Benefits of Diverse Agriculture on Crop Yield

Long-term impacts of crop rotation and farming practices in a changing climate

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Benefits of diverse agriculture on crop yield – long-term impacts of crop rotation and farming practices in a changing climate

Abstract

Using yield data from multiple long-term experiments (LTEs) covering a period ranging between 20 to 55 years, I first investigated how a specialized cropping system compared with diverse systems and whether a crop-livestock system provided added benefits compared to a stockless diverse system in terms of yield and stability (Paper **I**). Stockless diversification provided a valid alternative to crop-livestock systems to maintain high yields. Specifically, diverse stockless and crop-livestock systems enhanced winter cereal yield by 15% compared to the specialized system.

As a second step, I examined how crop rotation and fertilization affected yield responses in the long-term (Paper **II**). I found a beneficial effect of diverse crop rotation on cereal yield especially in combination with mineral fertilization.

I further investigated how cereal yields in contrasting crop rotations responded to past climatic variation, to identify the potential of diverse crop rotation to provide an adaptation to climate change (Paper **III**). Diversifying crop rotation represents an adaptation strategy for enhancing cereal yields, both at northern latitudes where precipitation and temperature is expected to increase, and at southern latitudes where precipitation is expected to decrease.

Finally, using soil collected in a LTE, I examined how contrasting crop rotations and soil compaction, a factor known to limit yield, influence the contribution of insect pollination to yield as well as the nitrogen acquisition of faba beans via nitrogen fixing (Paper **IV**). Insect pollination contributed to seed quality and the combination of crop monoculture and soil compaction reduced the capacity of the legume crop to biologically fix nitrogen.

In conclusion, diversification of cropping systems and of the crop rotation offers opportunities to maintain or enhance yield in a changing climate.

Keywords: Crop yield, diversification, crop rotation, fertilization, manure, climate variation, precipitation, insect pollination, biological nitrogen fixation.

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To my friends Gabrielle, Geneviève and Johanie,

who, during our high-school years, forced me to follow the protocols of experiments for which we knew the outcome in advance,
who held me back from testing something new when I argue we might make a discovery,
thanks to whom I actually passed my science degree.

What does not kill me makes me stronger.
Albert Camus

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 St-Martin, A., G. Vico, G. Bergkvist and R. Bommarco (2016). Diverse cropping systems enhance but do not always stabilize yield in conventional agriculture (accepted after minor revision at Agriculture, Ecosystems & Environment).

II St-Martin, A., L. Marini, G. Vico, G. Baldoni, A. Berti, A. Blecharczyk, K. Huss-Danell, I. Malecka-Jankowiak, F. Morari, Z. Sawinska, R. Bommarco. Long-term effects of crop rotation and manure fertilization on crop yield (manuscript).

III St-Martin, A., L. Marini, G. Vico, G. Baldoni, A. Berti, A. Blecharczyk, K. Huss-Danell, I. Malecka-Jankowiak, F. Morari, Z. Sawinska, R. Bommarco. Diverse crop rotation as adaptation to climate change: results from long-term field experiments across Europe (manuscript).

IV St-Martin, A., R. Bommarco (2016). Soil compaction and insect pollination modify impacts of crop rotation on nitrogen fixation and yield. *Basic and Applied Ecology* 17, 617-626. http://dx.doi.org/10.1016/j.baae.2016.07.001

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The contribution of Audrey St-Martin to the papers included in this thesis was as follows:

I Developed research questions and analytical framework, analysed the data and wrote the paper with assistance from Göran Bergkvist, Giulia Vico and Riccardo Bommarco.

II Developed research questions, analysed the data and wrote the paper with assistance from Lorenzo Marini, Giulia Vico and Riccardo Bommarco. Other co-authors provided data.

III Developed research questions and analytical framework, analysed the data and wrote the paper with assistance from Lorenzo Marini, Giulia Vico and Riccardo Bommarco. Other co-authors provided data.

IV Developed research questions, performed the experiment, analysed the data and wrote the paper with assistance from Riccardo Bommarco.

1 Introduction

1.1 Yield challenges

Maintaining stable food production in a changing world is a major challenge for agriculture. Evidence is growing that the yields of major crops in developed countries are declining or stagnating (Ray et al. 2012; Lin and Huybers 2012, Brisson et al. 2010). At the same time, environmental changes at global scale pose a major threat to crop production as temperatures increases and rainfall patterns change (Moore and Lobell, 2015; Lesk et al. 2016).

Intensification of agricultural practices has allowed crop yields to increase, mainly through technological improvements such as mechanization, use of synthetic fertilizers and plant protection products, as well as the development of new crop varieties (Calderini and Slafer, 1998; Gervois et al. 2008; Robinson et al 2002). This trend, however has resulted in the overall simplification of agricultural systems (Lemaire et al. 2014). This occurs when diverse crop-livestock systems are replaced by specialized crop production systems separated from the raising of livestock (Schiere et al., 2002). Abandonment of livestock reduces the soil organic matter input provided by fertilization using green or animal manure (Ball et al. 2005). It also reduces the need for complex crop rotation that includes perennial grass-clover leys and annual legumes (Karlen et al. 1994). A simpler crop rotation or a monoculture involves the repeated use of the same management practices and machinery, combined with growing crops with the same rooting patterns (Bennett et al 2012). Simplification of agricultural systems is also linked to simplification of the landscape structure, i.e. an increase in field size together with loss of field-edge vegetation and conversion of natural and semi-natural habitats to arable land. This reduces the potential for biotic interactions to benefit agricultural production (Power, 2010; Robinson et al. 2002; Tscharntke et al. 2005). To what extent the simplification of agricultural systems contributes to yield stagnation and decline is poorly understood. Similarly, how yields in these systems respond to change in climate requires investigation.

To tackle the problems that crop yields are facing, there is a need to consider solutions that go beyond traditional intensification relying exclusively on external input and technological improvement (Kates et al. 2012). Diversification offers a possibility to maintain and stabilize crop yield but the actual effects of diversification on yield remain poorly studied (Gaudin et al. 2015; Davis et al. 2012). Diversification occurs by increasing the number of crops in the rotation, by integrating perennial crops into the crop rotation, by incorporating alternatives to synthetic fertilizer such as green or animal manure in the fertilization plan, and by enhancing the contribution of biotic interactions such as insect pollination and biological nitrogen fixation that can have a positive impact on crop production (Kremen and Miles, 2012). So far, diversification has mostly been studied as a means to make the transition from conventional to organic or low-input agriculture (Lotter et al. 2003; Eltun et al. 2002) but its effects in conventional agriculture remain understudied, especially when considering the impact of a changing climate on yield.

