detrimental to crop yields (Luan et al., 2021; Vogel et al., 2019). It is thus critical to adapt cropping systems to climate change, so that climate-induced productivity losses are mitigated (Challinor et al., 2014; Khanal et al., 2021).

Specialized industrial cropping systems such as monocultures and short rotations are particularly vulnerable to disturbances and changes in climatic conditions. A limited crop portfolio is similarly affected by the same environmental conditions (Cadotte et al., 2011; Schindler et al., 2015), and thus most of the harvest can be lost if the climatic conditions are unfavourable for those crops. Moreover, industrial cropping system soils have a limited water and nutrient retention, which could otherwise hamper crop heat and water stress during unfavourable climatic conditions (Renwick et al., 2019; Sadok et al., 2021). Conversely, promoting crop diversity provides insurance against disturbances through richer crop portfolios, and water and nutrient retention through enhanced soil biophysical conditions (Schindler et al., 2015; Schmer et al., 2020; Sprunger et al., 2020). Yet, it is poorly understood how crop diversity mediates the response of crop yields to shifts in climatic conditions.

1.4 Potential benefits of crop diversity

Promoting crop diversity in cropping systems can be achieved in time, e.g., growing two or more crop species in the same field in sequence over a number of growing seasons in rotation, or in space, e.g., simultaneously growing two or more crop species sharing the same field, i.e., intercropping. While both intercropping and crop rotations promote biodiversity in cropping systems, in intercropping crop species are grown simultaneously and can directly compete with, facilitate, or complement each other over resource acquisition (Brooker et al., 2015). If the same crop mixture is repeated year after year, intercropping has limited benefits regarding reducing pest pressure. Rotating crop species can interrupt pest life cycles and reduce host availability over time (Bennett et al., 2012). In crop rotations, crop species do not directly compete or facilitate each other because they are grown separately over time. Crops in rotation can instead indirectly benefit or detriment the following crop (Peltonen-Sainio et al., 2011). If well planned, crop rotations can promote various ecosystem services that benefit the soil and crop productivity over time (Kremen & Miles, 2012).

The theory behind the benefits of crop diversification is based on the principle that plant diversity promotes ecosystem functions such as primary production and nutrient cycling (Hector et al., 1999; Loreau et al., 2001). Plant diversity can be expressed, e.g., as the number of plant species and their relative abundance in an ecosystem, i.e., species diversity, or as the number functional types, i.e., groups of plant species that acquire resources and respond to the environment in similar ways (Tilman et al., 1997). The mechanisms behind the benefits of plant diversity to primary production are tied to a combination of selection and niche-complementarity effects. The selection effect implies that by increasing the pool of available plant species, it is more likely to include plant species that have high productivity, or that benefit the productivity of other species (Loreau et al., 2001). Niche complementarity occurs when plant species in a defined environment have life cycles and traits that differ in expression over time, such as period of activity during certain time of the day or of the year, or over space, such as rooting depths (Postma & Lynch, 2012; Thorup-Kristensen et al., 2009). Increasing the number of functional types, i.e., functional richness, thus more directly promotes niche complementarity than species diversity. However, it is poorly understood how species diversity and functional richness in crop rotations modulate productivity in cropping systems. Evidence from field experiments in Europe showed that diverse crop rotations increase small grain cereal yields compared with monocultures (Marini et al., 2020). Yet, it is unclear how different levels of diversity in crop rotation affect different types of cereal yield.

In North America, 11 long-term experiments indicated that increasing species diversity in rotation can increase maize yield with improved benefits over time (Bowles et al., 2020), although the mechanisms driving such relationship are still poorly understood. Meta-analyses showed that crop rotation foster the capacity of soils to store nutrient and water, recycle nutrients, and increase N availability through leguminous crops, building up a legacy that can benefit harvests over time (Gardner & Drinkwater, 2009; McDaniel et al., 2014). We have yet to understand whether increasing crop species diversity, functional richness, or the addition of specific crops bring the largest benefits to yields, and how such benefits change over time.

Enhanced crop rotational diversity could also reduce the need to fertilise cropping systems, while retaining high productivity. The legacy effects of crop rotations, combined with selection and niche-complementarity effects of crop diversity, likely explain why increasing species diversity in crop rotations enhanced yields particularly under low N fertilisation regimes, as reported by a meta-analysis of European and African long-term experiments (MacLaren et al., 2022). Increasing functional richness in crop rotation promote diversity in rooting strategies and resource acquisition (Bardgett 2014, Griffith 2022). This allows a higher soil microbial activity, which foster nutrient recycling (Wang 2022, Zhang 2021), and a crop N uptake that is better distributed across soil layers and growing season (Duchene et al., 2020; Griffiths et al., 2022). These effects can potentially increase N acquisition and retention at the rotation level, i.e., considering all crop outputs and all N input per rotation cycle. It remains an open question how functional richness affects cereal productivity under contrasting fertilisation regimes. Further, it is unclear how crop diversity can mediate the relationship between productivity and explicit variations of N inputs at the rotation level.

Diversifying crop rotations also show promises to promote climate adaptation in cropping systems, but there is a lack of evidence that simultaneously and explicitly consider variation in crop diversity and climatic conditions. Crop rotations have higher productivity than monocultures under warm and dry conditions (Marini et al., 2020), but it is unclear how increasing crop rotational diversity, either as species diversity or functional richness, modulates yield response to climatic conditions. Long-term experiments in North America showed that increasing crop rotational diversity benefits cereal yields in particular under years of low productivity (Bowles et al., 2020), although climatic conditions were not explicit. The benefits of crop diversity to soil nutrient and water retention, combined with a diversity in response to environmental condition (Kremen & Miles, 2012; Loreau et al., 2021) are plausible mechanisms behind these findings. The increased nutrient and water availability benefits crop productivity, and reduces heat and water stress during adverse climatic conditions (Renwick et al., 2019; Sadok et al., 2021). Understanding how crop rotational diversity can prevent crop yield losses under explicit climatic conditions is important to foster climate change adaptation in cropping systems.

Despite evidence that diversified rotations can enhance cereal yields (Bowles et al., 2020; MacLaren et al., 2022), it is argued that diversified cropping systems cannot meet global food demands, as fewer staple cereals will be grown. Staple cereals are richer in carbohydrates and calories than most other crops, and reducing their global production could lead to further agricultural expansion to meet current food demands (Cassman & Grassini, 2020). Compared with cereals, other crops such as legumes and oil crops can provide more protein and/or fats, which are also essential for human nutritional needs. Yet, the nutritional output of all crops in the rotation is rarely assessed. In a long-term experiment in North America, diverse crop rotations produced less calories than a maize monoculture (Sanford et al., 2021). However, calories alone are not sufficient to meet nutritional needs. A balanced intake of macronutrients, i.e., carbohydrates, proteins, and fats, is needed (Dwivedi et al., 2017). It is unclear whether the combined yield of all crops in diversified rotations can produce more human-available nutritional outputs than cropping systems where only cereals are grown, how the outputs change as crop diversity is increased, and how the diversity-output relationship changes over time.

1.5 Knowledge gaps & main questions of the thesis

The overarching objective of this thesis is to explore how crop rotational diversity affects productivity at the crop and rotation level, and whether it can reduce the need for fertilizer and promote climate change adaptation, and if so, how. The main questions framing this thesis are:

1) How do different levels of crop rotational diversity modulate cereal yields over time, under contrasting fertilisation regimes? (**Paper I**)
2) How does increasing functional richness in crop rotations affect the relationship between crop calorie, carbohydrate, protein, and fat outputs and N inputs at the rotation level over time? (**Paper II**)
3) How does increasing crop rotational diversity affect the response of cereal yields to climatic conditions? (**Paper III**)
4) How does increasing functional richness modulate calorie and macronutrient outputs at the rotation level over time? (**Paper IV**)

2. Methods

2.1 Long-term experiments

To explore effects of crop diversity on cereal yields and rotation-level outputs over time (**Paper I-IV)**, we gathered historical yield data from 23 European and 11 North American long-term experiments (LTEs) - (Table 1). Each LTE is unique in its design and duration, but contains at least two rotations of contrasting crop species number, and at least 10 years of data, to ensure a minimum of two full rotation cycles for each rotation. All rotations include at least a cereal, because they are staple components in global human diets and hence often investigated in long term experiments. Cereals are winter or spring small grains, or maize, depending on the location. Other treatments, e.g., rates of fertilisation, tillage intensity, are either fully crossed among the rotations or comparable, e.g., fertiliser and pesticides applied as needed.

For **Paper I** and **Paper III**, we used the entire dataset except for two European sites, which were not available at the time of the analyses (Figure 1 and Supplementary Information (SI), Supplementary Table 1, **Paper I**; SI, Table S1, **Paper III**). For **Paper II** and **Paper IV**, we focused on the European LTEs, using data from 15 and 17 sites, respectively, in which all crops in each rotation treatment were grown in the same year (**Paper II**, SI, Table S1, and **Paper IV**, SI, Table S5 and Figure S5). The 15 out of 17 sites used in **Paper II** and **Paper IV** coincide, and the two remaining LTEs were not used in **Paper II** because they strongly affected the outcome of the analysis in one case, and generated data unbalance in the other.

Table 1 Site location per region and country. Range of functional richness (i.e., the number of functional groups present in a rotation) indicates the range between the lowest and the highest functional richness within the experiment; 1M indicates a cereal monoculture, and 1C a cereal-only rotation. Range of species diversity, measuring of crop rotational diversity based on the inverse Simpson's diversity index, indicates the range between the lowest and the highest rotational species diversity within the experiment. Fertilisation treatment indicates whether fertilisation treatments, e.g., contrasting rates of fertilisation, were present in the experiment

Region	**Country (State for United States)**	**Site (code)**	**Range of functional richness**	**Range of species diversity**	**Fertilisation treatment (yes/no)**	**Used in paper**
Europe	England	Broadbalk (BB)	1M – 1C	1 – 2.27	Yes	I, II, III, IV
Europe	England	Woburn (Wo)	2 – 2	2.27 – 5	Yes	I, III
Europe	Italy	Bologna (B)	1M – 1C	1 – 2	Yes	II, IV
Europe	Italy	Foggia (Fo)	1M – 2	1 –2	No	I, III
Europe	Italy	Padova (Pa)	1M – 3	1 – 3.6	Yes	I, III, IV
Europe	Italy	Perugia (Pe)	1M – 3	1 – 2	No	I, II, III, IV
Europe	Poland	Brody (Brd)	1M – 3	1 – 4.08	Yes	I, II, III, IV
Europe	Poland	Grabow (GO)	2 – 3	4.17 – 5	No	I, II, III, IV
Europe	Poland	Osiny (Os)	1M – 2	1 – 3	No	I, II, III, IV
Europe	Scotland	Tulloch Early (TuE)	2 – 3	1.8 – 2.57	No	I, II, III, IV
Europe	Scotland	Tulloch Late (TuL)	3 – 4	2.88 – 6	No	I, III, IV

Europe	Scotland	Woodland (W)	3 – 3	3 – 4.57	No	II, IV
Europe	Scotland	Woodside (WS)	3 – 3	2.57 – 4	No	I, II, III, IV
Europe	Spain	El Encin (EE)	1 – 2	1 – 2	No	I, III
Europe	Spain	La Canaleja (LCa)	1M – 2	4.5 – 6	No	I, II, III, IV
Europe	Sweden	Lanna Ley (LL)	2 – 3	4.5 –6	Yes	I, II, III, IV
Europe	Sweden	Lännäs LTE (LLTE)	2 – 4	1.38 – 3.38	No	I, III
Europe	Sweden	Ojebyn (Oj)	1M – 4	1 – 4.8	Yes	I, III
Europe	Sweden	Robacksdalen (Rb)	1M – 4	1 – 4.8	Yes	I, II, III, IV
Europe	Sweden	Stenstugu Ley (St)	2 – 3	4.5 –6	Yes	I, II, III, IV
Europe	Sweden	Säby Ley (SL)	2 – 3	4.5 –6	Yes	I, II, III, IV
Europe	Sweden	Säby LTE (SLTE)	1M – 2	1 – 6	Yes	I, II, III, IV
Europe	Sweden	Ås (As)	1M – 4	1 – 4.8	Yes	I, III
North America	Canada	Elora (El)	1M – 2	1 – 2.91	No	I, III
North America	Canada	Woodslee (WoSl)	1M – 2	1 – 2.67	Yes	I, III
North America	United States (CO)	Akron (Ak)	1C – 2	2.67 – 4	No	I, III
North America	United States (MD)	Beltsville (Bv)	2 – 3	3.13 – 4.5	No	I, III

North America	United States (SD)	Brookings (Br)	2 – 3	2 – 4	No	I, III
North America	United States (MI)	Hickory Corners (HC)	1M – 2	1 – 4.26	No	I, III
North America	United States (OH)	Hoytville (Hv)	1M – 3	1 – 3	No	I, III
North America	United States (MN)	Lamberton (La)	2 – 3	2 – 3.56	Yes	I, III
North America	United States (NE)	Mead (Me)	1M – 2	1 – 4.57	Yes	I, III
North America	United States (PA)	Rock Springs (RS)	1M – 2	1 – 4.57	Yes	I, III
North America	United States (OH)	Wooster (Wst)	1M – 2	1 – 3	No	I, III

2.2 Crop diversity indices

To quantify crop rotational diversity, we used two indices: species diversity, and functional richness.

Our species diversity index is based on Simpson's reciprocal diversity index (Simpson, 1949), which is generally used to describe the number of species (species richness), and their relative abundance in a set space. We modified the index to capture the time (years), instead of space, in which a species is grown. Since crop diversity in crop rotations is expressed over time, i.e., crop species are rotated over a defined number of growing seasons, we accounted for the crop species richness and their relative abundance over the length of the rotation, i.e., the number of growing seasons required to complete a rotation cycle (see Methods, **Paper I** and **Paper III**).

To measure crop diversity based on niche differentiation, we used functional richness (FR), a count of crop functional types in the rotation, as a more appropriate alternative to species diversity (Cadotte et al., 2011). We used four crop functional types: cereals, annual legumes, broadleaves (*Brassicaceae* and *Solanaceae*), and perennial leys (mixture of perennial grasses and legumes). Differentiating broadleaves into oil and root crops was unfeasible because it generated a level of functional richness with too few data points. The functional types differ in management practices, e.g., rate and timing of fertilisation, in resource acquisition strategies, e.g., length of roots and periods of activity, and in ecosystem functions, e.g., nitrogen fixation for annual legumes, prolonged soil cover for perennial leys. Cereal-only rotations and monocultures represent the lowest level of functional richness. As such, pairing one or more cereal species with one or more functional types increases functional richness. For example, a functional richness of 2 can be reached by pairing cereal species with legumes, and a functional richness of 3 indicates cereals paired with two additional functional types.

2.3 Handling of crop yield data

As indicator of cropping system performance, we used marketable yield data, measured as dry matter, for **Paper I** and **Paper III**, or the outputs of entire rotations, measured as calories, carbohydrates, proteins, or fats, for **Paper II** and **Paper IV** (Figure 1).

2.3.1 Cereal yields

For **Paper I** and **Paper III**, we focused on cereals. Different LTE had different cereals, based on local conditions and practices. We subset the cereals into maize, spring small grain cereal, and winter small grain cereal yields, as their yield ranges and response to environment differ substantially due to physiological and phenological differences (Fan et al., 2016; Kadam et al., 2014). Maize yield data was only available for North American LTEs and one European LTE, while small grain cereals were only available for European LTEs.

