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Organic Farming is an international, open access, academic, interdisciplinary journal, published by Librello.

Cover image
Harvesting winter wheat research plots at the University of Maine Rogers Farm Forage and Crop Research Facility in Old Town, Maine, USA (author: Ellen B. Mallory).

About *Organic Farming*

Focus & Scope

Organic Farming (OF; ISSN 2297–6485) is a new open access academic journal that publishes articles on advances and innovations in organic agriculture and food production to provide scholars and other groups with relevant and highly topical research in the field.

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Organic Farming welcomes contributions in diverse areas related to organic farming and food production, such as soil and plant management, crop breeding, regulation of pests and diseases, protection of soil, water, biodiversity and other resources, livestock health and management, marketing and acceptance of organic products, food quality and processing, policies and regulations.

The articles of *Organic Farming* will be immediately accessible upon publication and we aim at making this journal a valuable venue for the communication among scientists, but also between researchers, producers, policy makers, traders and consumers of organic products.

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Editorial

How Scientific Is Organic Farming Research?

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Opening the third volume of this journal provides a renewed opportunity to reflect on the current developments within the world of organic farming. As the most recent international data show, the organic sector continues to grow on a global scale, in terms of organic area, market share and number of producers [1]. Yet, for organic farming—as for any movement—expansion always entails the difficulty of maintaining identity. Achieving both, i.e. becoming 'bigger' and 'better', is the explicit goal of Organic 3.0 [2], the international initiative to advance and evolve organic farming. Launched in 2014, Organic 3.0 is now gaining increasing momentum, e.g. as a key topic at the upcoming Organic World Congress in India this autumn. The Organic 3.0 initiative proposes an ambitious plan for promoting "a widespread uptake of truly sustainable farming systems" [2]. One of the suggested pathways to achieve the goals of Organic 3.0 is improved and extended research and development.

So what kind of research is needed for the ambitious development goals of organic farming? A recently published comprehensive review of this question concludes that a multitude of research approaches will be needed for advancing organic farming [3]. In particular, while it is recognized that holistic, interdisciplinary system research will need to play the lead role (e.g. [4]), also component research, following more specialized and reductionist approaches, is seen as necessary. Interactions between researchers and farmers will need to span the full range, including classical on-farm research and participatory action research.

However, the organic sector is not isolated in the research world. Choosing which research approach is to be pursued, and, in fact, which questions should be asked, is not a boundless process. In particular, these choices are often influenced by interactions with colleagues who work in non-organic fields. Such interactions are often determined by competition for research resources, e.g. when it comes to defining the denomination of academic chairs, setting up strategic plans for the future direction of research institutes, or allocating funds to, or within, public research programmes.

This struggle is particularly difficult when it is poisoned by the underlying view still pertinent outside the organic sector that organic farming research is somehow 'unscientific'. Over the past few decades, organic research has responded to such critique, partly by moving towards more established research, and away from heterodox methodologies, by expanding and professionalising, by increasing its research output, and by progressively focussing more on peer-reviewed articles [5]. Organic research, at least partly, has also followed the trend towards increased disciplinary specialization. So over the past decades, organic scientists have engaged in the mainstream of scientific publication, and this has partly resulted in increased reputation and credibility from outside.

At the same time, agricultural science from outside the organic sector is—at least in part—becoming aware of the importance of applied participatory and farmer-led research, calling for research to become more practice-oriented, and partly adopting organic research approaches. In addition, research outcomes and innovations, generated in the organic sector, e.g. in the area of legume cropping, are being taken up in non-organic systems. But for a minority, little can be more perplexing than when its goals become pursued by the mainstream.

More recently the earlier critique against the organic heterodoxy has been turned on its head: Now the argument has become that because organic methodologies are more or less fully transferable to other systems, organic research has no methodologies of its own, and has therefore no identity or right of existence as a separate branch of science. In this view, organic can be subsumed under bigger headings, because innovations and methodologies generated by organic research are transferable to other systems. Issues concerning organic farming are suggested to be taken up by specialists of mainstream science. The parts of organic research deemed as scientific enough can be swallowed whole. What was once a department of organic farming, to all intents and purposes, soon becomes occupied with other things, and is pulled away from concentrating its attention on solutions for the organic sector. Organic issues become hidden and diluted. The gain in credibility and reputation through mainstreaming organic research is followed by an embrace that is not always a friendly one.