1.2 Simplification of agricultural systems and diversification

In Europe, the number of mixed crop-livestock farms decreased by 70% between 1975 and 1995 (Ryschawy et al. 2012). In some areas, the production of crop and livestock is regionally separated (Naylor et al. 2005). In this context, we need to evaluate the long-term effect of cropping system specialization on crop yield under conventional management. At the same time, we need to quantify the contribution of different diversification options. Integration of crop and livestock is used as a strategy to manage nutrients and water and to maintain production in dry climates (Allen et al. 2007). Are crop-livestock systems really needed to maintain or enhance yield in a changing world? Or would diversification without livestock and including a complex crop rotation provide similar benefits? With the cropping systems approach in long-term experiments (LTEs), the combined effect of different management practices can be investigated to offer an indication about how yield level and variation is affected. However, this approach does not allow disentangling the separate effects of crop rotation and fertilizer type on yield.

To further evaluate the consequence of diversification on crop yield, we need to disentangle the separate and combined effects of management practices associated to diverse cropping systems: diverse crop rotation and mixed fertilization (animal manure supplemented with mineral fertilizer). Does having both diverse crop rotation and mixed fertilization provide additional yield benefits, or would diversification through crop rotation but without manure from livestock be a valid alternative? A factorial approach to LTE is needed to identify the single and combined contribution of crop rotation and fertilization to yield.

With projected change in climate, crop production is expected to decline in some regions while in others, climate change might instead enhance yield by expanding the climatically suitable area for production of specific crops (Ewert et al. 2005; Bindi and Olesen, 2011). Yields in technically advanced crop production systems, where large-scale monoculture dominates, have been suggested to be more sensitive to climate change, especially drought (Lesk et al. 2016), so that yields are expected to decline in the future. There is a need to investigate the role that diversification could play in adapting cropping systems to climate change. So far, very little is known about the capacity of diverse crop rotation to influence yield responses to climate.

There is a need to investigate the contribution of crop rotation under different management practices. The intensive use of heavy farm machinery can lead to soil compaction and deterioration of soil structure (Hamza and Anderson, 2005). Simplification of the crop rotation can make the soil more sensitive to soil compaction. Soil compaction can reduce root growth, water and nutrient uptake and thereby reduce crop yield (Lipiec and Hatano, 2003). At the same time, integrating an active management of biotic interactions, such as insect pollination and biological soil processes, into farming systems has the potential to provide critical inputs to agriculture (Shennan 2008; Kremen and Miles 2012; Bommarco et al. 2013). Insect pollination can directly enhance crop yield (Garibaldi et al. 2014; Klein et al 2007). There is mounting evidence that the contribution of pollination to crop yield relies not only on the crop's dependency on pollinators, but also on how the crop is managed in the field. Therefore, the combined investigation of how crop rotation, soil compaction and insect pollination interactively influence yield provides a way to understand the capacity of biotic interactions to compensate yield loss arising from intensive management practices such as the ones leading to soil compaction. Manipulative greenhouse experiments, including soil collected from a LTE provide a promising approach to investigating these issues.

2 Thesis aims

The specific aims are to:

- Investigate how crop-livestock, specialized and stockless diverse cropping systems influence cereal yield and stability (**I**)

- Examine the effect of crop rotation and fertilization on long-term yield response and yield gain from fertilizer (**II**)

- Investigate how monoculture and diverse crop rotations respond to past climatic variation and to assess the potential of diverse crop rotation as an adaptation to climate change (**III**)

- Investigate how insect pollination contributes to yield and nitrogen acquisition of a legume grown under contrasting crop rotations and soil compaction (**IV**)

3 Materials and methods

An important consideration is how best to study the effects of contrasting cropping systems or crop rotations on yield. This requires a long-term perspective because diversification involves processes that develop over time, especially soils have a tendency to respond slowly to a change in management (Berti et al. 2016; Johnson et al. 2009). Therefore, the use of long-term field experiments (LTEs) is particularly well suited to address long-term effects of agricultural management. The strength of LTEs is that the treatment under investigation is kept constant over time, and they include a control. A limitation is that they are generally poorly replicated and that yield increase due to technological improvements, such as new cultivars, is confounded with yield changes due to the long-term processes and climatic trends. However, the long-term differences among treatments is still there to be analysed.

Because the effect of diversification on cropping systems and associated management practices is complex, I investigated yield from LTE from different angles (Table 1). I began by investigating yield response in a cropping system experiment, an approach where the cropping system itself is the experimental treatment (Paper **I**). The cropping system approach has been seldom investigated through LTEs, compared to the factorial approach (Berti et al, 2016).

To deepen my understanding, I then moved on to investigating the separate and combined effects of diversification via crop rotation and fertilization within cropping systems using factorial experiments. An important methodological consideration is the scale at which the effects of diversification are studied. Most experimental designs reported in the literature do not allow for generalization of the effect of, for instance, crop rotation on crop yield because experiments are performed at single locations and over a short time span

(Gaudin et al. 2015; Borelli et al. 2014; Grover et al. 2009; Johnson et al. 2009; Smith et al. 2007; Wilhelm and Wortmann, 2004; Lotter et al. 2003, Brown et al. 2002, Berzsenyi et al. 2000; Schmidt et al. 2000; Varvel, 2000; Yamoah et al. 1998). An option to better utilize and generalize the unique information drawn from LTEs is to use primary data, and to integrate multiple LTEs to investigate the more general effect of a management practice across multiple locations. With this approach, I investigated long-term yield response to contrasting crop rotations and to fertilization (Paper **II**), and I examined the yield response to past climate by investigating how temperature and precipitation encountered in 7 LTEs for a period ranging between 20 to 55 years influenced yield change (Paper **III**).

Another way to make use of LTE is to integrate them as part of another experiment. Soil from contrasting treatments within a LTE can be collected and integrated into a manipulative experiment. This allows expanding the range of more mechanistic questions to be answered, exploiting the experimental treatment of the LTE itself. In this way, I collected soil from a LTE where management as barley monoculture and ley rotation was ongoing since 1965 and integrated it to a greenhouse experiment. I subjected the soil to contrasting soil compaction and plants to absence or presence of pollinators to investigate the impact of crop rotation, soil compaction and pollination on yield and nitrogen acquisition of faba bean (Paper **IV**).

Table 1. Experimental design and treatments within each experiment included in the Papers

	Design	Treatments
Paper I	1 LTE with cropping system as experimental treatment	3 cropping systems: crop-livestock, specialized stockless, diverse stockless
Paper II	7 European LTEs with factorial design	2 diversity levels: monoculture vs diverse crop rotation
Paper III	6 European LTEs with factorial design	2 diversity levels: monoculture vs diverse crop rotation 3 fertilizer types: control, manure supplemented with mineral, mineral only
Paper IV	1 Manipulative greenhouse experiment	2 diversity levels: monoculture vs ley-rotation 2 compaction levels: low vs high 2 pollination levels: presence / absence

3.1 Long-term field experiments

3.1.1 Cropping system approach

To investigate the impact of farm specialization and associated simplification of the rotation on crop yield, I obtained data from a LTE located in Borgeby, Scania, Sweden (Paper **I**, Fig. 2). The experiment has been running since 1959 (Bergkvist and Öborn, 2011). In the experiment, three cropping systems are compared: 'crop-livestock', 'specialized', and 'diverse' (Fig. 1).