To reduce yield variations caused by differences in species identity and LTE local pedoclimatic conditions, we considered yield anomalies as yield measurement, i.e., mean-centred yield data based on crop- and LTE- specific long-term mean yield.

2.3.2 Outputs of entire rotations

When considering the outputs of the entire rotation we used calories and macronutrients. These outputs are more directly related to food security than crop yields (Cassidy et al., 2013; Headey & Ecker, 2013), and allow to combine the yields of different crops in a meaningful way. We transformed yield data into calories, and macronutrients (carbohydrates, proteins, and fats) per unit area, using nutritional tables relative to common retail products of the crops (**Paper IV**, SI, Table S2). Common retail products include flour for cereals, seed oil for oil crops, raw legumes, and raw root crops. We calculated rotation outputs as the sum of outputs from all crops included in the rotation (see Methods, **Paper II** and **Paper IV**).

Some crops in rotations were not intended for direct human consumption (hereafter forage crops). Depending on the site, these were ley, but also cereals, legumes, or broadleaves (**Paper IV**, SI, Table S1). Forage crops can be used as feed for livestock, with different final goals, or for non-food products, such as biofuels. For **Paper II**, we considered that forage crops were used for milk production. For **Paper IV** we explored two additional contrasting assumptions on the use of forage crops, with decreasing efficiency of conversion into human-accessible nutrients, i.e., beef or biofuel production. For biofuels, we assumed no human-accessible calories and macronutrients to be produced. We also assumed any residue from food production, generally used as feed, to be discarded. We then used nutritional tables to calculate calorie and macronutrient outputs of milk and beef, as described above.

Since all rotated crops are present in every growing season in separate plots, we could determine the whole rotation output on a yearly basis. Specifically, we weighted the output contributed by each crop in each year by the rotation length, i.e., the number of years needed to complete a full rotation cycle, and number of times that crop appeared in the rotation. This is equivalent to imagining that, out of one hectare under cultivation, the fraction cultivated with each crop corresponds to the frequency of that crop in the rotation. This approach allows a fair comparisons between long and short rotations or monocultures.

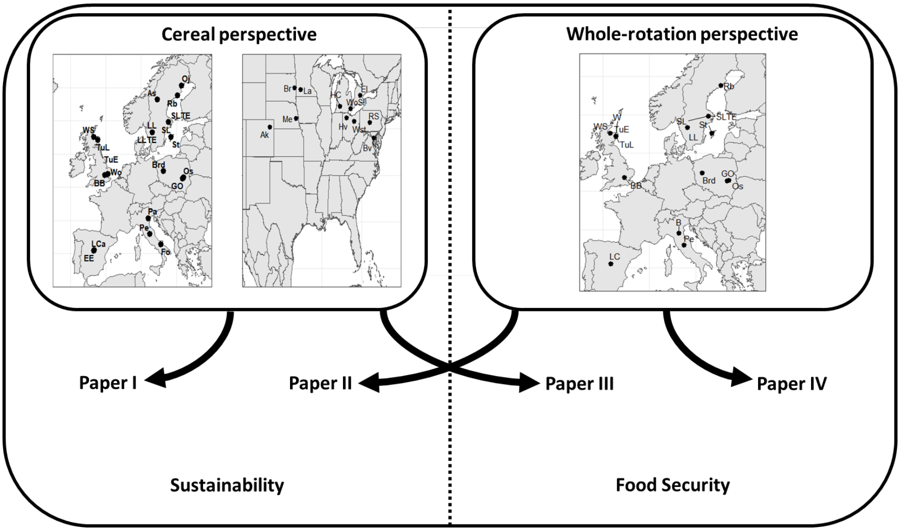

Figure 1 Conceptual diagram showing the overarching perspective used for the papers, and the location of the long-term experiments used in the papers. See Table 1 for clarifications of long-term experiment codes.

2.4 Nitrogen inputs

To explore how crop rotational diversity modulates the relationship between rotational outputs and N inputs, we summed all yearly N contributions from direct fertilisation (organic and inorganic), biological N fixation (when legumes are included in the rotation), and atmospheric N deposition (**Paper II**). We acquired inorganic and organic N contribution directly from our LTE data. To estimate biological N fixation, we used legume crop yields (measured as kgN/ha), and parameters based on the crop N uptake efficiency and total below-ground N fixation, including below-ground contribution such as rhizo-deposition and exudates (see Methods, **Paper II**). We calculated atmospheric N deposition by summing wet and dry atmospheric deposition of oxidized and reduced N (NOy and NHx, respectively). We acquired NOy and NHx data as annual 0.5° gridded simulation from the Atmospheric Chemistry and Climate Model Intercomparison Project (Lamarque et al., 2013).

2.5 Handling of climatic variables

To characterize the local climatic conditions, we used daily climatic data covering the growing season period of our maize, spring and winter small grain cereal data. We gathered daily gridded climatic data relative to the location and time period of each LTE, extracting data from the E-Obs database for Europe (Cornes et al., 2018), and the CONUS dataset for North America (Livneh et al., 2015). These data are available at spatial resolution of 0.1 ° x 0.1 ° for Europe and 0.0625 ° x 0.0625 ° for North America. Specifically, we extracted daily maximum temperature and daily precipitation total.

For spring small grain cereals and maize, i.e., crops that are sown during spring, we defined the growing season based on available LTE planting and harvesting information. For winter small grain cereals, i.e., crops that are sown during autumn and undergo winter dormancy, we assumed the (main) growing season to begin at the end of winter dormancy. We estimated the end of winter dormancy based on accumulated growing degree days and contribution of photoperiod (see SI, S1, **Paper III** for a detailed explanation).

To explore how crop rotational diversity modulates the response of cereal yields to climatic conditions (**Paper III**), we used two sets of climatic indices. The first one described conditions averaged over the entire growing season. Specifically, we used average maximum daily temperature and cumulated precipitation over the growing season because they had a high explanatory power of yield in our models. The second set focused on a part of the growing season when conditions were likely detrimental. Given the key role of water availability for crop development and yield, and that diversification might ameliorate water stress, we considered the duration (in days) and maximum temperature of the longest dry spell within the growing season. We define dry spells as the occurrence of consecutive days within a growing season with daily precipitation < 2 mm. We considered climatic indices relative to dry spells to capture short-term impacts on yields that are not detectable by climatic conditions averaged over the entire growing season (Troy et al., 2015).

To reduce the dependency of climatic conditions to local climates, for the analyses in **Paper III**, we considered climatic condition anomalies, i.e., we subtracted the LTE-specific long-term mean of each climatic variable from each year observation. Thus, the climatic indices included in **Paper III** are:

i) anomalies of cumulated precipitation, ii) anomalies of average maximum temperature, iii) anomalies of average length of the longest dry period, and iv) anomalies of the average maximum temperature of the longest dry period.

2.6 Statistical analyses

We used linear mixed effect models in all papers, but with different dependent and independent variables depending on the hypothesis to be tested. We quantified the response of cereal yield anomalies to crop diversity over time using crop rotational diversity expressed as either species diversity or functional richness, time since the beginning of the experiment (t), and their interaction, as fixed variables (**Paper I**). To account for possible diminishing returns of crop rotation diversity on y over time, we also included the quadratic effect of time and its interaction with crop rotation diversity (**Paper I**). To explore how crop rotation diversity modulates the response of cereal yield anomalies to climatic conditions over time, we used the same fixed variables, but with the addition of two sets of climatic indicators as fixed effects (as temperature indicator, anomalies of average maximum temperature or anomalies of average maximum temperature of the longest dry period; and, as water availability indicator, anomalies of cumulated precipitation or anomalies of average length of the longest dry period - **Paper III**). Since temperature and water availability can have compound effects on yields, we included an interaction term between them (**Paper III**). We also included the quadratic effect of temperature indicators (**Paper III**), because many biological plant processes have intermediate thermal optimum (Wang et al., 2017).

To explore how whole rotation outputs (i.e., calories, carbohydrates, proteins, or fats per hectare) change with functional richness over time, we used an additional model, with functional richness, time and their interaction as fixed effects (**Paper IV**). To explore how functional richness modulates the relationship between outputs and rotation total N inputs (N_{tot}) over time, we used functional richness, time, N_{tot}, and the interactions between functional richness and N_{tot} and functional richness and time as fixed effects (**Paper II**).

In all linear mixed effect models, we used calendar year and a variable that accounts for non-diversity related treatments and replicates, hereby experimental group, nested within the LTEs as random factors, to account for intercept variations explained by changes in spatial, temporal, and management conditions. For **Paper II**, we allowed the N_{tot} slope to vary with experimental group to account for variations in crop N uptake due to changes in soil conditions following treatments related to, e.g., tillage intensity and use of organic fertilisers. Further, for **Paper II**, we also included rotation identification code as a random effect, to account for intercept variation due to rotational composition, e.g., presence of N fixers.

3. Results & Discussion

Based on primary data of crop yields from LTEs, their nutritional content, biological N fixation parameters, annual N deposition estimates, and daily temperature and precipitation, we found that crop diversification can provide higher yields, and outputs of calorie and macronutrient, compared with cereal only monocultures. Simultaneously, crop diversification can reduce the need for fertiliser use and mitigate cereal yield losses to climate change. Specifically, increasing functional richness steadily increased cereal yields, particularly at low fertilisation levels, with increasing benefits over time, and with varying intensity depending on indicator crop (**Paper I**). Diversified crop rotations under low fertilisation could produce more cereal yields than rotations of the lowest levels of crop rotational diversity under high fertilisation (Figure 2). Using a cereal-only rotation with 200 kg N/ha of inputs as a baseline, functionally rich rotations produced similar calorie outputs but using 44% less N, 20 years since the rotations were implemented. The same reduction in N allowed functionally rich rotations to produce more proteins and fats but less carbohydrates than the baseline, after 20 years of production (Figure 3 and **Paper II**). Increasing functional richness more than compensated climate-induced cereal yield losses in monocultures (Figure 4, and **Paper III**). Functionally rich rotations gave higher calorie, protein, and fat outputs compared with cereal-only rotations, with increased benefit over time, when forage was used for milk production. They also produced similar carbohydrates to cereal-only rotations after 10 years of production, when forage was used to produce milk (Figure 5, and **Paper IV**).

3.1 Sustainability of diverse crop rotations

3.1.1 Crop rotational diversity substantially increase cereal yields, particularly under low fertilisation (**Paper I**)

Using data from 32 European and North American LTEs, we explored the effects of different crop rotational diversity levels, measured as crop species or functional richness, on cereal yields over time, and under contrasting external N fertilisation regimes. We found that crop species diversity and functional richness increased cereal yields over time. Yield declines occurred at the highest level of species diversity for winter-sown small grain cereals, while increasing functional richness steadily provided yield benefits (**Paper I**, Fig. 1 and Supplementary Table 3 and 4). We showed that diverse crop rotations can produce cereal yields at low fertilisation that are comparable with conventionally fertilised monocultures (Figure 2). The presence of specific crop functional types in rotation modulated the benefits of crop rotational diversity to cereal yields. For example, we found that maize yields benefitted from the presence of annual legumes and perennial leys, but not broadleaves (**Paper I**, Supplementary Table 5).

It is widely recognized that diverse crop rotations can enhance cereal yields and require less external inputs than monocultures (Bennett et al., 2012). However, these effects of diversity had previously been shown in single fully-crossed experiments that compare crop rotations with a corresponding monoculture (Bowles et al., 2022), do not take into account explicit crop diversity levels (Marini et al., 2020), or use only a single site (Gaudin et al., 2015; Sindelar et al., 2015). Our analysis, using 32 LTEs covering a gradient of crop diversity, allowed us to assess the robustness of the effects of crop diversity in modulating yields of several cereal species across a wide climatic gradient and a wide range of cereal species. By considering different levels of species and functional diversity we showed that functional richness, not species diversity, had the largest potential for yield advantages. We speculate niche complementarity is one of the main mechanisms behind this result, because it is more associated with functional richness than species diversity (Cadotte et al., 2011), e.g., two cereal species differ less in traits than a cereal species and a legume one. Our finding that crop rotational diversity increases cereal yields more at low than high fertilisation is in line with a meta-analysis that identified the presence of legumes as the driver of such an effect (MacLaren et al., 2022). We expand on this by showing that the benefits of crop rotational diversity on cereal yields at low fertilisation increase over time, and that they depend also on the indicator cereal crop, with maize gaining more benefits than small grain cereals (**Paper I**, Fig. 3).

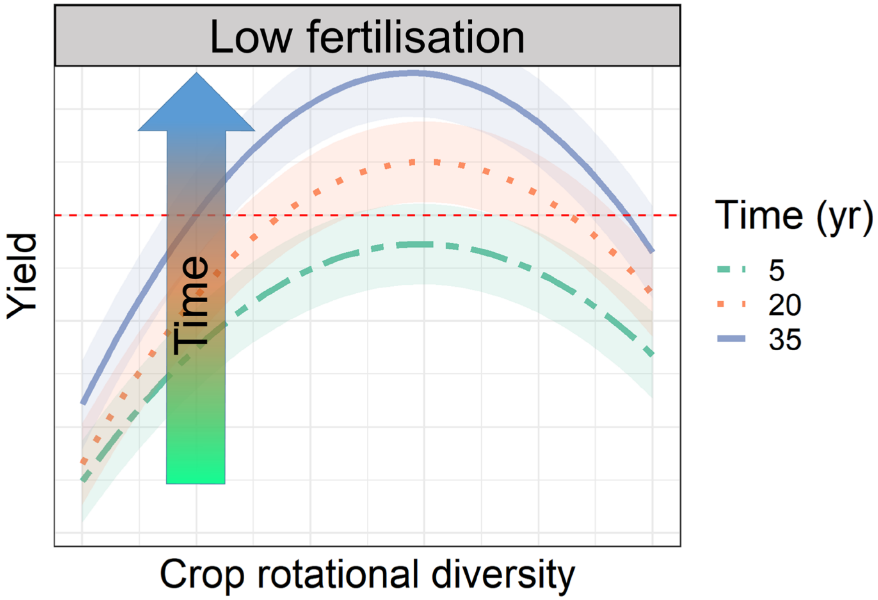

Figure 2 Graphical concept of yield as a function of crop rotational diversity under low fertilisation regime, i.e., application of fertilisation below local recommendation, and time since the rotations were implemented. The horizontal red dotted line represent the yield of the lowest crop rotational diversity when fertilisation rates are within or above local recommendations, in the first year of production. The curves are cereal yields produced by the rotations after 5, 20, and 35 years since they were implemented (dotted green lines, dotted orange lines, and solid blue lines, respectively).

3.1.2 Functionally rich rotations produce nitrogen-efficient calories and macronutrients (**Paper II**)

Using primary data from 15 European LTEs, we assessed how functional richness shapes the relationship between rotational outputs, measured as calories, carbohydrates, proteins, or fats, and N inputs, derived from inorganic and organic fertilisation, and from atmospheric N fixation and deposition, over time. We found that functionally rich rotations, i.e., including three crop functional types in the rotation, produce more calories, proteins and fats per unit of total N input, compared with cereal-only rotations, irrespective of N input level and with increasing difference over time for calories and proteins (**Paper II**, Figure 1). Functionally rich rotations produced similar carbohydrates to cereal-only rotations under low input regimes (less than 100 kg N/ha) or independently of N in the long term, and produced less carbohydrates in the short term under high input regimes (**Paper II**, Figure 1). Functionally rich rotations also had higher marginal gains of calorie, protein and fat per unitary increase of N inputs than cereal-only rotations, especially under low input regimes (less than 100 kg N/ha), with increasing difference over time (**Paper II**, Figure 2). Using 44% less N compared with a cereal-only rotation of 200 kgN/ha of inputs, 20 years old functionally rich rotations could produce as many calories, while producing 8% more proteins, and 92% more fats, but 21% less carbohydrates (Figure 3 and **Paper II,** Figure S1). We also found that intermediately functionally rich rotations, i.e., rotations with two crop functional types, could provide similar calorie and protein outputs to cereal-only rotations only when compared under low-input regimes (**Paper II**, Figure 1). Intermediately functionally rich rotations produced less carbohydrates, and more fats, per unit N than cereal-only rotations, irrespective of time and N levels (**Paper II**, Figure 1).