Reasons for these developments are manifold. One of them lies in the disincentives against organic research imbued in current research evaluation [6]. Systems investigated by organic farming researchers are typically highly complex meaning that research can take longer so that research output per unit time is lower than for simpler systems. Further, the interdisciplinary nature of organic research is often not favoured by the gatekeepers of specialised disciplinary science. However, there are also various developments that are slowly bringing about significant changes in the practice of research evaluation, including the open access movement, which has particular relevance for organic agriculture [6].

There are now opportunities to bring these various movements together (open access, critique on inappropriate science evaluation) and it is likely that organic farming in particular will benefit from these new developments. It is now necessary to take action, and seize the opportunity to diversify the research evaluation system. More generally, the organic community will need to develop strategies for expanding organic research while maintaining its organic identity, similar to, and beyond Organic 3.0. One of these strategies will be the provision of free breathing space for organic researchers outside existing pressures on research, to promote sustainable innovations for and within the organic sector.

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Research Article

Evaluating Split Nitrogen Applications and In-Season Tests for Organic Winter Bread Wheat

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Abstract: Achieving high grain yields and crude protein (CP) standards in organic winter wheat (*Triticum aestivum* L.) is challenging because ensuring that adequate nitrogen (N) is available at key periods of wheat growth is difficult in organic systems. Split application regimes and in-season N management tests may improve organic production. In field trials conducted over four site-years in Maine and Vermont, USA, N application regimes were analyzed for their effects on organic winter wheat, N uptake, grain yield, and CP. Tiller density and tissue N tests were evaluated as in-season decision tools. Eight treatments arranged in a non-factorial design differed in terms of N application timing (pre-plant (PP), topdress at tillering (T1), and topdress at pre-stem extension (T2)) and N rate. Treatments were: (1) an untreated check, (2) pre-plant N at a low rate of 78 kg N ha^{-1} (PP$_L$), (3) pre-plant N at a high rate of 117 or 157 kg N ha^{-1} (PP$_H$), (4) T1$_{78}$, (5) PP$_L$ + T1$_{39}$, (6) PP$_L$ + T2$_{39}$, (7) PP$_H$ + T2$_{39}$, and (8) PP$_L$ + T1$_{39}$ +T2$_{39}$. Responses to N treatments were variable among site-years, however some common results were identified. The PP-only treatments increased grain yields more than they increased CP. The T1$_{78}$ and PP$_H$ + T2$_{39}$ treatments were the most effective at increasing yield and CP, compared with the PP-only treatments. Tiller density and tissue N tests were good predictors of grain yield (r = 0.52, $p < 0.001$) and CP (r = 0.75, $p < 0.001$) respectively. Future work should test in-season decision tools using a wider range of tiller densities, and topdress N rates against tissue N measurements.

Keywords: grain crude protein; grain yield; hard red winter wheat; pre-plant N; plant N uptake

1. Introduction

An expanding market for locally produced bread flour in the northeastern United States has created demand for local, organic bread wheat. Economically, organic bread wheat can be a high-value crop for growers if production targets for grain yield and quality are met. Grain CP is a major indicator of quality as it dictates dough elasticity and workability [1]. On the bread wheat market, a grain CP of generally 120 g kg^{-1} or greater is desired because it gives dough strength and provides loaf volume [2]. Grain with lower CP can be sold as feed but typically receives a lower price [3].

Nitrogen plays a key role in supporting both grain yield and CP in bread wheat [4,5]. Nitrogen not only affects grain yield components such as heads m^{-2}, seeds head^{-1}, and kernel size [6], but is also needed to form the proteins for baking quality [7]. Early in the season, N uptake tends to influence vegetative growth, and therefore grain yield