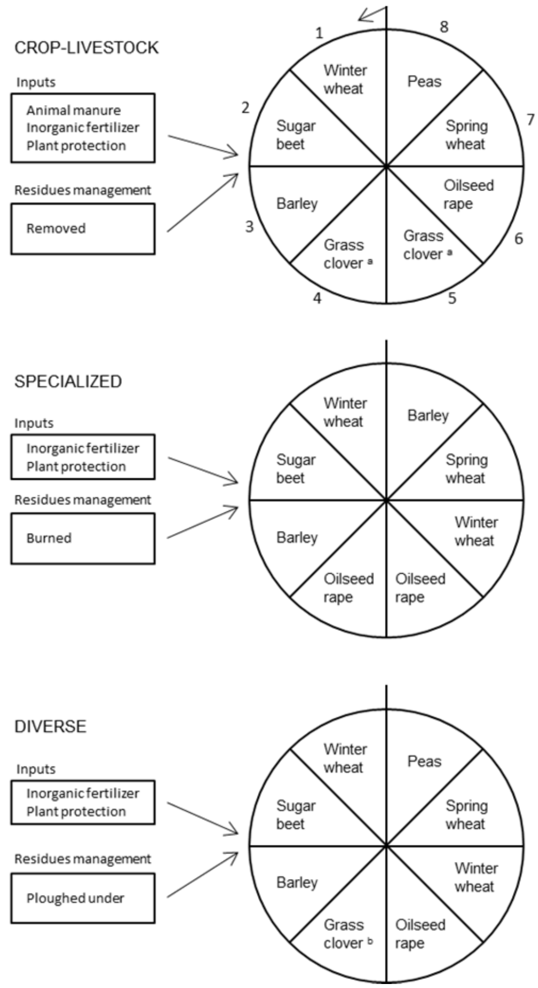

Figure 1. Schematic representation of the crop sequence, inputs and residues management in the three cropping systems in Borgeby. The numbers represent the position in the rotation so that a rotation cycle starts with winter wheat in the three cropping systems (Fig 1. in Paper **I**).

During the course of the experiment, the three cropping systems received the same crop-specific dose of mineral fertilizers. In addition to the mineral fertilization, the 'crop-livestock' treatment received 25 tons of animal manure per hectare in the autumn after each winter wheat harvest. In addition, harvest residues have been managed differently in each cropping system. In 'crop-livestock', residues are removed after most crops to mimic use of straw for animal husbandry. Residues are ploughed into the soil during the second year of ley and after winter oilseed rape. In the 'specialized' treatment, residues are burned after all crops except sugar beet, from which they are removed. In the 'diverse' treatment, residues are ploughed under except after barley where they are chopped and left on the soil surface, to preserve the under-sown grass-clover crop. Before sowing of all crops, the seed bed is prepared by harrowing. Plant protection is applied to all cropping systems equally, according to crop.

Figure 2. Long-term field experiment at Borgeby, Sweden. © Audrey St-Martin

The cropping system approach to LTE is a holistic approach using the whole cropping system as an experimental treatment (Oberle and Keeney, 1991). The experimental design at Borgeby does not allow for disentangling the effects of specific management practices, such as crop rotation or fertilization within the cropping systems. A factorial design is better suited to address these questions. These are explored further in Paper **II** and **III**.

3.1.2 Multiple factorial LTEs

I obtained data from 7 LTEs conducted at 7 different sites in Sweden, Poland and Italy. Sites differed widely in climate and other environmental factors. Ås has a subarctic climate. Öjebyn and Röbäcksdalen are bordering on a subarctic and a humid continental climate. Säby and Poznan have a humid continental climate (Fig. 3). Padova and Bologna have a humid subtropical climate (Fig. 4). Weather data was obtained from the closest meteorological station (Table 3 in Paper **III**).

Each experiment had a split-plot design including 2 treatments: a crop rotation and a fertilization treatment. The crop rotation treatment consisted of a cereal grown in monoculture and cereal grown as part of several diverse crop rotations. The fertilizer treatment consisted of several types and amounts of fertilizer application (Table 2 in Paper **II**).

Figure 3. Long-term experiment from the Department of Agronomy, Poznan University of Life Sciences, Poland. ©Andrzej Blecharczyk © Zuzanna Sawinska

Figure 4. Long-term experiment from the Department of Agronomy, Food, Natural resources, Animals and Environment, at Padova University. © Audrey St-Martin

Effect of crop rotation and fertilization on long-term yield response

To evaluate the consequence of farm specialization on crop yield, I investigated how crop rotation and fertilization affected yield response. For this, I used yield observations from 6 of the 7 above-mentioned LTEs where crop rotation diversity was set as a binary variable (either monoculture or rotation with at least 3 crops). Yield observations were selected from 3 fertilizer treatments: a control (unfertilized or low NPK), locally recommended high mineral NPK and the manure supplemented with mineral NPK (Table 2 in Paper **II**). The yield response and the yield gain from fertilizer were investigated. The yield gain from fertilizer is an indicator of nitrogen use efficiency. Only 6 of the 7 LTEs were used because 1 site did not include a manure treatment.

Assessing the potential of diverse crop rotation as adaptation to climate change

To assess how crop rotation diversity affects crop yield response to past climate, I used yield observations from the 7 above-mentioned LTEs where crop diversity was set as a binary variable (as above). I only selected yield observations from the treatment receiving the locally recommended high mineral NPK fertilizer rate. For each site, I calculated the growing season means of daily temperature and precipitation throughout the experiments based on weather data collected at each experimental site. Although cumulative precipitation might be more commonly used to analyze impacts of precipitation on yield, I used daily precipitation to facilitate comparisons because different sites have different length of the growing season. Spring and winter cereals were analysed separately because these two crop types cannot be assumed to respond to temperature and precipitation in the same way as they are sown and growning in the field at different times during the year.

3.1.3 Manipulative experimental approach

To test the impact of crop rotation, soil compaction and pollination on yield and nitrogen fixation of faba bean (*Vicia faba* var. minor L.) (Paper **IV**), I set up a greenhouse experiment where faba beans were grown on soil collected from the LTE in Röbäcksdalen, Sweden (Table 2 in Paper **III**). I established a compaction treatment of low and high compaction and a pollination treatment by bagging inflorescences, thereby allowing or preventing bumblebees from performing pollination (Fig. 5).