Previous experiments and meta-analyses showed that legumes and perennial leys in rotations can increase crop N retention and use efficiency, while promoting cereal yields (Lassaletta et al., 2014; Lehman et al., 2012; MacLaren et al., 2022; Nilsson et al., 2023). In our analysis, we move past analysing the outcome of adding single specific crop types, and explore how increasing functional richness shapes the relationship between the outputs and the N inputs of the entire rotations. We found that adding a single crop type to cereals limits the benefits of functional richness to outputs per unit N at intermediate to high total N inputs, whereas adding two functional types provided steadier benefits over large ranges of total N inputs. A meta-analyses of multiple LTEs, and our previous findings (**Paper I**), showed that the benefits of crop diversity are higher under low than high fertilisation regimes (MacLaren et al., 2022; Smith et al., 2023). Our results likely differ because we included biological N fixation and atmospheric N deposition among the sources of N, and included the outputs of all crops in rotation. Considering calories and macronutrients as outputs is also a novel approach, allowing us to infer that functionally rich rotations can provide overall more human-available energy and nutrient-balanced outputs than cereal-only rotations while requiring less N inputs.

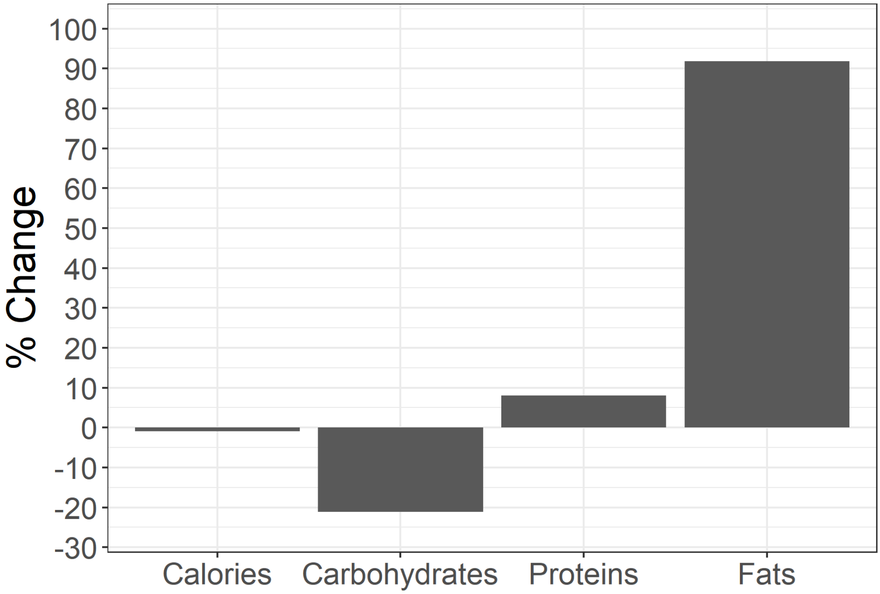

Figure 3 Percentage change in macronutrient outputs (carbohydrates, proteins, and fats) of a functionally rich rotation with a total nitrogen input of 112 kg/ha, compared with a cereal-only rotation with a total nitrogen input of 200 kg/ha, 20 years since the rotations were implemented.

3.2 Crop rotational diversity benefits crop production

3.2.1 Crop rotational diversity mitigates climate-induced cereal yield losses (**Paper III**)

To analyse how different levels of crop rotational diversity modulate cereal yield response to explicit climatic variables we combined primary yield data from 32 European and North American LTEs with daily gridded climatic dataset. We found that species diversity and functional richness mitigated yield losses under anomalously warm and dry conditions for maize, and anomalously warm and wet conditions for small grain cereals (Figure 4 and **Paper III**, Figure 1 and 3, Table 1 and 3). Similarly, species diversity and functional richness mitigated yield losses under anomalously long and warm dry spells (**Paper III**, Figure 2 and 4, Table 2 and 4). The yield loss mitigation was weaker at the highest species diversity for winter-sown small grain cereals, while increasing functional richness steadily mitigated yield losses (**Paper III**, Figure 1 to 4).

Previous analyses of yields of LTEs suggest that increasing crop rotational diversity can buffer climate-induced cereal yield losses (Bowles et al., 2020; Marini et al., 2020), but ours is the first comprehensive analysis that simultaneously accounts for gradients of crop diversity and climatic conditions in an explicit way. Thus, we could determine the level of species diversity or functional richness necessary to offset cereal yield losses associated with defined changes in climatic conditions. We could also explore how the benefit of crop diversity is modulated by variations in climatic conditions. Both aspects are important in understanding the climate adaptation potential of increasing crop diversity.

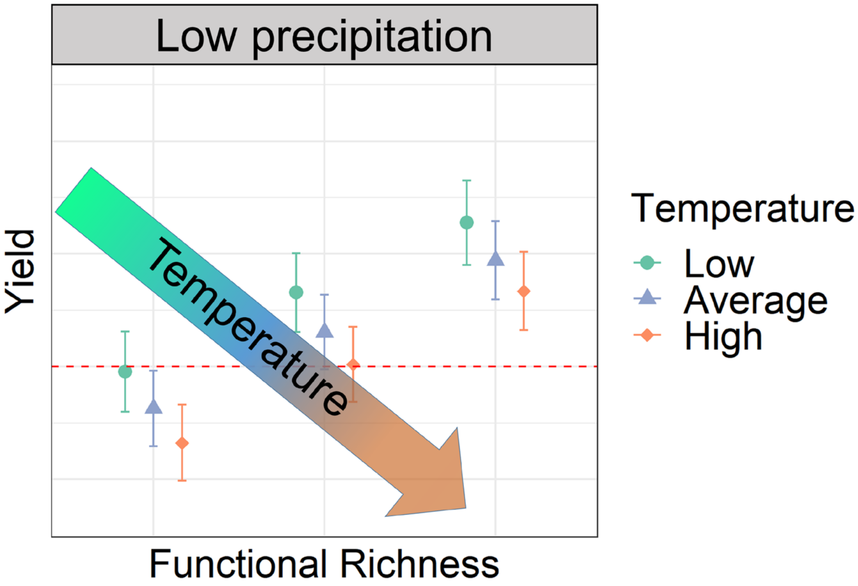

Figure 4 Graphical concept of yield as a function of functional richness under low precipitation, and temperature. The horizontal red dotted line represent the yield of the lowest functional richness under average precipitation and temperature. The symbols are cereal yields produced by the rotations under low, average, and high temperature (green circles, blue triangles, and red diamonds, respectively).

3.2.2 Functionally rich rotations increase calorie and macronutrient outputs, with benefits over time (**Paper IV**)

Using yield data from 17 European LTEs, we explored whether increasing functional richness increases whole rotation outputs, measured as calories, carbohydrates, proteins, and fats, over time, compared with cereal monocultures. We found that functionally rich rotations, i.e., including three crop functional types, could produce more calories, proteins and fats than cereal monocultures, and more so over time, if forage crops were used for milk production (Figure 5 and **Paper IV**, Figure 1a,c,d and Table 1a,c,d). Over the long term, functionally rich rotations also produced more carbohydrates than cereal monocultures and similar carbohydrates to cereal-only rotations (**Paper IV**, Figure 1b and Table 1b). Rotations of two functional crop types produced more fats, similar calories and proteins, and less carbohydrates than cereal monocultures, irrespective of time (**Paper IV**, Figure 1 and Table 1). Further, the presence of specific crop types in rotation largely affected caloric outputs, and balance of macronutrients (**Paper IV**, Table 2). Using forage crops for beef or biofuel production, instead of milk, reduced calorie and macronutrient outputs, cancelling the advantages (Paper IV, Supplementary Information, Table S3 and S4).

Our key finding that increasing functional richness enhanced calorie outputs contrasts findings from a North American experiment, showing that growing fewer cereals in rotation leads to reduced calories (Sanford et al., 2021). Our results are based on rotations spanning a wider range of functional richness and data from several LTEs, and include oil crops, which provide calorie-rich outputs thanks to their high fat content (**Paper IV**, Supplementary Information, Table S6). The finding of increased macronutrient outputs in functionally rich rotations compared with cereal monocultures are in line with a global analysis (Ricciardi et al., 2018). However, there crop diversity was estimated based on survey data estimations, whereas we could account for explicit variations in functional richness. A two-year field experiment in Northern India (Gora et al., 2022) also showed that functionally rich rotations can provide higher outputs of macronutrients than a cereal-only rotation. This is partially in contrast with our finding that functionally rich rotations produced less carbohydrates than cereal-only rotations over the short-term, or similar carbohydrates after 10 years of production. A possible explanation is that the diverse rotation in the Northern Indian included maize, which typically yields more than most cereals, and gains more yield benefits from crop rotational diversity than small grain cereals (**Paper I** and **Paper III**). Nevertheless, by combining data from several LTEs, we were able to account for crop diversity effects that accrue over time, and conclude that the pattern of increased macronutrients with functional richness holds in experiments with different pedoclimatic and management conditions, with increasing benefits over time, and over a wide geographic distribution.

Overall, we show that diversifying crop rotations through functional richness does not lead to food shortages, if crop choice is aimed at producing a balanced set of macronutrients.

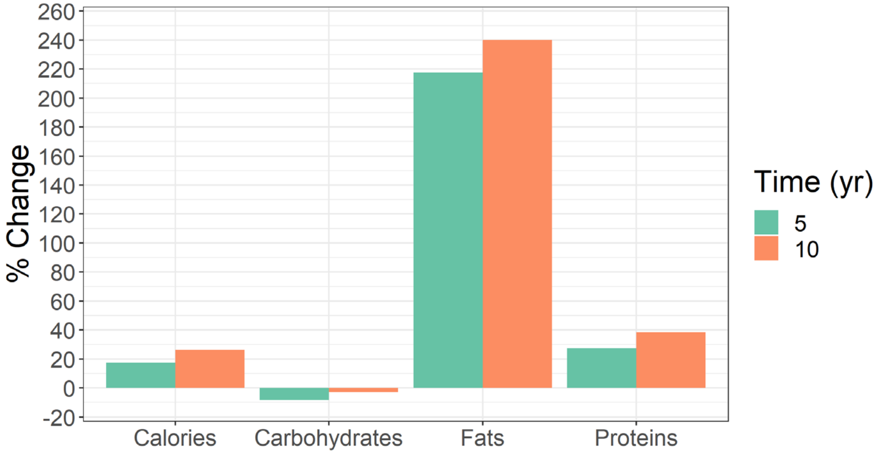

Figure 5 Percentage change in outputs (calories, carbohydrates, proteins, and fats) of a functionally rich rotation with, compared with a cereal-only rotation in its first year of production, when forage crops are used for milk production. Coloured bars are outputs produced by the rotation after 5 and 10 years since they were implemented (green bars, and red bars, respectively).

3.3 Mechanisms

Lacking data beyond crop yield we can only speculate on which mechanisms explain the outcomes of our analyses. It can be useful to differentiate mechanisms promoted by crop diversity that emerge for an indicator crop, i.e., that benefit cereal yields in our case (**Paper I**, **Paper III**), and those that emerge at the rotation level, i.e., benefitting the outputs of the whole rotation (**Paper II**, **Paper IV**).

Niche complementarity is a likely mechanism underlying cereal yield benefits of crop rotational diversity, as we found that functional richness gave steadier yield benefits than crop species diversity (**Paper I**, **Paper III**). Plant diversity, and more specifically niche complementarity have been shown to promote or facilitate multiple ecosystem functions that ultimately benefit or maintain crop yields (Storkey et al., 2015). For example, crop rotational diversity fosters below-ground microbial diversity, thus increasing soil quality, i.e., biophysical conditions and fertility (Mooshammer et al., 2022; Venter et al., 2016). This results in improved nutrient cycles, and water and nutrient retention (Sprunger et al., 2020; Wang et al., 2022). Further, increasing crop diversity can mitigate pest and pathogen impacts to crops, as natural enemies become more abundant, and rotating diverse crop can interrupt pest and pathogen life cycles (Bennett et al., 2012). At the rotation level, increasing crop rotational diversity promotes a richer crop portfolio, providing a diversity in response to environmental condition that could lower productivity losses in the face of detrimental conditions (Loreau et al., 2021). The presence of suitable hosts for pests and pathogens is also reduced as functionally diverse crop species are rotated in succession, which could benefit all crops in rotations (Bennett et al., 2012). These emergent mechanisms, facilitated by niche complementarity, could explain why of the total calorie and macronutrient outputs from all crops in the whole rotation is enhanced as functional richness increases (**Paper II**, **Paper IV**).

The higher nutrient availability and retention promoted by crop rotational diversity can explain why the yield benefits of both crop species and functional richness were more pronounced under low N fertilisation regimes (**Paper I**, Fig. 1 and 2). In such conditions, the presence of annual legumes in rotations of intermediate functional richness and annual and perennial legumes in functionally rich rotations is particularly important, as they largely contribute to the total N inputs (Figure 4). The variation in root traits and cropping period amongst the crops promote a more efficient use of nutrients over space and time (Duchene et al., 2020; Griffiths et al., 2022), which could benefit the whole rotational output independently of N levels. This, in addition to the crop portfolio effect and reduced impact of pests and pathogens, are possible reasons why increasing functional richness increased the outputs produced per unit N, independently of N input levels (**Paper II**, Fig 1). Nevertheless, increasing functional richness promoted higher marginal output gains per unit N under low N input regimes (**Paper II**, Figure 2). The large contribution of biologically fixed N to the total N inputs under low fertilisation regimes (Figure 4) suggests that legumes in functionally rich rotations are a crucial element for high marginal gains under low N input regimes.

Crop diversity also enhances soil water and nutrient retention, due to enhancements to microbial activity and soil organic matter (Renwick et al., 2019; Schmer et al., 2020; Sprunger et al., 2020). Improving water retention also reduces water stress when precipitations are scarce, and limit nutrient leaching under abundant precipitation (Renwick et al., 2019). Moreover, higher water retention facilitates evaporative cooling and thus reduce plant heat stress under warm periods (Sadok et al., 2021). These mechanisms could explain our results showing that species diversity and functional richness can mitigate cereal yield losses associated with detrimental climatic conditions (**Paper III**, Figure 1-4).