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more than protein, and these effects shift as the season progresses [8]. This relationship occurs because when N is available early in the season, it determines yield potential and once yield potential is set additional N increases grain protein content [9]. Nitrogen management systems have long been studied to determine the effects of application timing on winter wheat grain yield and CP. Woodward and Bly [4] found 165 kg N ha^{-1} of ammonium nitrate fertilizer applied pre-plant to hard red winter wheat raised yields but not CP, and the inverse effect was true when the application was split between fall and spring. Eilrich and Hageman [8] reported April applications of N, as Ca $(NO_3)_2$, on soft red winter wheat caused a 5% grain yield increase, whereas N applications in May did not increase grain yield but instead increased % grain N. A tradeoff between grain yield and CP can also occur due to factors such as limited moisture [10], cultivar, and environmental conditions [11]. As described by Fowler et al. [9], environmental or genotypic effects that increase grain yield must be met with increased amount of N to create a proportionally positive increase in CP. Brown and Petrie [12] found it possible to produce both high yields and acceptable CP in irrigated hard red winter wheat by providing both early-season and late-season N, and warned of the difficulties in achieving adequate CP without late-season N.

In organic cropping environments, overall N supply tends to be low [13–15] and the availability of N derived from organic sources such as animal manures and plant residues is less predictable than from inorganic sources [16]. Olesen et al. [17] reported that manure was more effective at increasing winter wheat grain yield while a grass-clover pre-crop was more effective at increasing grain protein due to differences in the timing of N availability. Solid animal and green manures are the most cost effective organic N sources but both must be applied before planting, the latter for logistical reasons and the former to reliably comply with the 90-day interval required by the National Organic Program Standards between raw manure applications and crop harvest [18]. Unfortunately wheat uptake of N applied at pre-plant tends to be low. Wuest and Cassman [19], for example, documented N recovery ranging from 30 to 55% for spring wheat. Recovery is likely lower in temperate climates because N in winter crops is susceptible to leaching and denitrification during the plant dormancy period [20,21]. The difficulty of ensuring late-season available N for winter wheat with pre-plant applications makes it challenging to achieve grain CP suitable for the bread flour market [12]. In an organic winter wheat study, Mallory and Darby [3] found that spring applied topdress N, in addition to pre-plant manure, increased grain CP by up to 2 percentage points. While no treatments reached the 12% CP milling standard in this study, had a variety with higher protein potential been used, that 2 percentage point increase might have increased CP to above 12%.

In conventional bread wheat production, split applications of N have been shown to increase grain yield and CP [22]—and to improve N utilization efficiency or grain weight per unit—of N from fertilizer [23,24]. The general concept is to reduce fall pre-plant N and to add spring topdress applications at one or two critical growth periods, such as spring tillering and just prior to stem extension, Zadok growth stages (GS) 25 and 30 [25], respectively. In humid regions of the U.S., in-season diagnostic tests are used successfully to guide topdress decisions for soft winter wheat [6,26]. The application rate of the first split is based on tiller density at GS25 whereas the second split is based on tissue N concentration at GS30. Low tiller density ($<$1000 tillers m^{-2}) indicates some or all fertilizer N application at GS25 is needed immediately to increase tiller numbers to support grain yields [20]. Alternatively, high tiller density ($>$1000 tillers m^{-2}) indicates additional N is not needed until GS30. Next, wheat tissue N at GS30 is used to assess crop fertilizer N requirements just prior to the period of highest N uptake [25] and has been identified as a beneficial indicator of the topdress rates needed to maximize yields in soft wheat systems. In Virginia, for example, Baethgen and Alley [26] identified 39.5 g kg^{-1} as the critical tissue N concentration at GS30 to achieve 90% of the maximum grain yield.

Few studies have analyzed split application regimes for organic winter wheat production [3] and to our knowledge none have used the in-season decision tools under organic conditions. The adoption of these practices by farmers has the potential to reduce N loss to the environment and increase the value of bread wheat through enhancing yield and quality. The objectives of this study were to: 1) evaluate the effects of pre-plant and split application treatments on grain N uptake, yield, and CP, on organic hard red winter wheat; and to 2) assess the potential of in-season tests to optimize grain yield and grain CP.