Figure 5. a) Bumblebee visiting faba bean flower in the greenhouse; b) soil from the i) ley rotation and ii) barley monoculture for the crop rotation treatment; c) pneumatic cylinder used to create the compaction treatment; and d) bagging of inflorescence to exclude pollinators © Audrey St-Martin

The experiment consisted of 80 experimental units (2 management types x 2 compaction levels x 2 pollination levels x 10 blocks) (Fig. 6). The treatments were randomized within the blocks and the blocks were randomly distributed in the greenhouse.

Seed yield of faba bean was assessed as total dry weight of seeds per plant. Yield components assessed were: number of seeds per plant, number of pods per plant, number of seeds per pod, individual seed weight and seed set. The individual seed weight was obtained by dividing the seed weight by the number of seeds per plant. Seed set was calculated as number of seeds per plant divided by number of pods per plant. The plants were weighed to obtain aboveground biomass. The roots were washed, dried at 65°C for 48 h and weighed to obtain belowground biomass. Growth partitioning was estimated by separately quantifying total biomass, aboveground biomass, belowground biomass, root-to-shoot ratio and reproductive-to-vegetative ratio. Reproductive-to-vegetative ratio was calculated as weight of seeds per plant divided by the sum of above- and belowground biomass. We estimated the proportion N acquired by faba bean via biological nitrogen fixation (BNF) using the natural abundance method (Shearer & Kohl, 1986). To assess the impact of the treatments on flower visitation by bumblebees, we observed the bumblebees on four occasions following individual bumblebees and recording which plants they visited.

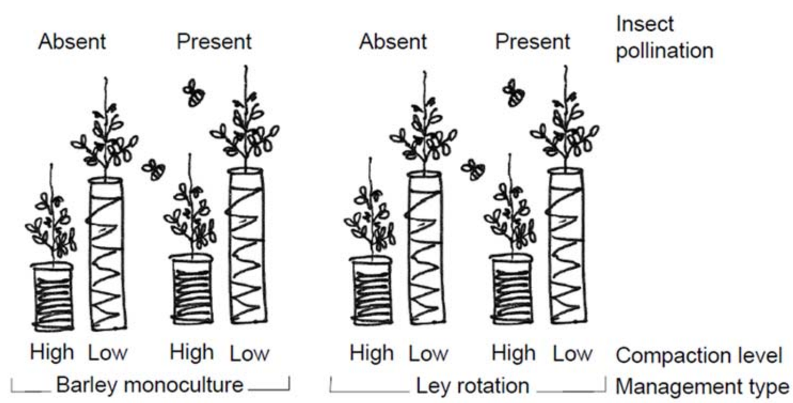

Figure 6. Schematic representation of the experimental design with soil from two management types (barley monoculture and ley rotation), two compaction levels (low and high) and two pollination levels (absence or presence of insect pollination).

3.2 Statistical analyses

Linear mixed models (LMM) are a useful approach to investigate hierarchical data such as LTEs. LMM allow the inclusion of fixed and random effects. Fixed effects are those of interest in a study, i.e. the treatments. Random effects consider variation arising from the experimental design. For example, blocks or sites are units of replication in the experiment that need to be included as random factors. If this is not considered, pseudoreplication arises i.e. "*the use of inferential statistics to test for treatment effects with data from experiments where replicates are not statistically independent*" (Hurlbert, 1984).

In Paper **I**, LMM were used to test for the effect of cropping system, time since the start of the experiment and their interaction on spring and winter wheat yield. Yield stability was investigated using the coefficient of variation (CV) and the stability index. The CV was calculated from a 4 year period, which represents half of the rotation cycle. I modified the stability analysis following Finlay and Wilkinson (1963) to account for the confounding effect of varietal and technological improvements as well as the change in fertilization rates during the course of the experiment (Fig. 7). Yields were therefore detrended to generate the stability index. I argue that regression of the residual treatment means against the yearly residual means allows a better representation of the response of the cropping systems to true environmental variability than the regression of the treatment yield means against yearly yield means. In fact, this approach allows excluding the effect of the overall trend in yield (i.e. the correlation between yields and years) and investigating the effect of the treatments.

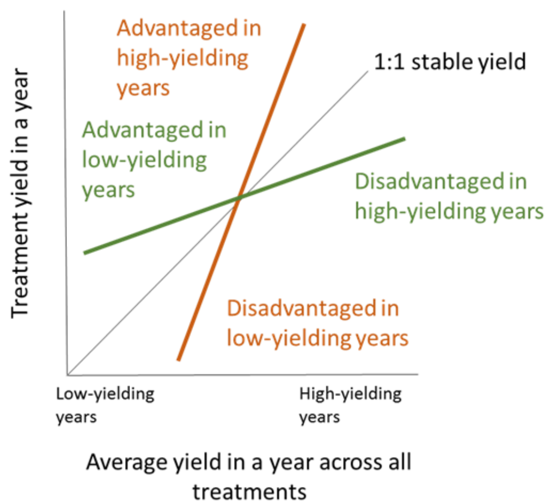

Figure 7. Schematic representation of the stability analysis.

In Paper **II**, LMM were used with multiple LTEs. This approach allows drawing conclusions that are not limited to one experiment in one location. It allows broader generalization because experiments can be considered to be selected randomly from a population of LTEs. To assess crop yield response to climatic factors, yields were detrended following Lobell et al. (2011), because estimation of the actual effect requires removal of the possibly confounding effects of non-climatic factors. Detrending yield was necessary to ensure the model is not biased from trends such as yield trends from technological

Figure 7. Schematic representation of the stability analysis.

improvement. To evaluate the role of climate on yield anomaly, an information-theoretic approach based on second-order Akaike's information criterion (AICc) was used. This approach compared the fit of all the possible candidate models obtained by the combination of investigated predictors using AICc. For each model *i* an Akaike's weight (w_i) was calculated, which is the probability that model *i* would be selected as the best fitting model if the data were collected again under identical circumstances (Burnham and Anderson 2002). Akaike's weight should be interpreted as a measure of model selection uncertainty.

In Paper **III**, a random coefficient mixed model was used to test the effects of crop rotation, fertilization, time since the start of the experiment and their interactions on yield of spring and winter cereals. Crop rotation, fertilization, time since the start of the experiment and their interaction were included as fixed effects; crop by site, block, and plot were included as nested random effects. The random coefficient model allows the explanatory variable to have a different effect for each crop by site. Yield gain from fertilizer was analysed with LMM to test for the effect of crop rotation, fertilization and their interaction as fixed effects. Crop by site, replication, and plot were included as random effects.

In Paper **IV**, LMM were used to test for the effect of crop rotation, soil compaction, pollination and all interactions on yield, yield components and the contribution of BNF to N acquisition in faba bean. Crop rotation, soil compaction and pollination were included as fixed effects, and block as a random effect.