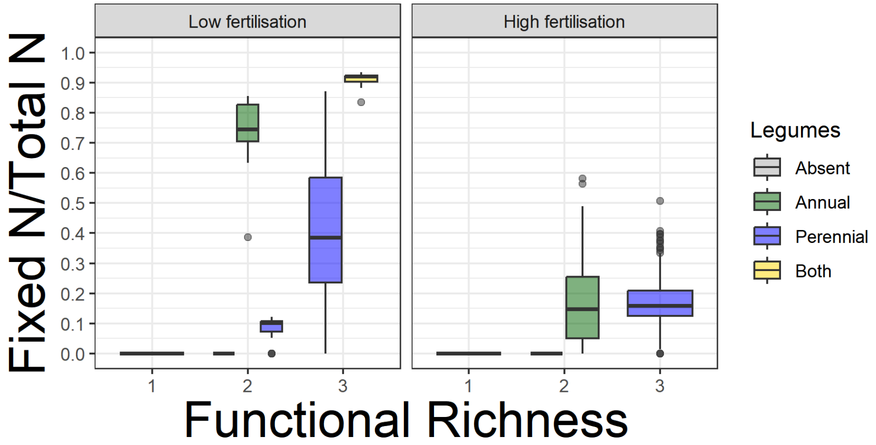

Figure 6 Ratio of biologically fixed N to total N inputs as a function of functional richness, and of presence and type of legumes, based on data from Paper II. Grey, green, blue, and yellow boxplots indicate when legumes in rotation are absent, annual, perennial, or both annual and perennial, respectively.

3.4 Advantages & limitations of LTEs

This thesis is based on the collation, harmonization and analysis of data from LTEs, across 32 sites. All experiments included a cereal crop, a minimum of two crop diversity treatments and were rainfed. In 17 of the LTEs, fertilisation had a fixed rate, while 15 LTEs presented variations in fertiliser treatments, e.g., inorganic fertilisation ranging from zero to rates above local recommendations, with presence or absence of organic fertilisers. Most sites were conventionally tilled, two sites were not tilled, and eight sites presented variations in tillage treatments, i.e., no tillage, reduced tillage, and conventional tillage. The treatments were replicated, except in 12 LTEs. The crops included in our data are typical of the regions in which the LTEs are located, e.g., grain legumes in southern Europe, and broadleaves and leys in northern Europe. Within the sites, the largest variation in species numbers and functional types across rotations was one to six species, and one to four functional types. The crop rotation designs in our data stem from local practices and needs, and thus represent realistic and applicable scenarios. However, the heterogeneity in crop rotation design across the LTEs did not allow to test for the role of specific crops. For example, we could not account for preceding crop effects in modulating cereal yields.

Historical data from LTEs is important in exploring trends and emerging properties and effects that are only observable over decades, such as climate change and soil dynamics. For example crop rotation effects on soil organic carbon can take more than 14 years to emerge (Schmer et al., 2020). However, the LTEs were originally designed and are managed to answer specific questions, which do not necessarily match those we have used the data for, particularly decades after the experiments begun. This inevitably leads to unbalanced designs across LTEs, where different aspects of managements are crossed together, such as tillage intensity and residue treatments, and thus add complexity that cannot be explained by crop diversity alone. Moreover, there occur changes over time of experimental design, soil and climatic conditions, and technology and crop varieties, which cannot be fully disentangled from accruing effects of crop diversity. In our analyses, these variations were exacerbated, as we used several LTEs that vary in location and cover different time periods and lengths.

These changes can pose some difficulties in the statistical analyses, such as temporal auto-correlation, and heterogeneity of variance across years. We accounted for intra- and inter-experimental variations of the LTEs by using linear-mixed effect models (Onofri et al., 2016). A large portion of the uncertainty in our models was explained by such variations, while the variables of interest had a low explanatory power, i.e., marginal R^2 values often below 0.10. Hence, our models cannot be used to calculate accurate future yield predictions, but they allowed us to make robust generalisations of the response of historical yields to changes in crop diversity levels, climatic conditions, N levels, and time, and their interactions.

Moreover, the logistic limitations imposed by the need to finance and run LTEs over long time periods result in relatively few variables being consistently measured. In our case yields and N inputs derived from fertilisation were available for all LTEs, while we lack detailed information, e.g., soil organic carbon, soil bulk density, N content in grain. For this reason, we could not explore which diversity-driven mechanisms affected the outcomes of the analyses.

3.5 Implications

With an increasing demand for food, growing hunger and malnutrition, and crop production threatened by ongoing climatic changes, it is important to rethink how we grow our food. We need sustainable alternatives to industrial crop production that can produce sufficient and nutritious food, while limiting environmental impacts and reduce climate-induced yield losses.

I show that crop rotational diversity enhanced staple cereal yields, with improved benefits over time, and especially under low regimes of fertilisation (**Paper I**). The benefits to cereal yields declined at high ranges of crop species diversity, while functional richness gave steady benefits. This implies that simply increasing crop species gives limited yield benefits, if the species are not complementary in functional traits, e.g., rooting depths and period of activity.

At the rotation level, functionally rich rotations allowed higher outputs produced per unit N inputs compared with cereal-only rotations (**Paper II**). Thus, increasing crop rotational diversity, particularly when expressed as functional richness, allows production of more human-available energy and nutrients than industrial cropping systems while requiring less N from fertilisers. Contrary to a previous analysis, I show that the European sustainability goal of reducing fertilizer use could be reached without sacrificing productivity in cropping systems (Beckman et al., 2020) through crop rotational diversity.

I also showed that functional richness steadily mitigated cereal yield losses caused by warming, and drier than average conditions for maize in North America, or wetter than average conditions for small grain cereals in Europe (**Paper III**). Functional richness also compensated for yield losses caused by long and warm dry spells (**Paper III**). Climate change is causing warming and increasing precipitation variability in many regions, while also increasing the frequency and intensity of dry spells. In light of this, my findings imply that crop rotational diversity can reduce cereal yield losses caused by increasingly common climatic conditions, thus promoting climate adaptation in cropping systems within temperate climates.

From a whole rotation perspective, I showed that functionally rich rotations produced higher outputs of calories and macronutrients than cereal-only rotations, with increasing benefits over time (**Paper IV**). The outputs produced by functionally rich rotations were more in line with dietary recommendations for the average adult than cereal-only rotations (**Paper IV**). Therefore, adopting rotations of high functional richness could foster food security, by increasing accessibility to nutrient-balanced diets. In summary, diversification of crop rotation provides benefits regarding food security, sustainability, and climate adaptation. Nevertheless, monocultures and short, simple rotations are prevalent in today's industrial agriculture.

3.6 Barriers to diversification

The choice of crop in cropping systems is largely determined by environmental conditions and interacting and self-reinforcing socio-economic factors (Magrini et al., 2018; Meynard et al., 2018). For example, non-staple crops and legumes for direct human consumption occupy a minimal portion of European agricultural land, due to low demands and investments in breeding, high cost of downstream production and logistics, and relatively inexpensive protein imports (European Commission, 2018; Ferreira et al., 2021; Magrini et al., 2018; Meynard et al., 2018). Also the production of highly marketable ultra-processed food can favour the choice of a limited set of crops. For example, corn is largely used in North America to produce artificial sweeteners in many processed foods and beverages (Ferder et al., 2010). While these factors limit the set of potential crops and thus crop diversity levels, as opposed to e.g., grassland experiments (Isbell et al., 2015), the similarity in crop choice between our data and typical farms, within a European and North American context, makes our results realistic and applicable to real scenarios. It remains to be explored whether our findings are also applicable in other pedoclimatic conditions, e.g., countries within tropical climate.

Diversifying crop production requires learning how to manage new crops, thus implying an increase in complexity in terms of cultivation practices, machinery types, and thus an increase in costs for production and labour. Further, compared with staple crops, alternative crops are less profitable, may be less adapted to local climates, and have less developed chains of distribution. These factors constitute economic risks and lock-ins often beyond the control of the farmers, which hinder the adoption of diversifying practices (IPES-Food, 2016; Magrini et al., 2018; Meynard et al., 2018). Despite these barriers, evidence indicates that diversified cropping systems can increase farm economic performance (Nilsson et al., 2022). Subsidies should support farmers in transitioning from input-intensive systems to diversified ones, as it may take years for the soils to recover from more industrial settings and to benefit from diversity effects, or for the alternative crops to increase in profitability. As adoption increase, logistic & storage of non-cereal crops need to be strengthened to lower the overall cost of production, as well as development of their varieties to further improve yields and resource use efficiency. In doing so, the short-coming of diversified cropping systems are likely to diminish over time, further promoting their adoption (IPES-Food, 2016).

4. Conclusion & future directions

I used data from LTEs spanning a wide set of crops and environmental conditions to explore how different levels of crop rotational diversity affect crop productivity at the crop and rotation level, the dependency on fertilisers and total nitrogen inputs, and climate adaptation potential. At the crop level, I showed that when crop rotational diversity is expressed as functional richness, it steadily benefits cereal yields, with increased benefits over time and under low fertilisation regimes, and reduces cereal yield losses associated with detrimental climatic conditions (**Paper I**). Other crops, e.g., legumes and broadleaves, respond differently to the surrounding environment, due to differences in physiology and trait expression. However, such crops were not represented in all diversity levels, and thus we could not explore how crop rotational diversity modulates yield responses to variation in fertiliser treatments and climatic conditions for non-cereal crops. Understanding these relationships could be important in designing crop rotations with the aim to diversify farm outputs, rather than maximizing cereal outputs.

At the rotation level, I showed that functionally rich rotations can produce more calories and macronutrients than cereal-only rotations, while requiring less inputs of nitrogen (**Paper II** and **Paper IV**). We assumed the calorie and macronutrient outputs per unit yield to be constant, as the data did not include information related to yield quality. In reality, crop quality depends on many factors, amongst which choice of crop varieties, management, and environmental conditions (Ebi et al., 2021). Given that crop rotational diversity modulates the response of crops to climatic conditions (**Paper III**), it is reasonable to assume that this can also affect crop quality. Given growing malnutrition and ongoing changes in the climate (FAO et al., 2022; IPCC, 2021), exploring the effects of crop rotational diversity while accounting for variation in yield quality is an important step in addressing food insecurity and understanding climate adaptation in cropping systems.

I also showed that the response of rotation outputs to unit N inputs increases with functional richness. However, the relationship between outputs and N inputs also depend on the environmental conditions (de Wit, 1992). For example, at the crop level, increasing precipitation can increase yield per unit N, and growing evidence indicates that diverse rotation could further increase this efficiency (Renwick et al., 2019). It is yet to be explored how changes in climatic conditions affects output-input relationships across different diversity levels, when outputs and inputs from the whole rotation are considered.

Open questions remain regarding how crop rotational diversity affects the year-to-year stability of the rotation outputs, i.e., an indication of how much the outputs remain constant over time. Output stability, commonly referred to yields, is a desirable quality in the face of climate change, as variations in climatic conditions explain more than one third of global yield variability (Ray et al., 2015). Two single LTE analyses showed that increasing crop rotational diversity can increase yield and caloric stability (Gaudin et al., 2015; Sanford et al., 2021). Conversely, a state level analysis in the US showed that crop diversity was negatively associated with caloric stability (Driscoll et al., 2022). However, crop diversity was quantified at the state level, thus not accounting for within-field variations in crop rotational diversity and not allowing to measure variations in functional richness. A field perspective from several LTEs, possibly accounting for crop functional richness, is an important next-step in exploring and generalising the potential of crop rotational diversity in promoting yields and output stability.

Due to variations experimental design across the LTEs, our data was too unbalanced to explore synergies or trade-offs between crop rotational diversity and other sustainable practices, e.g., organic fertilisers, relay cropping, reduced tillage. Understanding interactions between crop rotational diversity and other practices is important to identify which combinations of treatments minimize trade-offs between productivity and sustainability or climate adaptation, or maximize desired outcomes.

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Popular science summary

Like all living beings, we humans depend on a variety of food to survive and thrive. The way we grow the crops we eat has been simplified over the last couple of decades, generating wasteful cropping systems with unstable levels of production. In the past, it was common in cropping systems to grow several types of crops in rotation. At the present time most cropping systems are industrialized, where few selected crops are grown in bulk, mostly cereals, sustained by an intense use of resources that damage the environment, such as fertilisers and pesticides. This transformation and substantial technical advances guaranteed a rapid increase in crop yields and human population but also contributed massively to biodiversity loss, environmental pollution and climate change. Biodiversity provides beneficial functions that sustain the crops, for example by increasing nutrient and water availability in the soil. Climate change brings forth conditions that increasingly disrupt our crops, such that food production is threatened globally. We have an urgent need to rethink how we grow our food, so that we can steadily feed a growing number of hungry and malnourished people, without further polluting the environment and contributing to climate change.

Crop diversification means growing a diverse set of crops, differing in characteristics, needs and susceptibility to detrimental growing conditions. A way to implement diversification is to rotate these crops in the field year after year. This practice enhances the biodiversity we have lost in cropping systems as a result of simplification and restores beneficial environmental functions. In doing so, we can potentially reduce the dependency of cropping systems to polluting inputs such as fertilisers. But can diversified cropping systems produce enough to satisfy a continuously increasing food demand, while sinking our environmental footprint? Are these systems able to sustain

their production in the face of ongoing climatic changes? And how much diversity is necessary to reach this aim?
To answer these questions, I combined crop yield information from several long-term field experiments, where crop rotations of varying diversity levels were managed and compared over several decades. I specifically analysed how cereal yields changed with the rotational diversity, i.e. number of crop species or types of crops grown, under contrasting fertilisation, and with variations in climatic conditions. I also analysed how the calories, carbohydrates, proteins and fats provided by all crops in rotation changed with increasingly diverse rotations, and with varying nitrogen availability.

Focusing on cereal productivity, I found that crop rotations with at least three different types of crops, such as cereals, legumes, and root crops, increased yields compared with rotations of only cereals, with increasing benefits over time, and particularly so when few fertilisers were used. Those rotations also reduced yield losses caused by damaging climatic conditions. When looking at the production of the whole rotations, I found that crop rotations with at least three different types of crops produced more energy and nutrients than rotations of only cereals, with growing benefits over time and while using less nitrogen. In summary, crop rotation diversity can simultaneously provide large quantities of food, reduce the need to fertilise, and reduce vulnerability to climate change.

Populärvetenskaplig sammanfattning

Som alla levande varelser är vi människor beroende av olika typer av föda för att överleva och leva. Hur vi odlar de grödor vi äter har förenklats under de senaste decennierna, vilket har lett till oeffektiva odlingssystem med instabila produktionsnivåer. Förr i tiden odlades flera olika typer av grödor i växelbruk. Idag är de flesta odlingssystem industrialiserade och utgörs av några få utvalda grödor i bulk, främst spannmål. Dessa odlingssystems produktion upprätthålls av en intensiv användning av resurser som skadar miljön, till exempel gödselmedel och bekämpningsmedel. Detta skifte, och betydande tekniska framsteg, har garanterat en snabb ökning av skördarna och den mänskliga befolkningen, men har också i hög grad bidragit till förlust av biologisk mångfald, miljöföroreningar och klimatförändringar. Biodiversitet bidrar med funktioner till odlingssystemet som gynnar grödorna, till exempel genom att öka tillgången på näring och vatten i jorden. Klimatförändringarna leder i sin tur till förhållanden som i allt högre grad stör våra grödor, vilket gjort att livsmedelsproduktionen idag hotas globalt. Vi har ett akut behov av att tänka om när det gäller hur vi odlar vår mat, så att vi stadigt kan föda ett växande antal hungriga och undernärda människor, utan att ytterligare förorena miljön och späda på klimatförändringarna.