2. Materials and Methods

2.1. Study Site and Experimental Design

The field experiment was conducted in 2012 and 2013 in Maine (ME) and Vermont (VT). In ME, the site was a certified organic field (MOFGA Certification Services, LLC) at the University of ME Rogers Farm Forage and Crop Research Facility (44°56' N, 68°42' W) in Old Town. The site was converted to organic production in 2007. The soil was a Melrose fine silt loam (coarse-loamy over clayey, mixed illitic, superactive, frigid Oxyaquic Dystrudepts) with a pH of 6.2, 3.3% organic matter, 11.8 kg ha^{-1} soil test phosphorus (P) by Modified Morgan, 547 kg ha^{-1} soil test potassium (K), and 23 kg ha^{-1} soil test sulfur (S) based on 2,241,702 kg ha^{-1} of soil in a plow layer (16.9 cm deep), as determined per the standard methods of the ME Soil Testing Service. In ME, the 2012 experiment was preceded by a season of tilled fallow to control perennial weeds and was planted to corn silage (*Zea mays* L.) in 2010. Immediately following winter wheat harvest a cover crop of mustard (*Sinapis arvensis* 'Ida Gold') was established and allowed to grow for 4 weeks. It was then incorporated into the soil two weeks before the 2013 experiment was initiated. The 2013 experiment was initiated in the same field in an area adjacent to the 2012 experiment that, in 2012, was cropped with winter wheat. The VT experiments were located at Borderview Research Farm in Alburgh (45°0' N, 73°18' W). In 2012, the soil was a Benson Rocky silt loam (loamy-skeletal, mixed, active, mesic Lithic Eutrudepts) with a pH of 6.9, 3.8% organic matter, 4.5 kg ha^{-1} soil test P (Modified Morgan), 81.8 kg ha^{-1} soil test K, and 20.2 kg ha^{-1} soil test S, determined as above. The prior crops were winter wheat and no-till sunflowers (*Helianthus annuus* L.) in 2011 and 2010, respectively. In 2013, the soil was a Benson Rocky silt loam (loamy-skeletal, mixed, active, mesic Lithic Eutrudepts) with a pH of 7.5, 5.2% organic matter, 9.6 kg ha^{-1} soil test P (Modified Morgan), 90.7 kg ha^{-1} soil test K, and 22.4 kg ha^{-1} soil test S, determined as above. The prior crop was spring wheat and this site had been in grass-legume sod for 14 to 15 years before being converted to annual cropping of minimum-tilled sunflowers in 2011.

Field plots were 1.8 m by 13.4 m, arranged in a randomized complete block design with four replications. Treatments were designed to evaluate the effectiveness of different N application options that organic farmers in the northeastern region would use to influence grain yield and CP. Table 1 provides a description of the treatments, which differed in terms of N application timing and N rates, but which were not a factorial arrangement of these two factors. Treatments differed in terms of total available N applied depending on pre-plant N application rate and whether topdress applications were made. The different N application timings were pre-plant (PP), topdress at tillering or Zadok 25 (T1), and topdress at stem elongation or Zadok 30 (T2). Dairy manure (*Bos taurus*) was used as the pre-plant N source to reflect the fact that farmers in the northeastern region and elsewhere rely on manure and green manures for pre-plant applications. The target rates for the pre-plant timing were 78 and 117 kg ha^{-1} of available N, with the exception of VT-2013 where a high rate of 157 kg ha^{-1} of available N was used. The PP$_L$ was chosen to represent the standard practice for organic hard red winter wheat in the area. Solid dairy manure was used in ME-2012, ME-2013, and VT-2013, and composted solid dairy manure was used in VT-2012. Estimated available N for dairy manure was calculated as 25% of the total organic N [27] and 40–50% of the total inorganic N [28,29] with a limit of 11.2 kg inorganic N ha^{-1} assuming anything greater was lost over the winter. This limit was based on prior organic winter wheat research conducted over four site-years where the difference in crop N uptake in the early spring at tillering between the pre-plant dairy manure treatment and a no-N check was on average only 7 kg ha^{-1} and never exceeded 10 kg ha^{-1} at any individual site-year. Organic producers in ME and VT have a limited window in the springtime to apply manure due to soil conditions and the National Organic Program 90-day rule [18]. Chilean nitrate (CN) was used because it was the preferred N source for topdressing among regional farmers at the time of trial initiation and it was not feasible to use the same pre-plant materials for topdressing. Chilean nitrate is also the least expensive per unit N of allowable materials that is accessible to farmers in ME and VT. The CN topdress N source is a mined sodium nitrate product (16-0-0) that was approved for use at the time of trial initiation under organic certification in the USA to supply up to 20% of crop N needs [30]. The CN rates in this study exceeded the 20% limit in some plots for experimental purposes.