The underlying statistical assumptions of linear models were graphically validated so that all data were analysed assuming normality and homogeneity of variance. Statistical analyses in Paper **I** were generated using SAS software, version 9.3 of the SAS System for Windows (SAS Institute, Cary NC, USA) and in Paper **II, III** and **IV** using the statistical language R (R Core Team, 2012).

4 Results

There is increasing pressure on agriculture with imperative to produce more food, reduce negative environmental impacts, prevent yield decline and adapt to climate change. In this thesis, I investigated how diversification in cropping systems and in crop rotations contributed to cereal yields throughout 8 LTE covering experimental periods ranging between 20 and 55 years.

4.1 Long-term field experiments

Overall yield response of contrasting cropping systems

I investigated how cropping systems 'crop-livestock', 'specialized', and 'diverse', and associated management practices affected yield response. I found that diversification enhance crop yield both at the cropping system level as well as at the level of the crop rotation (Paper **I**, **II**, **III**).

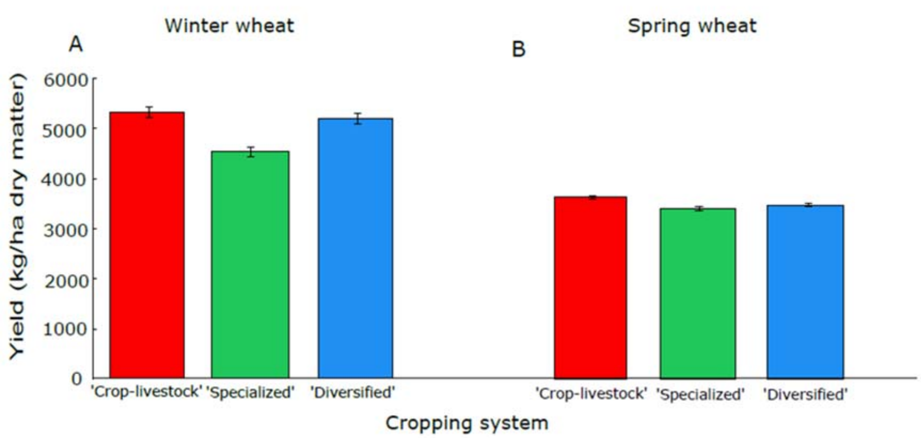

Figure 8. Overall yield mean of the three cropping systems over 52 years of experiment for A) winter wheat and B) spring wheat (n= 302 and 289 respectively). Means ± standard error are shown.

In my investigations, yields in specialized system and in monoculture were lower than in diverse cropping systems or diverse crop rotations (Fig. 8, 10, 11, 14, 15).

Long-term effects of cropping systems on yield

When examining the development over time in the LTE in Borgeby, Sweden, yields in contrasting cropping systems did not differ (Paper **I**, Fig. 9) but data from 6 LTEs across Europe shows that management with diverse crop rotation contribute to maintaining high yield (Paper **II**, Fig. 10).

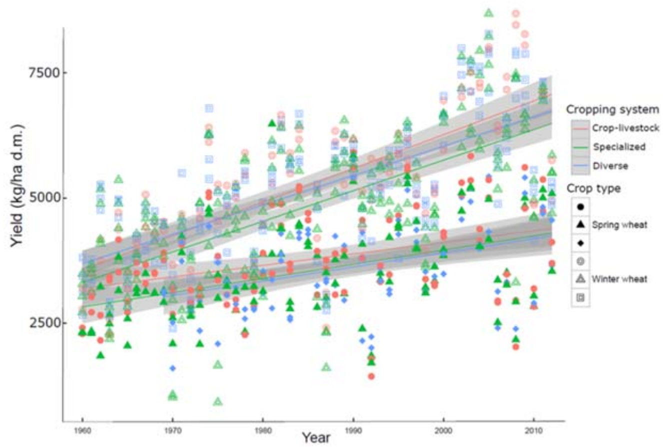

Figure 9. Yield under three cropping systems in the Borgeby LTE in Sweden (crop-livestock: red;, specialized: green; diverse: bleu) for two crops (winter wheat: hollow; spring wheat: solid). Shaded areas around the lines represent 95% confidence interval.

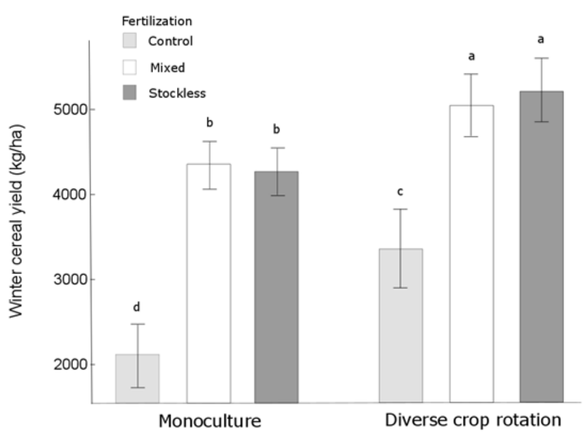

Figure 10. Effect of crop rotation and fertilization in 6 LTEs across Europe. Error bars represent 95% confidence interval. Bars with identical letters are not significantly different at $p<0.05$ (Tukey HSD).

When examining 6 LTEs, I found that yields in monoculture underwent a slower increase over time than did yield in diverse crop rotations (Paper **II**, Fig. 11).

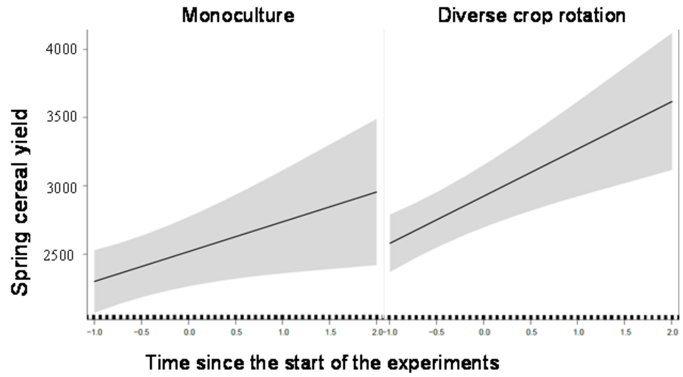

Figure 11. Effect of crop rotation on spring cereal yield development in 6 LTE across Europe. Shaded areas around the lines represent 95% confidence interval.

There was no evidence for yield decline over time for crop grown in monoculture in the 6 LTEs investigated.

Yield effect of crop-livestock system compared to stockless diversification

When investigating diversification options at the cropping system level, I found that crop-livestock and stockless diverse systems are both equal at delivering high cereal yield, with a tendency of crop-livestock to deliver higher yield for spring cereals (Paper **I**). Data from 6 LTEs shows that mixed fertilization (combining manure with mineral fertilizer) associated with crop-livestock system does not provide added yield benefit compared to stockless fertilization relying exclusively on mineral fertilizer (Fig. 12) and that the yield gain from fertilizer was greater under stockless fertilization (Paper **II**).