Diversifierad odling innebär bland annat att man odlar flera olika grödor med olika egenskaper, behov och känslighet för ogynnsamma odlingsförhållanden. Detta kan göras genom att rotera dessa grödor på fältet år efter år. Denna metod ökar den mångfald som vi har förlorat när odlingssystemen förenklades och återställer viktiga funktioner i odlingssystemet. På så sätt kan vi potentiellt minska odlingssystemens beroende av förorenande insatsmedel, som gödselmedel. Men kan diversifierade odlingssystem producera tillräckligt med mat för att tillgodose en ständigt ökande efterfrågan på livsmedel, samtidigt som vi minskar vårt

miljöavtryck? Kan dessa system upprätthålla sin produktivitet trots de pågående klimatförändringarna? Och hur mycket diversifiering krävs för att nå detta mål?
För att besvara dessa frågor kombinerade jag information om avkastning från flera långliggande fältförsök, där olika växtföljder jämförts under flera decennier. Jag analyserade särskilt hur spannmålsavkastningen förändrades med diversifiering av växtföljden, dvs. antalet växtarter eller typer av grödor som odlades, med olika gödsling och med variation i klimatförhållandena. Jag analyserade också hur kalorier, kolhydrater, proteiner och fetter från alla grödor i växtföljden förändrades med ökande diversitet i växtföljden och med variation i kvävetillförsel.

Med fokus på spannmålsproduktiviteten fann jag att växtföljder med minst tre olika typer av grödor, t.ex. spannmål, baljväxter och rotfrukter, ökade avkastningen jämfört med växtföljder med enbart spannmål, med ökande fördelar över tid, och särskilt när om gödselnivån var låg. Dessa växtföljder minskade också de skördeförluster som orsakades av otjänliga klimatförhållandena. När jag tittade på produktionen i hela växtföljden fann jag att växtföljder med minst tre olika typer av grödor producerade mer energi och näringsämnen än växtföljder med enbart spannmål, med ökande fördelar över tid samtidigt som mindre kvävetillförsel användes. Sammanfattningsvis kan diversitet i växtföljden ge stora mängder mat samtidigt som det minskar behovet av gödsling och sårbarheten för klimatförändringar.

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ARTICLE

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Increasing crop rotational diversity can enhance cereal yields

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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.

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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 itself[1]. 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 species[6], 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[6,13] 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 pressure[6]. 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 present[18]. 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 rotations[19], focus on a single site[20,21], are short term[22,23], or only include a single indicator crop[24] 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 community[27]. 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 CRD[32], 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 soils[33]. 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 properties[34] that may take longer to accrue[35]. 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 traits[21]. 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

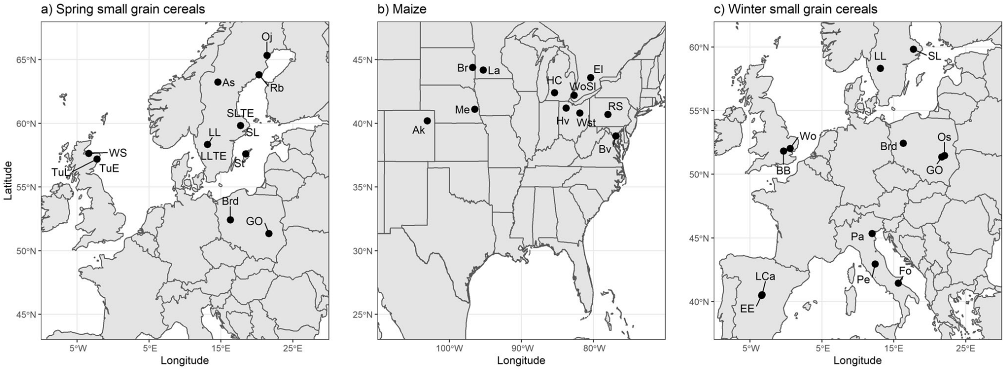

Fig. 1 Location of long-term agricultural experiments of each indicator crop. Spring-sown small grain cereals (**a**) were grown in Europe, maize (**b**) in North America, and winter-sown small grain cereals (**c**) in Europe. See Supplementary Table 1 for the clarification of site codes and additional 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 maize[40].

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 N_2, 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 diversity[28]. Certain groups of crops are particularly efficient at breaking a sequence of agronomically and ecologically similar cereal species[43]. 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 R^2 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 fertilisation[18]. 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

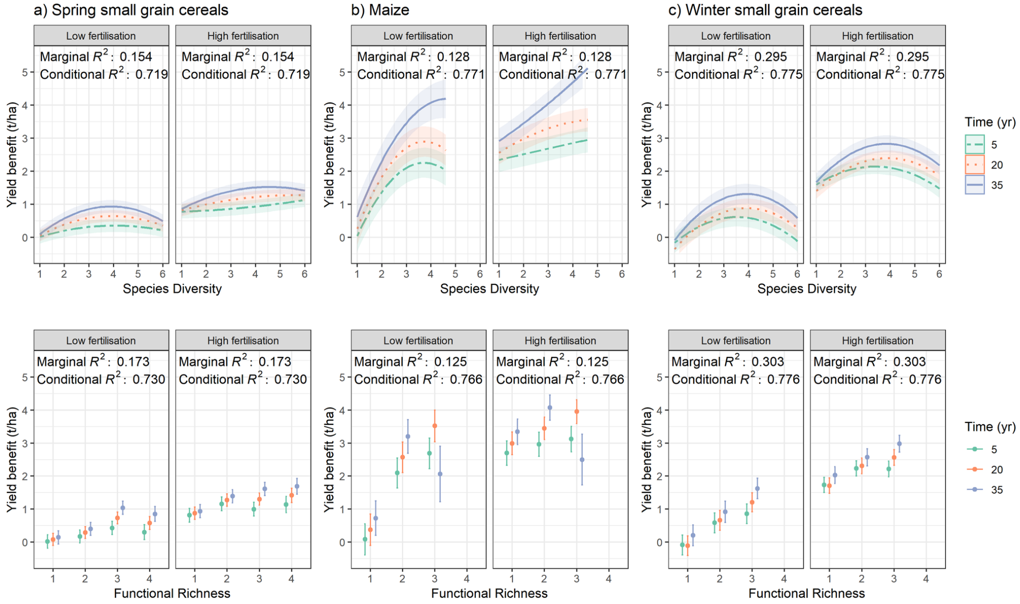

Fig. 2 Yield benefit for each indicator crop from crop rotational diversity (CRD) and external nitrogen (N) fertilisation. The y-axis presents model-predicted yield benefit compared to monocultures at year = 0, based on mean-centred observations, i.e., the difference from to the long-term within-site 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 strategy[46], 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

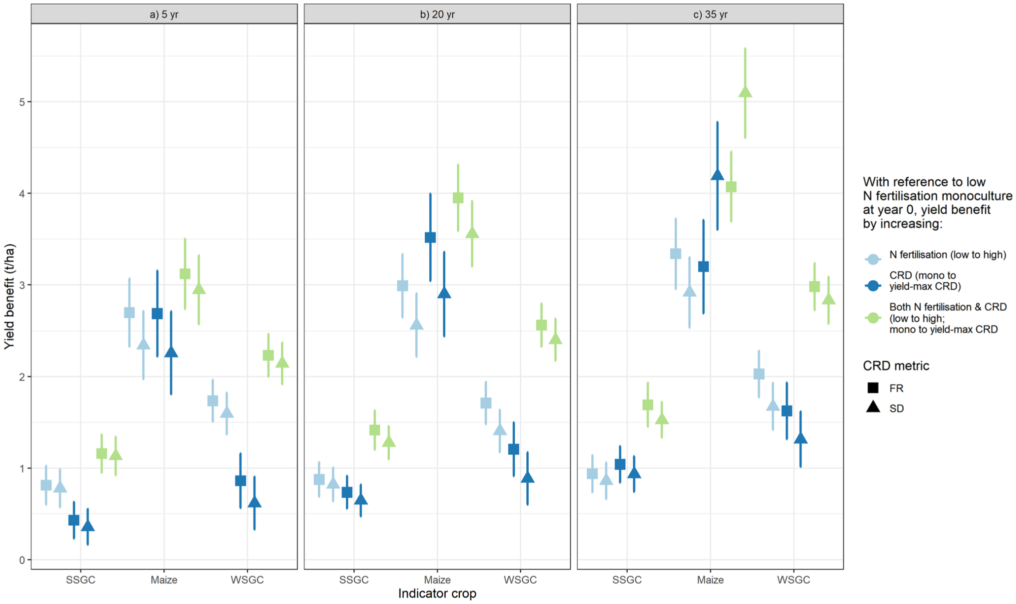

Fig. 3 Comparison of yield benefits from increasing either crop rotational diversity (CRD), external nitrogen (N) fertilization or both over time. Mean (±95% CI) yield benefits, with reference to monocultures at year = 0 receiving low external N inputs, from only increasing N fertilisation to high (maintaining 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 area[18], particularly when the other crops in the rotation support a diverse plant-based diet[47].

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 quality[54], 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 wheat[58]. 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 input[18] 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 diversity[27]. 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 effects[21] 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 diversity[34], 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 yield[69], a substantial part of which is currently bound for animal feed or biofuels[70], and alternatively analyse multifunctionality[71] and the total production of energy, proteins and nutrients of the entire rotation matching human dietary needs[72]. 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 population[47], 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 weather[24] 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 Encín, 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 index[77] 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 $1/(p_{\mathrm{maize}}^2 + p_{\mathrm{soy}}^2 + p_{\mathrm{w.wheat}}^2) = 1/((2/4)^2 + (1/4)^2 + (1/4)^2) = 2.7$. 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 indices[24]. 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–alfalfa[21]).

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 found[19].

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 1.1-26[78] in R version 4.1.0[79]. 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 Criterion[80] 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:

$$\begin{aligned} Yield = {} & \beta_0 + \beta_{SD} SD + \beta_t t + \beta_{SD^2} SD^2 + \beta_{SDt} SD\,t + \beta_{SD^2t} SD^2 t \\ & + \beta_{fert_H} fert_H + \beta_{fert_H SD} fert_H SD + \beta_{fert_H SD^2} fert_H SD^2 \end{aligned} \tag{1}$$

$$\begin{aligned} Yield = {} & \beta_0 + \beta_{SD} SD + \beta_t t + \beta_{SD^2} SD^2 + \beta_{t^2} t^2 + \beta_{SDt} SD\,t \\ & + \beta_{SD^2t} SD^2 t + \beta_{SDt^2} SD\,t^2 + \beta_{SD^2t^2} SD^2 t^2 + \beta_{fert_H} fert_H \\ & + \beta_{fert_H SD} fert_H SD + \beta_{fert_H SD^2} fert_H SD^2 \end{aligned} \tag{2}$$

$$\begin{aligned} Yield = {} & \beta_0 + \beta_{SD} SD + \beta_t t + \beta_{SD^2} SD^2 + \beta_{t^2} t^2 + \beta_{SDt} SD\,t \\ & + \beta_{SD^2t} SD^2 t + \beta_{SDt^2} SD\,t^2 + \beta_{fert_H} fert_H + \beta_{fert_H SD} fert_H SD \\ & + \beta_{fert_H SD^2} fert_H SD^2 \end{aligned} \tag{3}$$

$$Yield = \beta_0 + \sum_{i=2}^{4} \beta_{FR_i} FR_i + \beta_t t + \sum_{i=2}^{4} \beta_{FR_i t} FR_i t + \beta_{fert_H} fert_H + \sum_{i=2}^{4} \beta_{fert_H FR_i} fert_H FR_i \tag{4}$$

$$\begin{aligned} Yield = {} & \beta_0 + \sum_{i=2}^{3} \beta_{FR_i} FR_i + \beta_t t + \sum_{i=2}^{3} \beta_{FR_i t} FR_i t + \beta_{t^2} t^2 \\ & + \sum_{i=2}^{3} \beta_{FR_i t} FR_i t^2 + \beta_{fert_H} fert_H + \sum_{i=2}^{3} \beta_{fert_H FR_i} fert_H FR_i \end{aligned} \tag{5}$$

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:

$$Yield = \beta_0 + \beta_{leg_Y} leg_Y + \beta_{ley_Y} ley_Y + +\beta_{bl_Y} bl_Y \tag{6}$$

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 R[81], 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 1.6.2-1[82]) and ggeffects package (version 1.1.1) for plots[83]. We used map data associated with the package rnaturalearth (version 0.1)[84] to produce Fig. 1 and all plots were created using ggplot2 package[85].

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. Strock, 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.

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Increasing crop rotational diversity can enhance cereal yields
Supplementary Information

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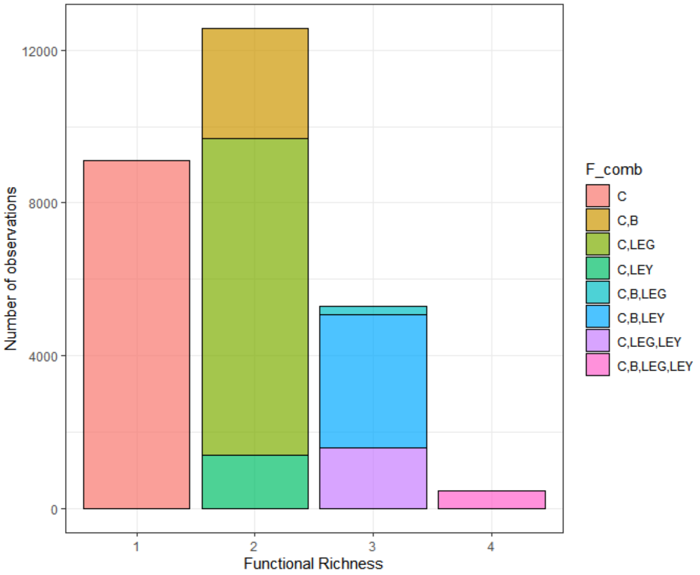
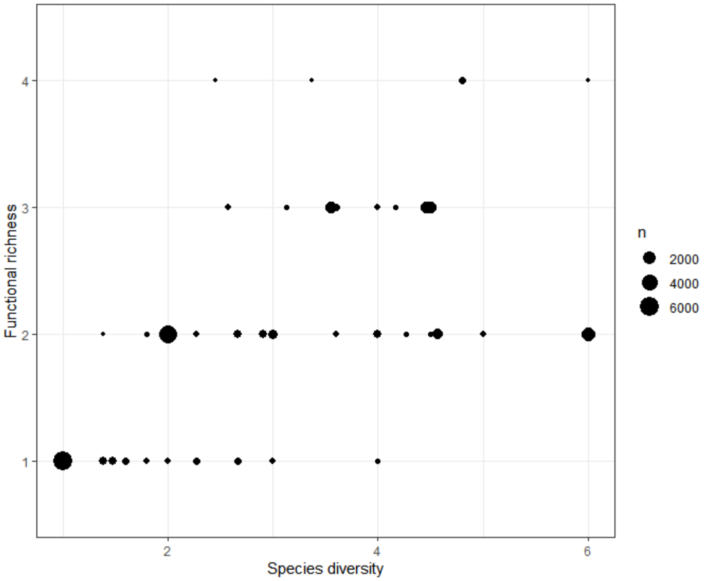
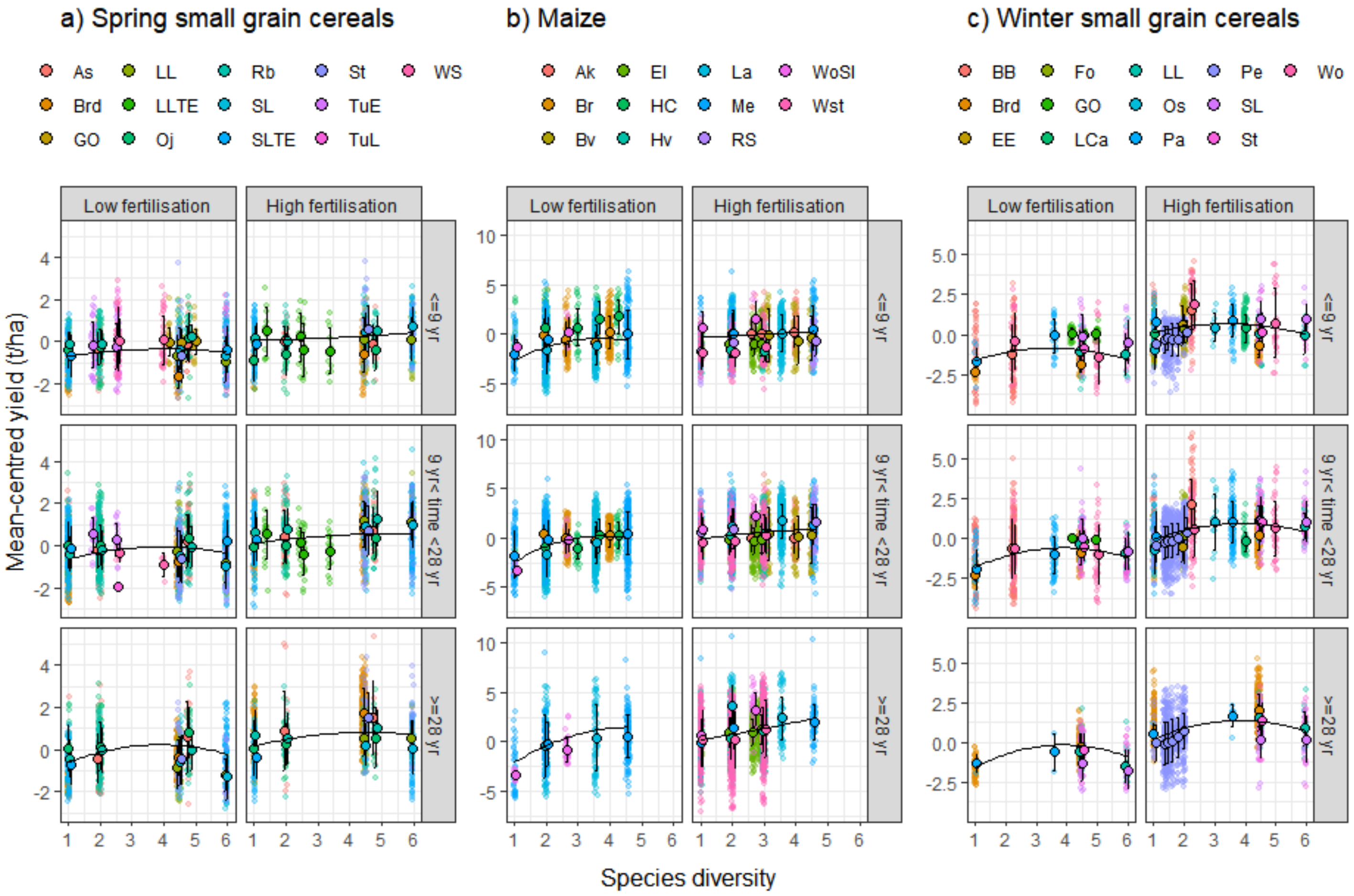