Table 1. Treatment descriptions for organic winter wheat N management study conducted in Maine (ME) and Vermont (VT) in 2012 and 2013.

Treatment		Topdress N rate† (kg ha^{-1})		
	Pre-plant (PP) manure target total available N rate	GS25‡ Tillering (T1)	GS30‡ Pre-stem extension (T2)	Total estimated available N applied
Check	0	0	0	0
PP$_L$	78	0	0	78
PP$_H$	117§	0	0	117¶
T1$_{78}$	0	78	0	78
PP$_L$ + T1$_{39}$	78	39	0	117
PP$_L$ + T2$_{39}$	78	0	39	117
PP$_H$ + T2$_{39}$	117§	0	39	156¶
PP$_L$ + T1$_{39}$ + T2$_{39}$	78	39	39	156

† Applied as Chilean nitrate.

‡ Zadoks scale for growth staging cereals [25].

§ Pre-plant N rate was 157 kg ha^{-1} in VT-2013.

¶ Total estimated available N in VT-2013 was 157 and 196 kg ha^{-1} for the PP$_H$ and PP$_H$ + T2$_{39}$ treatments, respectively.

2.2. Management Practices

Prior to experiment initiation, one composite soil sample was collected from each trial location to verify adequate P, K, and S levels [31]. Dates of field operations, topdress applications, and sampling are provided by site-year in Table 2. In ME, one day before wheat seeding, manure was applied by hand and incorporated within 4 hours using a Perfecta® II Field Cultivator (Unverferth Manufacturing Co, Inc. Kalida, OH, USA). In VT, manure was applied by hand and immediately incorporated with a Perfecta® II Field Cultivator on the same day as wheat seeding. Manure application rates are presented in Table 3.

Plots that did not receive pre-plant manure were not amended with P and K because soils had adequate nutrient levels, based on pre-plant soil testing. In ME, hard red winter wheat (variety AC Morley) was seeded at a density of 350 viable seeds m^{-2} and row spacing of 17.7 cm using an Almaco cone seeder with double-disk openers (Almaco Inc., Nevada, IA, USA) after which plots were packed with a Brillion 1.5 m Sure Stand grass seeder (Landoll Co., Marysville, KS, USA). In VT, hard red winter wheat (variety Harvard) was seeded at a rate of 335 viable seeds m^{-2} in 2012 and 306 viable seeds m^{-2} in 2013 with a Sunflower 9412 3.0 m grain drill (Sunflower Manufacturing, Beloit, KS, USA) double disc opener outfitted with a row spacing of 17.8 cm. Topdress N applications were applied by hand at wheat developmental stages on dates outlined in Table 2 and at rates indicated in Table 3.

Table 2. Summary of field operations, topdress applications, and biomass sampling in the organic winter wheat N management study conducted in Maine (ME) and Vermont (VT) in 2012 and 2013.

Operation	Wheat growth stage†	ME-2012	ME-2013	VT-2012	VT-2013
Manure application, PP‡	-	19 Sept 2011	14 Sept 2012	27 Sept 2011	24 Sept 2012
Wheat seeding	-	20 Sept 2011	15 Sept 2012	27 Sept 2011	24 Sept 2012
Wheat biomass sampling no. 1	Tillering, GS25	19 Apr	30 Apr	12 Apr	19 Apr
Topdress N application, T1	Tillering, GS25	20 Apr	30 Apr	12 Apr	19 Apr
Wheat biomass sampling no. 2	Pre-stem extension, GS30	30 Apr	13 May	26 Apr	03 May
Topdress N application, T2	Pre-stem extension, GS30	02 May	13 May	26 Apr	03 May
Wheat biomass sampling no. 3	Soft dough, GS85	06 Jul	03 Jul	02 Jul	09 Jul
Wheat harvest	Maturity, GS93	25 Jul	1 Aug	11 Jul	19 Jul

† Zadoks scale for growth staging cereals [25].

‡ PP, pre-plant; T1, topdress at tillering; and T2, topdress at pre-stem extension.

Table 3. Material and nutrient application rates for N sources applied as pre-plant and topdress to winter wheat in Maine (ME) and Vermont (VT) in 2012 and 2013.