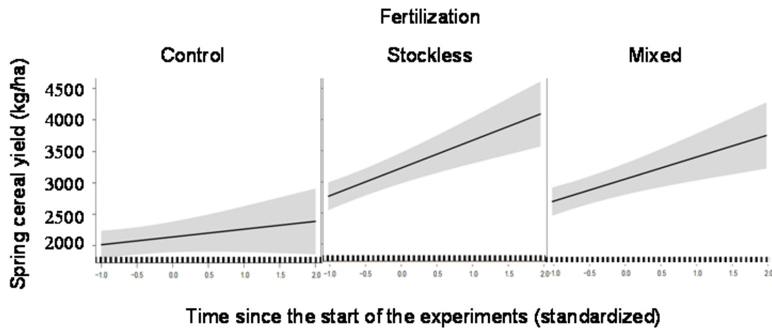

Figure 12. Effect of fertilization on spring cereal yield development in 6 LTE across Europe. Shaded areas around the lines represent 95% confidence interval.

Interestingly, diverse cropping systems and diverse crop rotation provided yield benefits even under high mineral fertilization (Paper **I** and **II**).

Effect of diversification on yield response to past climatic variation

Investigating the effect of year-to-year variation on yield of contrasting cropping systems in Borgeby, I found that the three cropping systems were equally stable with regards to winter wheat. The crop-livestock system tended to deliver higher yield in high-yielding years for spring wheat (Paper **I**, Fig. 13).

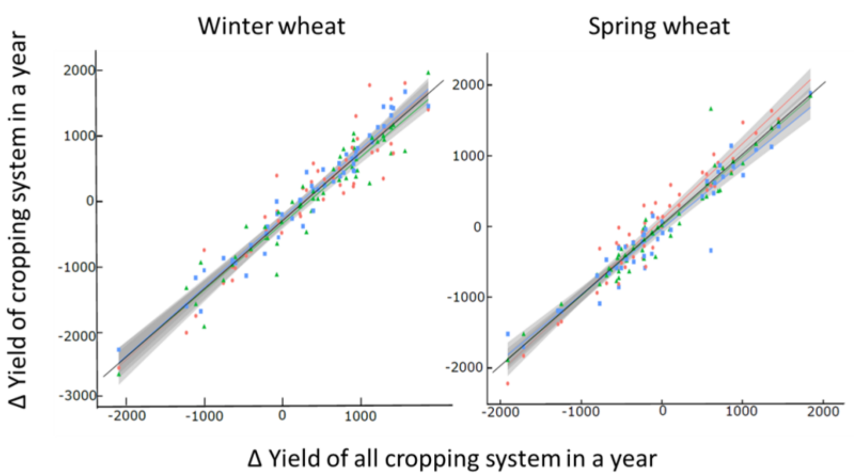

Figure 13. Stability analysis of winter and spring wheat yields in the Borgeby LTE, Sweden. Black line represents 1:1. Individual data points are the mean of 2 replicates.

When investigating the yield response in contrasting crop rotations to the range of temperature and precipitation encountered in 7 LTEs across Europe, I found a positive effect of increasing growing season precipitation and a negative effect of increasing growing season temperature on spring cereal yields (Paper **III**).

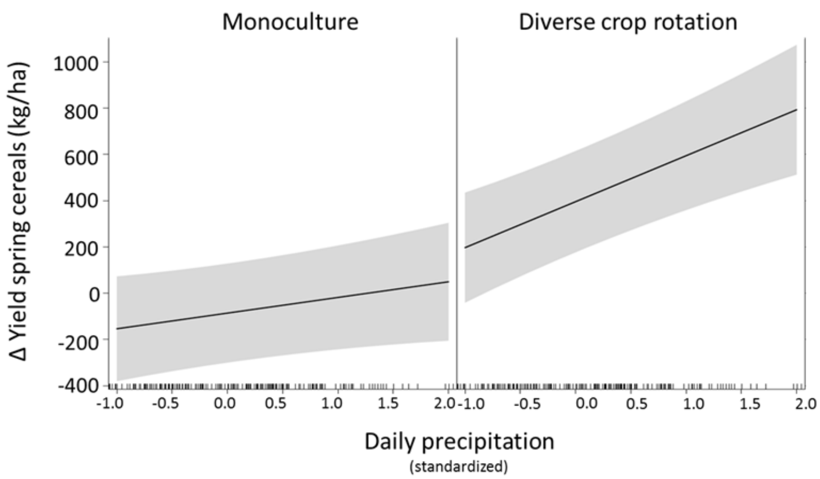

Figure 14. Change in yields and daily precipitation over the growing season for spring cereals grown in monoculture and in diverse rotation in 5 LTEs. Shaded areas around the lines represent 95% confidence interval.

In northern latitudes where spring cereals are grown, the positive effect of precipitation was more pronounced in diverse crop rotation than in monoculture (Fig. 14). Data from 5 LTEs suggests that diverse crop rotation is a potential adaptation to expected climate change for northern latitudes.

I found a negative effect of both increasing temperature and precipitation during the growing season on winter cereal yields (Paper **III**).

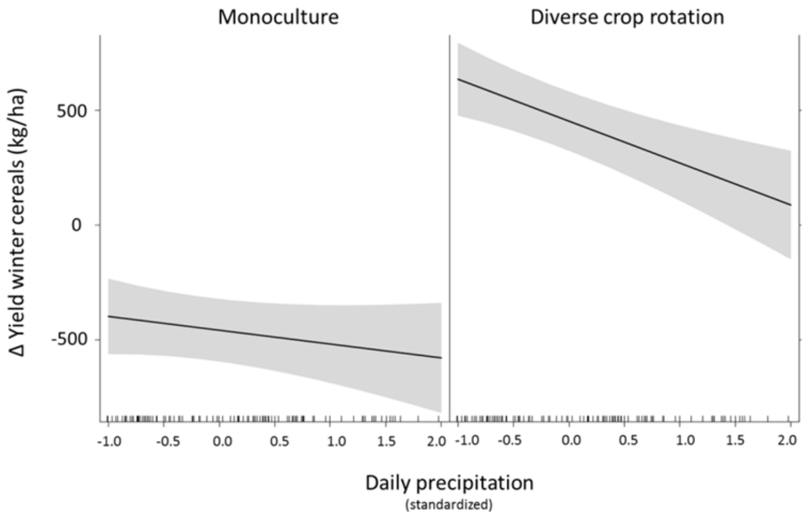

Figure 15. Change in yields and daily precipitation over the growing season for spring cereals grown in monoculture and in diverse rotation in 5 LTEs. Shaded area around lines represent 95% confidence interval.