Supplementary Figure 1. Identity of functional richness levels. Distribution of crop functional groups (C=cereal, B=broadleaf, LEG=legume, LEY= biennial or perennial ley) within each level of functional richness, i.e., number of yield observations for each crop functional group included in the rotation, across the whole dataset.



Supplementary Figure 2. Relationship between species diversity (inverse Simpson's diversity index) and functional richness (number of crop functional groups included in the rotation) across all indicator crop datasets. The size of the circle is relative to the sample size (n) of yield observations for each species diversity x functional richness combination.


a) Spring small grain cereals
As
Brd
GO
LL
LLTE
Oj
Rb
SL
SLTE
St
TuE
TuL
WS
b) Maize
Ak
Br
Bv
El
HC
Hv
La
Me
RS
WoSl
Wst
c) Winter small grain cereals
BB
Brd
EE
Fo
GO
LCa
LL
Os
Pa
Pe
SL
St
Wo
Low fertilisation
High fertilisation
<=9 yr
9 yr< time <28 yr
>=28 yr
Mean-centred yield (t/ha)
Species diversity
4
2
0
-2
10
5
0
-5
5.0
2.5
0.0
-2.5
1
2
3
4
5
6

Supplementary Figure 3. Summary of the raw data used for our models. Distribution of mean-centred yields (calculated as the observed yield minus the long-term within-site average across all crop rotational diversity (CRD) treatments for an indicator crop species) for each indicator crop group (a-c). We modelled across CRD levels (species diversity based on the inverse Simpson's diversity index; x axis), sites (colours) at low (left panels) and high (right panels) external N fertilisation and grouped by three different time periods (rows) in years (yr) since the beginning of each experiment. Mean yields (also based on mean-centred yields per site, fertilisation, diversity and time periods) are represented by the black outlined circles coloured by site, and error bars represent standard deviations from the means. See Supplementary Table 1 for site codes. Black curves represent model predicted mean-centred yields for each indicator crop group after 5 (top), 20 (middle) and 35 (bottom) years since the beginning of the experiment (as in Fig. 2 but not compared to monocultures at year = 0).

Supplementary Table 1. Site information. Site location indicated as country and geographic coordinates. Fertilisation levels are relative to common practices, where high = at or above local recommendations, low = below local recommendations. Fertilisation type indicates whether inorganic, organic or both fertilisations were used for the specified fertilisation level. Application rates of fertilisers and crop protection chemicals were either the same between diversity treatments or variable based on need. Tillage regimes were always the same for comparisons between diversity levels and included: CT = Conventional Tillage, NT = No Tillage, RT = Reduced Tillage. Years indicates the difference between the last and first year of sampling per site +1. Seasonal mean temperature and total precipitation refer to the long-term average growing season temperature and mean of the total growing season precipitation to which yield data refer (sources of daily meteorological data: EObs [1]; Livneh *et al.* [2] for North America). The LTEs growing seasons were determined as the average period from sowing to harvest dates for spring-sown crops (S), and the average period from the end of winter dormancy to harvest for winter-sown crops (W). We set the end of winter dormancy as the day when the growing degree days corrected by photoperiod (calculated as in Olesen *et al.* [3]) began to increase steeply, i.e., increased of at least 4 °C with respect to the previous day. Annual mean temperature and precipitation are reported within parenthesis.

Site (code)	**Country (state/province for North America)**	**Lat., °**	**Lon., °**	**Study design (replication)**	**Fertilisation level and type**	**Application rates between CRD comparisons**		**Till**	**Years**	**Seasonal (annual) mean T, °C**	**Seasonal (annual) total P, cm**	**Source**
						Fertiliser	**Crop protection**					
Akron (Ak)	US (CO)	40.2	-103.1	Randomised block (3)	High Inorganic	Same	Variable	NT	23	S=19.6 (10.7)	S=27.7 (40.1)	4; 5
Beltsville (Bv)	US (MD)	39	-76.9	Split plot (4)	High Organic	Variable	Same (nill)	CT	20	S=22.4 (13.6)	S=52.3 (111.6)	6; 7
Broadbalk (BB)	England	51.82	-0.35	Split-split plot (1)	High Organic & Inorganic; Low Organic & Inorganic;	Same	Same	CT	21	W=12.3 (10.3)	W=40 (67.7)	8
Brody (Brd)	Poland	52.43	16.3	Split plot (4)	High Organic & Inorganic; Low Inorganic	Same	Same	CT	63	S=16.6 W=14 (8.6)	S=24.4 W=29.2 (53.9)	9
Brookings (Br)	US (SD)	44.4	-96.8	Randomised block (4)	Low Inorganic	Variable	Same	NT	16	S=18.1 (6.5)	S=43.4 (65.8)	10
El Encin (EE)	Spain	40.48	-3.37	Split-split plot (4)	High Inorganic	Variable	Variable	CT; NT; RT	14	W=13.3 (14.7)	W=16.9 (30.8)	11; 12; 13
Elora (El)	Canada (ON)	43.6	-80.4	Split plot (4)	High Inorganic	Same	Same	CT; NT;	36	S=13.7 (7.1)	S=59.3 (91.5)	14; 15

								RT				
Foggia (Fo)	Italy	41.45	15.6	Strip-strip plot (3)	High Inorganic	Same	Same	CT; RT	17	W=14.7 (17.6)	W=33.6 (65.2)	16; 17; 18
Grabow (GO)	Poland	51.35	21.66	Randomised block (4)	Low Organic	Same	NA	CT	10	S=13 W=15 (8.9)	S=29.7 W=27.5 (56.6)	19
Hickory Corners (HC)	US (MI)	42.4	-85.4	Randomised block (4)	Low Inorganic	Same (nill)	Same (nill)	CT	16	S=18.2 (10.1)	S=61.7 (101.9)	20; 21
Hoytville (Hv)	US (OH)	41.2	-83.8	Split plot (3)	High Inorganic	Same	NA	CT; NT; RT	51	S=19.5 (9.8)	S=43 (86.5)	22
La Canaleja (LC)	Spain	40.53	-3.33	Split plot (1)	High Inorganic	Variable	Variable	CT; NT	14	W=13 (14.6)	W=18.9 (37.7)	23
Lamberton (La)	US (MN)	44.2	-95.3	Split plot (3)	High Organic & Inorganic; Low Inorganic	Same	Variable	CT; RT	27	S=18.7 (7.2)	S=45.3 (70.6)	24; 25
Lanna Ley (LL)	Sweden	58.33	13.12	Split plot (1)	High or Low Organic	Same	Variable	CT	46	S=13.9 W=13.5 (6.6)	S=24.1 W=24.8 (57.1)	26
Lännäs LTE (LLTE)	Sweden	58.33	13.12	Split plot (1)	High Organic	Variable and Same	Variable	CT	33	S=14.24 (7.2)	S=26.83 (58.5)	26
Mead (Me)	US (NE)	41.1	-96.5	Split plot (5)	High or Low Inorganic	Same	Same	CT; NT	31	S=20.7 (10.1)	S=47.3 (74.4)	27; 28
Ojebyn (Oj)	Sweden	65.35	21.38	Split plot (1)	High Organic & Inorganic; Low Inorganic	Variable and Same	Variable	CT	43	S=12 (2.4)	S=22.5 (54.9)	26
Osiny (Os)	Poland	51.46	22.05	Block design (1)	High Inorganic	Variable	Variable	CT	24	W=14.9 (8.7)	W=25 (56)	29
Padova (Pa)	Italy	45.35	11.97	Split plot (3)	High Organic & Inorganic; Low Inorganic	Variable	Variable	CT	30	W=14.8 (14.2)	W=29.9 (80.6)	30
Perugia (Pe)	Italy	42.96	12.38	Split plot (3)	High Inorganic	Same	Variable	CT	40	W=12.8	W=30.6	31

										(14.4)	(73.2)	
Robacksdalen (Ro)	Sweden	63.82	20.28	Split plot (1)	High Organic & Inorganic; Low Inorganic	Variable and Same	Variable	CT	42	S=11.8 (3)	S=23.6 (63.6)	26
Rock Springs (RS)	US (PA)	40.7	-78	Split plot (4)	High Organic & Inorganic	Variable	NA	CT	17	S=17.3 (9.9)	S=49.4 (109.5)	32
Stenstugu Ley (St)	Sweden	57.6	18.43	Split plot (1)	High or Low Organic	Same	Variable	CT	45	S=13.8 W=14 (7.2)	S=18.9 W=18.3 (54.7)	26
Säby Ley (SL)	Sweden	59.82	17.7	Split plot (1)	High or Low Organic	Same	Variable	CT	42	S=14 W=14.1 (6.1)	S=23.5 W=23.3 (55.4)	26
Säby LTE (SLTE)	Sweden	59.82	17.7	Split plot (2)	High or Low Organic	Variable	Variable	CT	45	S=14.1 (6.1)	S=23.5 (55.6)	26
Tulloch Early (TuE)	Scotland	57.18	-2.25	Randomised block (2)	Low Organic	Variable	Same (nill)	CT	16	S=11.4 (8.2)	S=33.7 (82.3)	33
Tulloch Late (TuL)	Scotland	57.18	-2.25	Randomised block (2)	Low Organic	Variable	Same (nill)	CT	12	S=11.7 (8.5)	S=38.6 (83.8)	33
Woburn (Wo)	England	52.02	0.58	Split-split plot (1)	High or Low Inorganic	Same	Same	CT	21	W=12.5 (10.5)	W=36.7 (60.4)	8
Woodside (WS)	Scotland	57.63	-3.4	Randomised block (2)	Low Organic	Variable	Same (nill)	CT	12	S=11.8 (8.5)	S=31.6 (71.1)	33
Woodslee (WoSl)	Canada (ON)	42.21	-82.75	Split plot (1)	High or Low Inorganic	Same	Variable	CT	58	S=19.2 (9.4)	S=41.7 (86)	34; 35; 36
Wooster (Wst)	US (OH)	40.8	-81.9	Split plot (3)	High Inorganic	Same	NA	CT; NT; RT	52	S=18.8 (9.6)	S=48.2 (95.2)	22
Ås (As)	Sweden	63.25	14.57	Split plot (1)	High Organic & Inorganic; Low Inorganic	Variable and same	Variable	CT	44	S=11.1 (2.9)	S=25.0 (51.7)	26

Supplementary Table 2. Crop rotation information. Cropping sequence of all crop rotations per site (see Supplementary Table 1 for site name and information) whereby '-' indicates a shift in time (usually a year) when the next crop is grown, '+' indicates multiple crop species grown together in the same field and '/' indicates that the two crops either side are grown in alternate years. Mean yield and standard errors refer to the long-term raw yield mean of each indicator crop per rotation and per external N input level for the period to which the yield data refer (see Supplementary Table 1 data years). Yields are based on 100 % dry weight. Mean yields were calculated regardless of management (except N input), therefore could include yields from different tillage. The number of observations relates to yield observations in our dataset for each indicator crop per rotation and per external N input level. Note that some rotations will have observations for more than one indicator crop. Also listed are the CRD metrics, species diversity (SD; based on the inverse Simpson's diversity index) and functional richness (FR; the number of functional groups included in the rotation) and a list of the specific functional groups present in the rotation (whereby, C = cereal, B = broadleaf, Ley = ley and LEG = legume). Crop abbreviations are as follows: A = alfalfa, Be = beans, B = barley (phenology not known), C = canola, CP = chickpea, F= fallow, FB = fava bean, L = ley, Lg = legume (specific species changed over the years), M = maize, Mi = millet, O = oats, OSR = oilseed rape, Pe = pea, Po = potato, R = rye, RC = red clover, S = soy, Saff = safflower, SB = spring barley, SF = sunflower, Sg = sorghum, SuB = sugarbeet, SW = spring wheat, Swe = swede, Ti = timothy, US = the following crop was undersown with the previous crop, V = vetch, W = wheat (phenology not known), WB = winter barley, WR = winter rye, WT = winter triticale, WW = winter wheat.