Material and nutrient application rates	Pre-plant manure target N rate†								Topdress Chilean nitrate target N rate†	
	ME-2012		ME-2013		VT-2012		VT-2013			
	78	117	78	117	78	117	78	157	39	78
Dry matter (%)	28.3		26.6		19.8		20.2			
Material (Mg ha^{-1})	72	108	56	84	45	67	40	74	0.25	0.49
Organic N (kg ha^{-1})	260	390	193	290	275	412	307	563	0	0
Inorganic N (kg ha^{-1})	46	68	25	38	32	48	23	42	39	78
Estimated available N‡ (kg ha^{-1})	76	109	59	84	80	114	86	152	39	78
Total P (kg ha^{-1})	150	224	173	259	91	137	73	134	0	0
Total K (kg ha^{-1})	286	429	325	488	154	232	101	184	0	0

† Estimated available N (kg ha^{-1}).

‡ Estimated available N was calculated as 25% of the total organic-N [27] and 40-50% of the total inorganic N for dairy manure [27–29] with a limit of 11.2 kg inorganic N ha^{-1}.

2.3. Measurements and Analytical Procedures

Tiller density was determined at tillering for the PP-only treatments by counting wheat shoots with three or more leaves in eight 0.3-m sections of row per plot. These treatments were sampled to measure pre-plant N effects on tillering because all other N applications came at or after tillering. Leaf tissue N concentration at pre-stem extension was measured via destructive sampling that took place in one half of each plot. Plants were clipped at 2 cm from the soil surface from three 0.3-m sections of rows (avoiding border rows). On consecutive sampling dates, sample areas were positioned 0.3 m away from the preceding sample area. Samples were bulked to represent a total sample area of 0.9 m of row per plot. Plants were dried at 60° C, weighed, and ground through a 2-mm mesh. Total N concentration was determined by combustion for a 250-mg subsample using a Leco CN2000 analyzer (Leco Corp., St. Joseph, MI, USA) in ME, whereas in VT, a 100-gram sample was submitted to Cumberland Valley Analytical Services (Hagerstown, MD, USA), for Near Infrared Reflectance spectroscopy. Plant N uptake by wheat and weed biomass was determined at three wheat developmental stages: tillering, pre-stem extension, and soft dough (GS85, or "peak biomass")—all using the same methods as for leaf tissue N sampling. Weed pressure was very low so no weed control measures were taken. Weed samples were collected from the sample area and included in plant N calculations when weed biomass composed $>$2% of the total plant biomass. Plant N uptake was calculated by multiplying plant above ground biomass by % N. At soft dough, the number of spikes per bulk sample was counted and recorded.

Grain was harvested between 25 July and 1 August from a 1.5 m by 9.1 m harvest area with a Wintersteiger small-plot combine (Ried, AT) in ME, and between 11 and 19 July, from a 1.4 m by 5.5 m harvest area with a Almaco SPC50 plot combine (Almaco, Inc., Nevada, IA, USA) in VT. Grain was cleaned with a small Clipper (Clipper, A.T. Ferrell Co., Bluffton, IN, USA) to remove weed seeds and inert material. Grain samples were weighed. Moisture was measured (GAC 2100, DICKEY-john Corp., Auburn, IL, USA) and adjusted to 135 g kg^{-1} on cleaned samples to determine grain yield. Grain was subsampled (100 g) and ground (2 mm mesh). In ME, grain CP was determined on a 250-mg sub-subsample by multiplying Leco N by 5.7 N, according to American Association of Cereal Chemists (AACC) method 46–30.01 [32], and adjusted to 120 g kg^{-1} grain moisture. In VT, grain CP was determined on a 250-mg sub-subsample using a Perten Inframatic 8600 Flour Analyzer (PertenElmer Co., Hägersten, SWE). Combustion and NIR techniques are both accepted methods for CP determination [33]. Thousand kernels weights (TKW) were collected in ME. One thousand seeds per plot were counted using a seed counter (Count-A-Pak Seed Totalizer, Seedburo Equipment Co., Des Plaines, IL, USA), weighed, and adjusted to 135 g kg^{-1} moisture. Weather data were collected at these research sites unless otherwise noted.