In Southern latitudes, where winter cereals are grown, the positive effect of decreasing precipitation during the growing season tended to be more pronounced in diverse crop rotation than in monoculture (Fig. 15). Data from 3 LTEs suggests that diverse crop rotation is a potential adaptation to expected climate change for southern latitudes.

4.2 Manipulative experimental approach

Effect of biotic interactions on yield of a crop grown in contrasting crop rotation under contrasting soil compaction level

Finally, investigating the contribution of insect pollination to crop yield of a legume grown in contrasting crop rotation and at contrasting compaction level, I found insect pollination to enhance yield and high soil compaction and ley rotation to reduce yield (Fig. 16).

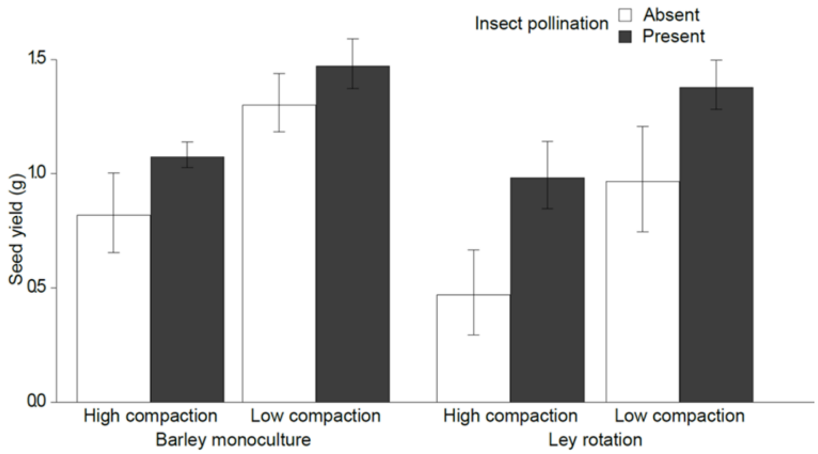

Figure 16. Effect of crop rotation, soil compaction and insect pollination on seed yield of faba bean in a greenhouse experiment. Means ± standard error are shown (n=79).

I found a positive effect of insect pollination and a negative effect of high soil compaction and ley rotation on crop yield. Interestingly, I found that insect pollination compensate the negative effect of ley rotation on yield component (individual seed weight, Fig. 17), indicating the importance of insect pollination for seed quality.

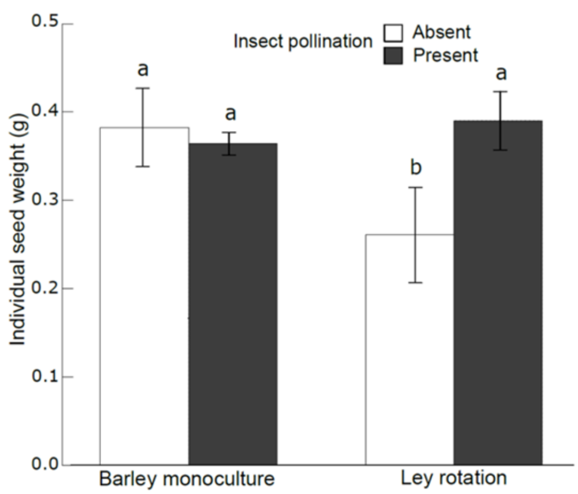

Figure 17 Effect of crop rotation and insect pollination on individual seed weight of faba bean in a greenhouse experiment. Means ± standard error are shown (n=79). Bars with identical letters are not significantly different at $p<0.05$ (Tukey HSD).

Regarding the contribution of biological nitrogen fixation (BNF) to N acquisition, I found that under high soil compaction, the % N derived from BNF was more important in the ley rotation than in the monoculture (Fig. 18A). Surprisingly, I also found that a greater proportion of N was derived from BNF for plants exposed to insect pollination in the ley rotation than in the monoculture (Fig. 18B).

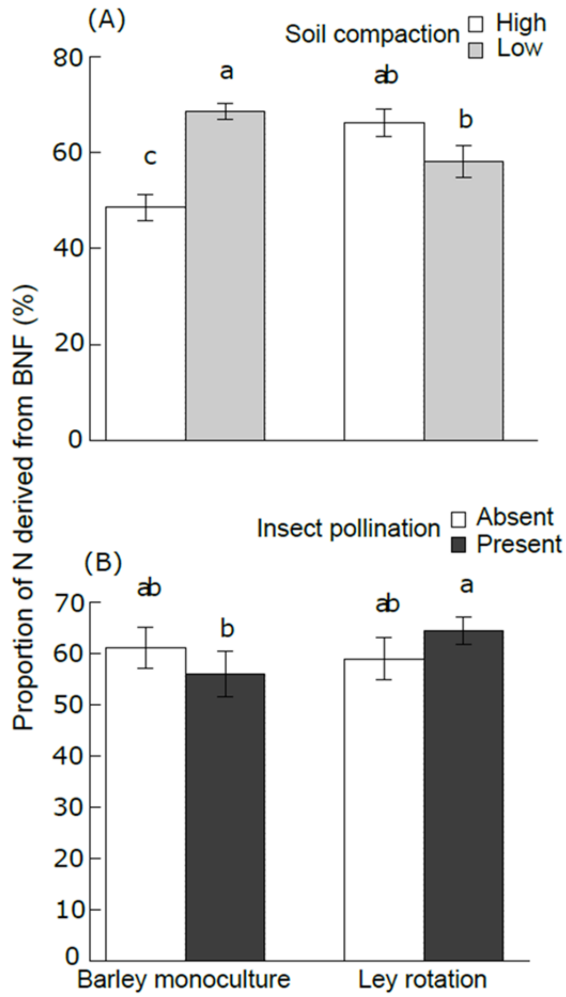

Figure 18. Effect of A) crop rotation and soil compaction and B) crop rotation and insect pollination on the proportion of N derived from BNF by faba bean in a greenhouse experiment. Means ± standard error are shown (n=31). Bars with identical letters are not significantly different at $p<0.05$ (Tukey HSD).

5 Discussion and conclusion

Yield decline and stagnation reported by investigations on global crop production (Ray et al. 2015) as well as the challenges brought by climate change (Lobell et al. 2011; Bindi and Olesen, 2011) call for the development of adaptive crop production systems. In this thesis, I provided evidence of whether diversification of agriculture might be such a strategy and its contribution to crop yield. I compared diversification in crop-livestock, diverse stockless and specialized cropping systems. I investigated the long-term effect of diverse crop rotation compared to monoculture and its contribution as climate change adaptation. I examined how biotic interactions contribute to crop yield and N acquisition in contrasting crop rotations.