Site code	**Cropping sequence**	**Indicator crop**	**Mean yield ± SE, t ha^{-1} (observations)**		**SD**	**FR**	**Functional groups present**
			Low N input	**High N input**			
Ak	M-F-WW	M	-	2.40 ± 0.15 (124)	3	1	C
	M-Mi-WW	M	-	1.91 ± 0.16 (63)	3	1	C
	M-Mi-F-WW	M	-	2.50 ± 0.19 (66)	4	1	C
	M-Mi-WW-WW	M	-	2.26 ± 0.20 (53)	2.67	1	C
	M-Saff-F-WW	M	-	2.07 ± 0.17 (64)	4	2	C, B
	M-Mi-Pe-WW	M	-	2.35 ± 0.21 (63)	4	2	C, LEG
Bv	M+R-S+V	M	-	3.78 ± 0.27 (74)	4	2	C, LEG
	M+R-S-WW+V	M	-	3.98 ± 0.30 (76)	4.5	2	C, LEG
	M+R-S-WW-A-A-A	M	-	4.22 ± 0.31 (72)	3.13	3	C, LEG, LEY
BB	WW	WW	3.26 ± 0.11 (210)	5.7 ± 0.1 (126)	1	1	C
	O-M-WW-WW-WW	WW	3.64 ± 0.11 (270)	6.42 ± 0.12 (162)	2.27	1	C
Brd	SB	SB	1.24 ± 0.04 (252)	3.23 ± 0.05 (504)	1	1	C
	WR	WR	1.82 ± 0.04 (252)	4.07 ± 0.06 (504)	1	1	C
	Po-SB-WT-A-A-WW-WR	SB	2.22 ± 0.05 (252)	3.95 ± 0.06 (504)	4.45	3	C, B, LEY
	Po-SB-WT-A-A-WW-WR	WR	3.05 ± 0.06 (252)	4.83 ± 0.06 (504)	4.45	3	C, B, LEY

Br	M-S	M	5.16 ± 0.17 (64)	-	2	2	C, LEG
	M-SF-SW-S	M	5.48 ± 0.16 (64)	-	4	3	C, B, LEG
	M-S-SW+RC	M	4.42 ± 0.17 (64)	-	3.6	2	C, LEG
	M-O-WW-S	M	5.33 ± 0.16 (64)	-	4	2	C, LEG
	M-S-SW-Pe	M	5.69 ± 0.21 (64)	-	4	2	C, LEG
	M-M-S-SW	M	4.40 ± 0.14 (128)	-	2.67	2	C, LEG
	M-S-SW-SF	M	5.02 ± 0.17 (64)	-	4	3	C, B, LEG
	M-C-WW-S	M	5.09 ± 0.16 (64)	-	4	3	C, B, LEG
	M-Pe-WW-S	M	5.13 ± 0.17 (64)	-	4	2	C, LEG
	M-S-SW-S	M	5.45 ± 0.18 (64)	-	2.67	2	C, LEG
EE	WW	WW	-	1.58 ± 0.12 (84)	1	1	C
	Pe/V-WW	WW	-	2.06 ± 0.13 (84)	2	2	C, LEG
El	M	M	-	7.16 ± 0.08 (296)	1	1	C
	M-M-A-A	M	-	7.78 ± 0.10 (296)	2	2	C, LEY
	M-M-S-S	M	-	7.25 ± 0.09 (296)	2	2	C, LEG
	M-M-S-WW	M	-	7.48 ± 0.09 (288)	2.67	2	C, LEG
	M-M-O-SB	M	-	7.47 ± 0.09 (295)	2.67	1	C
	M-M-S-WW+RC	M	-	7.56 ± 0.09 (294)	2.91	2	C, LEG
	M-M-O+RC-SB+RC	M	-	7.76 ± 0.10 (287)	2.91	2	C, LEG
Fo	WW	WW	-	3.51 ± 0.17 (54)	1	1	C
	Lg-WW	WW	-	4.11 ± 0.19 (54)	2	2	C, LEG
GO	M-O+B+W+Pe-L-L-SW/WW	SW	1.68 ± 0.12 (28)	-	4.17	3	C, LEG, LEY
	M-O+B+W+Pe-L-L-SW/WW	WT	4.35 ± 0.06 (12)	-	4.17	3	C, LEG, LEY
	M-O+B+W+Pe-L-L-SW/WW	WW	2.38 ± 0.09 (20)	-	4.17	3	C, LEG, LEY
	M-SB-O+B+W+Pe-Pe-SW/WW	SW	1.70 ± 0.11 (28)	-	5	2	C, LEG
	M-SB-O+B+W+Pe-Pe-SW/WW	WT	4.14 ± 0.05 (12)	-	5	2	C, LEG
	M-SB-O+B+W+Pe-Pe-SW/WW	WW	2.43 ± 0.08 (20)	-	5	2	C, LEG
	M-O+B+W+Pe-SW-RC-SW/WW	SW	1.68 ± 0.11 (28)	-	4.55	2	C, LEG
	M-O+B+W+Pe-SW-RC-SW/WW	WT	3.90 ± 0.05 (12)	-	4.55	2	C, LEG
	M-O+B+W+Pe-SW-RC-SW/WW	WW	2.40 ± 0.07 (20)	-	4.55	2	C, LEG
HC	M	M	2.64 ± 0.19 (64)	-	1	1	C

	M+RC	M	3.38 ± 0.23 (63)	-	2	2	C, LEG
	M-S	M	4.05 ± 0.21 (63)	-	2	2	C, LEG
	M-S-WW	M	3.70 ± 0.22 (64)	-	3	2	C, LEG
	M+RC-S-WW+RC	M	4.78 ± 0.23 (64)	-	3.6	2	C, LEG
	M+RC+R-S-WW+RC+R	M	4.93 ± 0.21 (63)	-	4.26	2	C, LEG
Hv	M	M	-	6.93 ± 0.10 (453)	1	1	C
	M-S	M	-	7.45 ± 0.09 (454)	2	2	C, LEG
	M-O-L	M	-	7.92 ± 0.11 (452)	3	2	C, LEG
LCa	WW	WW	-	0.95 ± 0.05 (170)	1	1	C
	F-WW-Lg-WB	WW	-	1.29 ± 0.06 (170)	4	2	C, LEG
La	M-S	M	4.72 ± 0.09 (720)	7.25 ± 0.08 (681)	2	2	C, LEG
	M-S-O+A-A	M	5.71 ± 0.08 (731)	7.59 ± 0.07 (725)	3.56	3	C, LEG, LEY
LL	SW-F-OSR-WW-O-SB	O	2.05 ± 0.1 (91)	3.63 ± 0.09 (92)	6	2	C, B
	SW-F-OSR-WW-O-SB	SB	2.01 ± 0.1 (90)	3.77 ± 0.12 (90)	6	2	C, B
	SW-F-OSR-WW-O-SB	WW	2.81 ± 0.11 (88)	4.65 ± 0.11 (88)	6	2	C, B
	L-L-OSR-WW-O-SB	O	2.49 ± 0.1 (92)	3.91 ± 0.1 (92)	4.5	3	C, B, LEY
	L-L-OSR-WW-O-SB	SB	2.4 ± 0.13 (89)	3.58 ± 0.12 (90)	4.5	3	C, B, LEY
	L-L-OSR-WW-O-SB	WW	3.43 ± 0.11 (88)	5.07 ± 0.12 (88)	4.5	3	C, B, LEY
LLTE	SB-L-L-L-L-L	SB	-	3.07 ± 0.20 (31)	1.38	2	C, LEY
	SB-L-L-L-SB-SB+OSR+Lg	SB	-	2.74 ± 0.15 (32)	2.45	4	C, B, LEG, LEY
	SB-L-L-SB-Po-SB+OSR+Lg	SB	-	2.21 ± 0.16 (31)	3.38	4	C, B, LEG, LEY
	SB-L-SB-Po-SB-Po	SB	-	2.17 ± 0.20 (30)	2.57	3	C, B, LEG
Me	M	M	4.63 ± 0.13 (310)	6.93 ± 0.17 (155)	1	1	C
	M-S	M	6.45 ± 0.12 (310)	7.51 ± 0.17 (155)	2	2	C, LEG
	M-O+RC-Sg-S	M	7.43 ± 0.11 (310)	8.24 ± 0.16 (155)	4.57	2	C, LEG
	M-S-Sg-O+RC	M	6.73 ± 0.14 (310)	7.69 ± 0.18 (155)	4.57	2	C, LEG
Oj	SB	SB	2.67 ± 0.08 (168)	2.51 ± 0.17 (42)	1	1	C
	SB-SB-Lg+O	SB	2.84 ± 0.08 (162)	2.57 ± 0.18 (41)	2	2	C, LEG
	SB-SB-L-L	SB	2.65 ± 0.07 (164)	2.79 ± 0.14 (45)	2	2	C, LEY
	SB-L-L-Pe+O-Po-R	SB	3.17 ± 0.12 (84)	2.97 ± 0.17 (40)	4.8	4	C, B, LEG, LEY

Os	WW	WW	-	4.90 ± 0.29 (23)	1	1	C
	OSR-WW-SW	WW	-	6.54 ± 0.31 (23)	3	2	C, B
Pa	WW	WW	2.46 ± 0.11 (90)	4.6 ± 0.11 (180)	1	1	C
	M-SuB-M-WW-A-A	WW	3.58 ± 0.13 (90)	5.3 ± 0.09 (180)	3.6	3	C, B, LEY
Pe	WW	WW	-	4.19 ± 0.09 (120)	1	1	C
	WW-Sg	WW	-	5.14 ± 0.10 (93)	2	1	C
	WW-FB	WW	-	5.22 ± 0.10 (93)	2	2	C, LEG
	WW-M	WW	-	4.94 ± 0.09 (120)	2	1	C
	WW-WW-M	WW	-	4.58 ± 0.07 (240)	1.8	1	C
	WW-WW-WW-M	WW	-	4.45 ± 0.06 (357)	1.6	1	C
	WW-WW-WW-WW-M	WW	-	4.42 ± 0.05 (471)	1.47	1	C
	WW-WW-WW-WW-WW-M	WW	-	4.36 ± 0.04 (582)	1.38	1	C
	WW-OSR/SuB	WW	-	5.02 ± 0.10 (93)	2	2	C, B
	WW-SF	WW	-	5.09 ± 0.10 (93)	2	2	C, B
	WW-Pe/CP	WW	-	5.13 ± 0.10 (93)	2	2	C, B
Rb	SB	SB	2.02 ± 0.06 (168)	2.88 ± 0.15 (42)	1	1	C
	SB-SB-Lg+O	SB	2.34 ± 0.07 (167)	2.94 ± 0.18 (42)	2	2	C, LEG
	SB-SB-L-L	SB	2.09 ± 0.07 (167)	2.83 ± 0.14 (41)	2	2	C, LEY
	SB-L-L-Pe+O-Po-R	SB	2.38 ± 0.1 (84)	3.41 ± 0.17 (42)	4.8	4	C, B, LEG, LEY
RS	M	M	-	8.09 ± 0.19 (136)	1	1	C
	M-S	M	-	8.66 ± 0.17 (136)	2	2	C, LEG
	M-O+WW-RC+Ti-RC+Ti	M	-	9.13 ± 0.17 (136)	4.57	2	C, LEY
St	SW-F-OSR-WW-O-SB	O	2.1 ± 0.11 (88)	4.1 ± 0.12 (88)	6	2	C, B
	SW-F-OSR-WW-O-SB	SB	1.83 ± 0.11 (86)	2.89 ± 0.12 (84)	6	2	C, B
	SW-F-OSR-WW-O-SB	WW	3.03 ± 0.11 (86)	4.82 ± 0.12 (86)	6	2	C, B
	L-L-OSR-WW-O-SB	O	2.64 ± 0.11 (88)	3.50 ± 0.11 (88)	4.5	3	C, B, LEY
	L-L-OSR-WW-O-SB	SB	2.37 ± 0.14 (85)	2.96 ± 0.10 (84)	4.5	3	C, B, LEY
	L-L-OSR-WW-O-SB	WW	3.57 ± 0.11 (86)	4.34 ± 0.10 (86)	4.5	3	C, B, LEY
SL	SW-F-OSR-WW-O-SB	O	2.32 ± 0.12 (82)	4.01 ± 0.13 (82)	6	2	C, B
	SW-F-OSR-WW-O-SB	SB	2.35 ± 0.11 (82)	4.1 ± 0.12 (82)	6	2	C, B

	SW-F-OSR-WW-O-SB	WW	3.17 ± 0.15 (78)	4.96 ± 0.15 (78)	6	2	C, B
	L-L-OSR-WW-O-SB	O	2.85 ± 0.12 (80)	4.08 ± 0.13 (80)	4.5	3	C, B, LEY
	L-L-OSR-WW-O-SB	SB	2.78 ± 0.14 (79)	3.83 ± 0.12 (80)	4.5	3	C, B, LEY
	L-L-OSR-WW-O-SB	WW	3.76 ± 0.15 (78)	5.03 ± 0.15 (78)	4.5	3	C, B, LEY
SLTE	O	O	3.4 ± 0.09 (176)	3.56 ± 0.09 (176)	1	1	C
	SB	SB	3.21 ± 0.07 (174)	3.76 ± 0.07 (176)	1	1	C
	SW	SW	2.97 ± 0.07 (174)	3.55 ± 0.07 (174)	1	1	C
	F-OSR-WW-O-SB-SW	O	3.54 ± 0.09 (180)	3.9 ± 0.09 (178)	6	2	C, B
	F-OSR-WW-O-SB-SW	SB	3.57 ± 0.07 (180)	4.1 ± 0.08 (179)	6	2	C, B
	F-OSR-WW-O-SB-SW	SW	3.46 ± 0.07 (186)	4.04 ± 0.08 (185)	6	2	C, B
TuE	L-L-L-O-Swe-US O	O	5.03 ± 0.19 (32)	-	2.57	3	C, B, LEY
	L-L-L-O-Swe-US O	U/S O	3.27 ± 0.18 (32)	-	2.57	3	C, B, LEY
	L-L-L-L-O-US O	O	5.08 ± 0.19 (32)	-	1.8	2	C, LEY
	L-L-L-L-O-US O	U/S O	3.46 ± 0.19 (31)	-	1.8	2	C, LEY
TuL	L-L-L-O-Swe-US O	U/S O	2.71 ± 0.32 (24)	-	2.57	3	C, B, LEY
	US W-US Be-US B-US O-L-Po	U/S O	2.78 ± 0.32 (20)	-	6	3	C, B, LEG
	Po-US Be-US B-US O-L-US W	U/S O	2.87 ± 0.31 (21)	-	6	3	C, B, LEG
Wo	R-M-B-WW-R	WW	3.18 ± 0.23 (62)	5.02 ± 0.23 (64)	5	2	C, LEG
	L-L-L-WW-R	WW	3.78 ± 0.2 (66)	5.37 ± 0.22 (66)	2.27	2	C, LEY
WS	L-L-L-O-Po-US O	O	3.35 ± 0.22 (23)	-	2.57	3	C, B, LEY
	L-L-L-O-Po-US O	U/S O	2.64 ± 0.21 (23)	-	2.57	3	C, B, LEY
	L-L-O-Po-US O-L-Swe-US O	O	3.07 ± 0.24 (23)	-	4	3	C, B, LEY
	L-L-O-Po-US O-L-Swe-US O	U/S O	2.91 ± 0.24 (22)	-	4	3	C, B, LEY
WoSl	M	M	1.22 ± 0.12 (55)	5.05 ± 0.19 (58)	1	1	C
	M-O+A-A-A	M	3.81 ± 0.17 (58)	7 ± 0.23 (58)	2.67	2	C, LEY
Wst	M	M	-	7.19 ± 0.12 (443)	1	1	C
	M-S	M	-	7.17 ± 0.12 (450)	2	2	C, LEG
	M-O-ley	M	-	8.07 ± 0.13 (436)	3	2	C, LEG
As	SB	SB	2.73 ± 0.08 (161)	3.39 ± 0.19 (42)	1	1	C
	SB-SB-Lg+O	SB	2.57 ± 0.07 (157)	3.19 ± 0.21 (40)	2	2	C, LEG

SB-SB-L-L	SB	2.56 ± 0.07 (162)	3.3 ± 0.22 (41)	2	2	C, LEY
SB-L-L-Pe+O-Po-R	SB	3.05 ± 0.14 (82)	3.51 ± 0.21 (41)	4.8	4	C, B, LEG, LEY

Supplementary Table 3. Summary of statistics for Species Diversity (SD; based on the inverse Simpson's diversity index) and its interaction with fertilisation as explanatory factor. Model estimates, their standard errors (SE) and significance (*p*) from the linear mixed effect model 4 for a) spring small grain cereals, b) maize, and c) winter small grain cereals. The marginal and conditional R^2 show the variation explained by the fixed factors alone and by the entire model including fixed and random factors, respectively [36].