2.4. Statistical Analysis and Calculations

Data were analyzed with the statistical program R [34] using a mixed model Analysis of Variance (ANOVA) with block as a random effect and treatment and site-year as fixed effects. The "nlme" package [35] was used to test the significance of site-year, treatment, and site-year by treatment interactions. The ANOVA assumption of equal variance was verified with Levene's test using the 'car' package [36]. Residual values were used to assess normal distribution with the Shapiro-Wilk Normality test. When residuals did not conform to equal variances and normality, a Box-Cox power transformation was used using the 'MASS' package [37]. The treatments were arranged in an incomplete factorial to test only treatments of specific interest to farmers in the region. The data were analyzed with a means separation using Fisher's Protected Least Significant Difference (LSD) using the 'multcomp' package [38]. Plant N uptake effects were analyzed by date as not all treatments were measured at every date thereby precluding a repeated measures analysis. Grain N yield was determined by multiplying grain N (%) by grain yield (kg ha^{-1}). The difference method was used to calculate apparent nitrogen recovery (ANR) for PP-only treatments by subtracting the plant N uptake of the check treatment from the plant N uptake of the PP-only treatments divided by the estimated amount of plant available N applied pre-plant [39]. Apparent nitrogen recovery was similarly calculated for topdress treatments by subtracting the plant N uptake of the PP-only treatments from the plant N uptake of the topdress treatment divided by the estimated amount of plant available N applied at topdress. Coefficient of variation (CV) was calculated as a function of square root of error mean square divided by the site-year mean for each response variable. In-season test data were analyzed with linear regression using treatment means over site-years because the tests should show relationships between variables over a range of sites, seeding rates, and varieties. These analyses were used to determine the correlations between: 1) grain yield and tiller density at GS25, 2) grain yield and tissue N concentration at GS30, and 3) CP and tissue N concentration at GS30.

3. Results

3.1. Weather

Monthly mean temperature and precipitation amounts for the four site-years are presented in Table 4. During seeding and pre-plant applications in September, all site-years except ME-2012 experienced greater than the 30-year normal precipitation. In VT-2012 approximately 24 mm of rainfall occurred 2 days after the pre-plant application and could have caused N leaching. For all site-years, March was warmer than average and there was a period of drier than average weather beginning in March and extending through April. The VT-2013 site-year experienced wetter than normal precipitation during the months of May and June but the majority of rainfall occurred at least a week after the T2 treatment application. In July, weather conditions turned dry, especially in ME-2012 and VT-2013 when rainfall was 65 and 59 mm less than the 30-year average, respectively.

Table 4. Monthly mean air temperature measured at 1.5 m from the ground, rainfall from September through November of the seeding year and from March through July of the harvest year at the experiment sites in Maine (ME) and Vermont (VT) compared with average climate data for 1981 to 2010.

	Maine			Vermont		
	2012	2013	30-year aver.	2012	2013	30-year aver.
Month	Mean temperature (°C)					
September†	16.1	13.5	13.9	17.1	16	16.1
October†	9.4	9.8	7.8	10.1	11.3	8.9
November†	5.0	0.7	2.2	6.3	3.1	3.9
March	2.3	0.3	-1.4	4.3	0.1	-0.6
April	6.8	5.1	5.3	7.2	6.4	7.2
May	12.7	11.9	11.4	15.8	15.1	13.3
June	15.9	16.9	16.4	19.4	17.8	18.9
July	20.0	20.8	19.7	21.9	22.1	21.7
	Rainfall (mm)					
September†	48	204	96	141	136	91
October†	109	179	101	89	105	91
November†	66	40	112	36	17	79
March	50	66	104	38	26	56
April	93‡	36	96	67	54	71
May	109	107	99	99	122	89
June	153	152	103	82	234§	94
July	25	112	90	96	48	107

† Seeding year.

‡ Precipitation data was not available for 26 April 2012 in ME.

§ June 2013 precipitation data for the VT site was taken from the National Weather Service, South Hero, VT (44.65° N 73.31° W).

3.2. Plant Nitrogen Uptake

Plant N uptake data were analyzed over site-years (Table 5). In ME-2013, weeds comprised 6% of aboveground biomass at the soft dough stage and 11% of total plant N uptake, and thus weed N uptake was included in plant N uptake (Table 5). However, there were no significant differences among treatments in either weed biomass or weed N uptake ($p = 0.157$ and 0.132, respectively; data not shown). In all other site-years, weed biomass never exceeded 2% of the aboveground biomass, thus plant N uptake reported in the results directly represents wheat N uptake.