Diversification of cropping systems

The experimental design in Borgeby allowed investigating the effect of diversification within conventional agriculture in the cropping system context. I found that crop-livestock and stockless systems were equal in delivering high yield in the long-term. This suggests that stockless diversification provides a valid alternative to crop-livestock systems to maintain high yields. Diversification provides an opportunity for land managers to increase crop productivity and secure continued high yields, even under conventional management. Investigations of the CV and stability analysis suggest that conventional management with application of inorganic fertilizer protect cropping systems in the face of environmental variability. It should be noted that the stability of each cropping system is very much dependent on the other systems included in the analysis. This means that the outcome could have been different if other cropping systems had been included. An extension to Paper I would be to evaluate the stability of each cropping systems for all the crops included in each system, not limiting it to one crop type. Another extension would be to include an analysis of profitability parallel to the yield analysis.

Through diversification of farm activities, integration of crop-livestock has been identified to mitigate the effects of price fluctuations on crop or input (Ryschawy et al. 2012). Crop-livestock systems have also been found to mitigate the effect of climate variability on farm performance due to the flexibility gained from the production of a wider range of agricultural products (Bell et al. 2014). The Borgeby experiment was designed more than 50 years ago to answer questions about farm specialization but without the concern regarding the effect of synthetic inputs on the environment. The design of future cropping system experiments should allow management practices such as inorganic fertilization, weed management and plant protection to vary between systems. That would allow identifying which cropping system might be better at delivering nutrients, or at dealing with weeds and disease.

Diversification of crop rotation and fertilization

In 6 LTEs located across Europe covering an experimental period ranging between 20 and 55 years, I found yield benefits of diverse crop rotation on long-term cereal yields. Both stockless and mixed fertilization delivered high cereal yield in the long-term. The results further suggest that diverse crop rotation gives added benefits under high mineral input. Investigating if the combination of diverse crop rotation and animal manure fertilization provide added benefit to crop production is important in the context of regional separation of crop and livestock (Naylor et al. 2005). The results suggest that crop rotation supplemented with adequate mineral fertilization can replace the benefit of mixed fertilization from traditional crop-livestock systems. A general limitation of crop rotation studies is the difficulty to identify if the effect on yield is due to N deriving from the presence of legumes, due to changes is soil properties or due to disease break (Cuvardic et al. 2004; Angus et al. 2015; Johnson et al. 2009). One limitation to comparing mineral fertilizer to manure is the difficulty to design comparable treatments.

Diversification and climate change

Investigating how yields from monoculture and diverse crop rotations in multiple long-term experiments have responded to past climatic variation provides an important step in understanding how these practices are likely to respond to projected future climates. The observed year-to-year variation in 7 LTEs across Europe was much greater than the observed trend in climate. Therefore, we could estimate yields reacting to, for example, an increase in growing season temperature and how crop rotation affected this response. I found that, at northern latitudes, where precipitation and temperature is expected to increase, and at southern latitudes where precipitation is expected

to decrease, diversifying crop rotation represents an adaptation strategy for enhancing cereal yields.
Alternative approaches are based on simulating the effects of projected climates on crop yield by incorporating effects of temperature and precipitation on crop growth processes in mechanistic and deterministic crop models (Kristensen et al. 2011). It would be interesting to compare model outputs with results from LTEs testing effects of crop rotation and climate such as the ones investigated here. The focus of this analysis was on average temperature during a fixed growing season. This does not take into account that the length of the growing season can have changed during the course of the experiments. Climatic factors that are more specific in explaining crop growth and yield formation than average temperature and precipitation over the growing season, might better explain variation in yield in a variable climate. Integrating more details about growing season and crop phenology could reveal additional patterns of crop rotation responses to climate (Peltonen-Saino et al 2011). Climatic extremes can have larger effects than average conditions (Lesk et al. 2016; Lobell and Field, 2007).

Diversification and biotic interactions

Plant response to soil compaction and monoculture affected pollination benefits on individual seed weight and size as well as contribution of BNF to N acquired by the plant. However, these effects were not reflected at the yield level. Variation in seed size may affect the capacity of seedling to establish in the field (Marshall, 1986). This could have important implications for seed growers where plants grown under stress benefit from pollinators for seed quality. The lowest proportion of N derived from BNF found in the highly compacted monoculture suggests that soil compaction exacerbates the negative effect of monoculture on BNF. By managing soils such that soil quality is deteriorated, farmers reduce legumes' ability to supply N, potentially increasing dependency on mineral N inputs and at the same time reducing key ecosystem services delivered by legumes in the agroecosystem. Furthermore, we found an interactive effect of management type and pollination on the proportion of N derived from BNF which suggests that enhanced seed yield by pollination increases plant demand for N. The relationship between pollinators and rhizobia is unclear and we have no leads in the literature to explain mechanisms for this pattern. Further studies are clearly needed to identify how insect pollination moderates N fixation.
Limitations to this approach include a lower realism in terms of yield as opposed to a field experiment, because the experiment was conducted in a greenhouse. Second, the contrast between soil from the monoculture and the

ley rotation cannot be generalized to represent differences between actual monoculture and crop rotations since, for that a population of monocultures and rotation would need to be sampled. An extension of this work would be to examine plant response from different fields with same management history.

Future directions

Investigating the yield response in multiple LTEs provides the opportunity to reflect on the scale at which effects are studied in agriculture (Levin, 1992). An important contribution of this thesis is to provide an example of using primary yield data from multiple long-term agricultural field experiments to explore the combined effects of climatic factors and an adaptation strategy on crop yield under a changing climate. By designing and using data from LTEs, we are investigating the scale of time. When using data from LTEs collected at multiple sites, we investigated the scale of space. According to Levin (1992), "*the observed variability of a system will be conditional on the scale of description*". For the effects of crop rotation on crop yield, this suggests that we might not come further in our understanding if we continue to study effects in single experiments, at single locations. Levin argues that "*by changing the scale of description, we move from unpredictable, unrepeatable cases to a collection of cases whose behaviour is regular enough to allow generalizations*". Indeed, the literature is rich of experimental studies that have investigated either the effect of crop rotation or of fertilization or both, but whose conclusions can serve only in predicting the outcome of these practices under the specific climatic and edaphic conditions under which they were studied (but see Hijbeek et al. 2017; Ladha et al. 2003). A next step would be to first compile the different method used to investigate the effect of climate change on yield and their results. Then try to understand how the scale of observation influences the description of the patterns observed.

Integration of farm economic performance in farm with contrasting levels of diversification (either in terms of cropping system or crop rotation) would provide a next step in investigating the applicability of diversification. A potential limitation to the adoption of diverse crop rotation has been that diverse crop rotation lowers the revenues of diversified operations in a given year compared to the selection of few high priced crops and reduces the benefits associated with economies of scale (Bradshaw et al. 2004). Therefore, integration of economic aspects is key to appreciating the more general benefits of crop diversification.

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