Indicator crops	*a) Spring small grain cereals*			*b) Maize*			*c) Winter small grain cereals*		
Predictor	**Estimate**	**SE**	***p***	**Estimate**	**SE**	***p***	**Estimate**	**SE**	***p***
Intercept (t ha^{-1})	-8.98×10^{-1}	1.24×10^{-1}	<0.001***	-4.43	3.45×10^{-1}	<0.001***	-2.09	1.90×10^{-1}	<0.001***
SD (t ha^{-1})	2.23×10^{-1}	5.06×10^{-2}	<0.001***	1.97	1.70×10^{-1}	<0.001***	7.42×10^{-1}	6.77×10^{-2}	<0.001***
SD^2 (t ha^{-1})	-1.11×10^{-2}	3.45×10^{-3}	0.001**	-3.97×10^{-2}	2.74×10^{-2}	0.147	-6.09×10^{-2}	1.23×10^{-2}	<0.001***
Year (t ha^{-1} yr^{-1})	-2.71×10^{-2}	7.42×10^{-3}	<0.001***	-2.51×10^{-1}	3.04×10^{-2}	<0.001***	-1.14×10^{-1}	1.09×10^{-2}	<0.001***
Year^2 (t ha^{-1} yr^{-2})	NA	NA	NA	1.22×10^{-3}	5.60×10^{-4}	0.029*	1.26×10^{-3}	2.28×10^{-4}	<0.001***
High Fertilisation (t ha^{-1})	1.02	1.01×10^{-1}	<0.001***	4.05	2.90×10^{-1}	<0.001***	1.94	1.75×10^{-1}	<0.001***
SD × Year (t ha^{-1} yr^{-1})	1.59×10^{-2}	1.66×10^{-3}	<0.001***	5.78×10^{-2}	1.47×10^{-2}	<0.001***	2.43×10^{-2}	2.97×10^{-3}	<0.001***
SD^2 × Year (t ha^{-1} yr^{-1})	-2.08×10^{-3}	2.52×10^{-4}	<0.001***	-1.12×10^{-2}	2.87×10^{-3}	<0.001***	-1.50×10^{-3}	4.04×10^{-4}	<0.001***
SD × Year^2 (t ha^{-1} yr^{-2})	NA	NA	NA	-1.22×10^{-3}	3.43×10^{-4}	<0.001***	-2.40×10^{-4}	2.81×10^{-5}	<0.001***

SD^2 × $Year^2$ ($t\ ha^{-1}\ yr^{-2}$)	NA	NA	NA	3.07×10^{-4}	7.37×10^{-5}	<0.001***	NA	NA	NA
SD × High Fertilisation ($t\ ha^{-1}$)	-2.99×10^{-1}	5.02×10^{-2}	<0.001***	-2.05	1.44×10^{-1}	<0.001***	-2.02×10^{-1}	6.18×10^{-2}	0.001**
SD^2 × High Fertilisation ($t\ ha^{-1}$)	4.71×10^{-2}	7.37×10^{-3}	<0.001***	2.97×10^{-1}	2.55×10^{-2}	<0.001***	2.40×10^{-2}	1.03×10^{-2}	0.020*
No. of observations		*8492*			*11833*			*7198*	
Marginal R^2		*0.154*			*0.128*			*0.295*	
Conditional R^2		*0.719*			*0.771*			*0.775*	

Supplementary Table 4. Summary of statistics with Functional Richness (FR; number of crop functional groups included in the rotation) and its interaction with fertilisation as explanatory factor. Model estimates, their standard errors (SE) and significance (*p*) from the linear mixed effect model 5 for a) spring small grain cereals, b) maize, and c) winter small grain cereals based on mean-centred observations, i.e., the difference from to the long-term within-site average across all CRD treatments. The marginal and conditional R^2 show the variation explained by the fixed factors alone and by the entire model including fixed and random factors, respectively [36].

Indicator crops	*a) Spring small grain cereals*			*b) Maize*			*c) Winter small grain cereals*		
Predictor	**Estimate**	**SE**	***p***	**Estimate**	**SE**	***p***	**Estimate**	**SE**	***p***
Intercept ($t\ ha^{-1}$)	-7.46×10^{-1}	1.10×10^{-1}	<0.001***	-2.98	2.88×10^{-1}	<0.001***	-1.66	1.75×10^{-1}	<0.001***
FR2 ($t\ ha^{-1}$)	1.35×10^{-1}	3.93×10^{-2}	0.001**	1.97	8.48×10^{-2}	<0.001***	6.00×10^{-1}	1.05×10^{-1}	<0.001***
FR3 ($t\ ha^{-1}$)	3.28×10^{-1}	4.87×10^{-2}	<0.001***	1.90	1.34×10^{-1}	<0.001***	7.64×10^{-1}	8.50×10^{-2}	<0.001***
FR4 ($t\ ha^{-1}$)	2.10×10^{-1}	7.45×10^{-2}	0.005**	NA	NA	NA	NA	NA	NA
Year ($t\ ha^{-1}\ yr^{-1}$)	4.20×10^{-3}	2.94×10^{-3}	0.154	1.64×10^{-2}	2.23×10^{-2}	0.463	-2.06×10^{-2}	1.13×10^{-2}	0.069
$Year^2$ ($t\ ha^{-1}\ yr^{-2}$)	NA	NA	NA	1.26×10^{-4}	4.43×10^{-4}	0.776	7.59×10^{-4}	2.16×10^{-4}	0.001**
High Fertilisation ($t\ ha^{-1}$)	7.92×10^{-1}	8.02×10^{-2}	<0.001***	2.61	2.44×10^{-1}	<0.001***	1.82	1.66×10^{-1}	<0.001***
FR2 × Year ($t\ ha^{-1}\ yr^{-1}$)	3.53×10^{-3}	1.41×10^{-3}	0.012*	7.22×10^{-3}	6.83×10^{-3}	0.291	1.55×10^{-2}	8.59×10^{-3}	0.071
FR3 × Year ($t\ ha^{-1}\ yr^{-1}$)	1.62×10^{-2}	1.42×10^{-3}	<0.001***	1.66×10^{-1}	1.94×10^{-2}	<0.001***	3.95×10^{-2}	5.77×10^{-3}	<0.001***
FR4 × Year ($t\ ha^{-1}\ yr^{-1}$)	1.42×10^{-2}	2.90×10^{-3}	<0.001***	NA	NA	NA	NA	NA	NA
FR2 × $Year^2$ ($t\ ha^{-1}\ yr^{-2}$)	NA	NA	NA	2.07×10^{-4}	1.43×10^{-4}	0.148	-3.52×10^{-4}	2.01×10^{-4}	0.079

FR3 × Year^2 (t ha^{-1} yr^{-2})	NA	NA	NA	-5.20×10^{-3}	7.11×10^{-4}	<0.001***	-5.95×10^{-4}	9.65×10^{-5}	<0.001***
FR4 × Year^2 (t ha^{-1} yr^{-2})	NA	NA	NA	NA	NA	NA	NA	NA	NA
FR2 × High Fertilisation (t ha^{-1})	1.93×10^{-1}	3.85×10^{-2}	<0.001***	-1.74	6.98×10^{-2}	<0.001***	-1.72×10^{-1}	8.62×10^{-2}	0.046*
FR3 × High Fertilisation (t ha^{-1})	-2.23×10^{-1}	4.78×10^{-2}	<0.001***	-2.18	1.01×10^{-1}	<0.001***	-4.64×10^{-1}	6.45×10^{-2}	<0.001***
FR4 × High Fertilisation (t ha^{-1})	4.54×10^{-2}	7.68×10^{-2}	0.554	NA	NA	NA	NA	NA	NA
No. of observations		*8429*			*11833*			*7198*	
Marginal R^2		*0.173*			*0.125*			*0.303*	
Conditional R^2		*0.730*			*0.766*			*0.776*	

Supplementary Table 5. Summary of statistics for inclusion of annual legumes, annual broadleaves, and biennial or perennial ley in the rotation as dependent variable. With linear mixed effect model estimates, their standard errors (SE) and significance (*p*) for spring small grain cereals, maize, and winter small grain cereals. Mean-centred observations, i.e., the difference from to the long-term within-site average across all CRD treatments were used. The marginal and conditional R^2 show the variation explained by the fixed factors alone and by the entire model including fixed and random factors, respectively [36].

Indicator crops	*a) Spring small grain cereals*			*b) Maize*			*c) Winter small grain cereals*		
Predictor	**Estimate**	**SE**	***p***	**Estimate**	**SE**	***p***	**Estimate**	**SE**	***p***
Intercept (t ha^{-1})	-4.32×10^{-1}	1.28×10^{-1}	0.001**	-1.05	1.98×10^{-1}	0.001**	-7.20×10^{-1}	1.39×10^{-1}	0.001**
Ley (t ha^{-1})	3.02×10^{-3}	4.46×10^{-2}	0.946	5.52×10^{-1}	4.62×10^{-2}	0.001**	5.48×10^{-2}	6.27×10^{-2}	0.382
Legumes (t ha^{-1})	2.06×10^{-1}	4.46×10^{-2}	<0.001***	7.35×10^{-1}	4.24×10^{-2}	0.001**	4.16×10^{-1}	5.01×10^{-2}	0.001**
Broadleaves (t ha^{-1})	3.40×10^{-1}	2.84×10^{-2}	<0.001***	9.93×10^{-2}	1.20×10^{-1}	0.409	4.51×10^{-1}	5.10×10^{-2}	0.001**
No. of observations	*8429*			*11833*			*7198*		
Marginal R^2	*0.0267*			*0.0329*			*0.0547*		
Conditional R^2	*0.528*			*0.481*			*0.597*		

Supplementary Table 6. Stepwise model selection for yield responses of the three indicator crop groups to Species Diversity (SD; based on the inverse Simpson's diversity index) over time (Years since experiments begun) and with high or low fertilisation. Polynomials of Year and SD and interaction terms were explored as part of the model selection process and only models that were based on hypotheses were included. Values of Akaike Information Criteria (AIC) and p refer to models that exclude the predictor shown on the leftmost column. For each step, single predictors with non-significant contribution to the model were removed based on the lowest AIC, and the process was repeated until all predictors showed statistical significance ($p < 0.05$). Asterisks on AIC and p values indicate predictors that were removed from the final model at a given step.

Full model: Yield ~ SD + SD^2 + Year + Year^2 + Fertilisation + SD : Year + SD^2 : Year + SD : Year^2 + SD^2 : Year^2 + Fertilisation : SD + Fertilisation : SD^2 + (1|Site:M.group) + (1|Site:C.year)		*a) Spring small grain cereals*		*b) Maize*		*c) Winter small grain cereals*	
Predictors dropped from model	**Step**	**AIC**	***p***	**AIC**	***p***	**AIC**	***p***
None	1	18249	-	37196	-	16987	-
SD : Year	1	18253	0.014	37210	<0.001	16993	0.005
SD^2 : Year	1	18250	0.066	37210	<0.001	16986	0.337
SD : Year^2	1	18247	0.695*	37207	<0.001	16986	0.220
SD^2 : Year^2	1	18247	0.562	37212	<0.001	16985	0.967*
Fertilisation : SD	1	18282	<0.001	37395	<0.001	16996	0.001
Fertilisation : SD^2	1	18287	<0.001	37330	<0.001	16990	0.020
None	2	18247	-	NA	NA	16985	-
SD : Year	2	18301	<0.001	NA	NA	17049	<0.001
SD^2 : Year	2	18309	<0.001	NA	NA	16997	<0.001

SD : Year^2	2	18246	0.292*	NA	NA	17055	<0.001
Fertilisation : SD	2	18280	<0.001	NA	NA	16994	0.001
Fertilisation : SD^2	2	18286	<0.001	NA	NA	16988	0.020
None	3	18246	-	NA	NA	NA	NA
Year^2	3	18245	0.817*	NA	NA	NA	NA
SD : Year	3	18336	<0.001	NA	NA	NA	NA
SD^2 : Year	3	18312	<0.001	NA	NA	NA	NA
Fertilisation : SD	3	18280	<0.001	NA	NA	NA	NA
Fertilisation : SD^2	3	18285	<0.001	NA	NA	NA	NA
None	4	18245	-	NA	NA	NA	NA
SD : Year	4	18334	<0.001	NA	NA	NA	NA
SD^2 : Year	4	18310	<0.001	NA	NA	NA	NA
Fertilisation : SD	4	18278	<0.001	NA	NA	NA	NA
Fertilisation : SD^2	4	18283	<0.001	NA	NA	NA	NA

Supplementary Table 7. Stepwise model selection for yield responses of the three indicator crop groups to Functional Richness (FR) over time (Years since experiments begun) and with high or low fertilisation. Polynomials of Year and interaction terms were explored as part of the model selection process and only models that were based on hypotheses were included. Values of Akaike Information Criteria (AIC) and *p* refer to models that exclude the predictor shown on the leftmost column. For each step, single predictors with non-significant contribution to the model were removed based on the lowest AIC, and the process was repeated until all predictors showed statistical significance ($p < 0.05$). Asterisks on AIC and *p* values indicate predictors that were removed from the final model at a given step.

Full model: Yield ~ FR + Year + Year^2 + Fertilisation + FR : Year + FR : Year^2 + FR : Fertilisation + (1|Site:M.group) + (1|Site:C.year)		*a) Spring small grain cereals*		*b) Maize*		*c) Winter small grain cereals*	
Predictors dropped from model	**Step**	**AIC**	***p***	**AIC**	***p***	**AIC**	***p***
None	1	18200	-	37524	-	17136	-
FR : Year	1	18220	<0.001	37597	<0.001	17179	<0.001
FR : Year^2	1	18199	0.199*	37582	<0.001	17171	<0.001
Fertilisation : FR	1	18284	<0.001	38161	<0.001	17190	<0.001
None	2	18199	-	NA	NA	NA	NA
Year^2	2	18197	0.977*	NA	NA	NA	NA
FR: Year	2	18341	<0.001	NA	NA	NA	NA
Fertilisation : FR	2	18282	<0.001	NA	NA	NA	NA
None	3	18197	-	NA	NA	NA	NA
FR : Year	3	18339	<0.001	NA	NA	NA	NA

Fertilisation : FR	3	18280	<0.001	NA	NA	NA	NA

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In this thesis, the potentiality of crop rotational diversity effects on crop production, fertiliser dependency, and climate adaptation were evaluated. The results indicate that increasing crop rotational diversity can enhance cereal yields and rotation outputs while reducing the need for nitrogen fertilisers, and reducing climate-induced yield losses. Diversifying crop rotation appears as a sustainable practice that can promote food security.

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