The PP-only treatments (PP$_L$ and PP$_H$) generally did not increase N uptake compared with the untreated check. The exception was in ME-2012 at tillering when the PP$_L$ treatment increased N uptake by 7.1 and 4.6 kg N ha^{-1} compared with the check and PP$_H$ treatments, respectively. Delaying all N applications until tillering (T1$_{78}$) increased plant N uptake at soft dough by 48.4 kg N ha^{-1} compared with applying the equivalent amount of N at pre-plant (PP$_L$).

The PP$_L$ + T1$_{39}$ treatment consistently increased N uptake compared with the PP-only treatments, and uptake was on average 20% and 28% greater at pre-stem extension and at soft dough, respectively. Topdressing supplemental N at pre-stem extension (PP$_L$ + T2$_{39}$ and PP$_H$ + T2$_{39}$) increased plant accumulated N compared with their respective PP-only treatments, by an average of 30%. Topdressing twice (PP$_L$ + T1$_{39}$ + T2$_{39}$) resulted in N uptake at soft dough that was similar to the PP$_L$ + T1$_{39}$ and PP$_L$ + T2$_{39}$ treatments but greater than PP$_H$ + T2$_{39}$ by 27.5 kg N ha^{-1}. Apparent N recovery rates of the PP-only treatments were the lowest among all treatments and were 15% on average (data not shown). The ANR of topdress treatments ranged from 60 to 89%. Both the T1$_{78}$ and PP$_L$ + T2$_{39}$ treatments had ANR values greater than 80%.

3.3. Tiller and Spike Densities

Due to significant treatment by site-year interactions for tiller density, spike density, and the other response variables listed in Table 6, the data were analyzed and are presented by site-year (Table 7). Tiller densities averaged 1371, 738, 906, and 890 tillers m^{-2} in ME-2012, ME-2013, VT-2012, and VT-2013, respectively, and were not influenced by PP-only treatments (data not shown).

The PP-only treatments also did not influence spike density except in ME-2013, where the PP$_L$ treatment produced 38% more spikes than the check. The addition of topdress N increased spike density in most cases in ME-2012; T1$_{78}$ vs. PP$_L$, and PP$_L$ + T1$_{39}$ vs. PP$_L$, and PP$_L$ + T1$_{39}$ + T2$_{39}$ vs. PP$_L$ + T2$_{39}$ treatments increased spike density by 48, 47, and 34%, respectively. In ME-2013 and VT-2013, the PP$_L$ + T1$_{39}$ + T2$_{39}$ treatment also increased spike density relative to the PP$_L$ + T1$_{39}$ treatment by 25 and 43%, respectively. Spike densities were unaffected by treatments in VT-2012, which had higher %CV than the other site years (Table 7).

3.4. Grain Yield

Average grain yields by site-year were 5.22, 2.41, 3.09, and 4.44 Mg ha^{-1} for ME-2012, ME-2013, VT-2012, and VT-2013, respectively (Table 7). Yields in ME-2013 and VT-2012 were approximately 1.08 and 1.63 Mg ha^{-1} lower, respectively, than average yields from trials conducted with the same varieties and locations in those years whereas VT-2013 average grain yields were 0.57 Mg ha^{-1} higher than the local equivalent [40].

Table 5. Mixed model ANOVA and LSD results of mean plant N uptake for wheat at different growth stages as affected by pre-plant and topdress N treatments in Maine (ME) and Vermont (VT) in 2012 and 2013. Treatment means presented are the means of the 4 site-years.

		Plant N Uptake (kg N ha^{-1})				
Effects and sources of variation		Tillering		Pre-stem extension		Soft dough
Site-year						
ME2012		41.5†		37.1		73.4
ME2013		10.5		26.8		91.1
VT2012		31.9		47.4		125
VT2013		18		51.8		231
Treatment						
Check		22.3^{a} ‡		34.6^{a}		93.7^{a}
PP$_{L}$†		29.4^{b}		39.2^{ab}		111.7^{ab}
PP$_{H}$		24.8^{a}		39.5^{ab}		102.2^{a}
T1$_{78}$		-		43.3^{bc}		160.1^{e}
PP$_{L}$ + T1$_{39}$		-		47.3^{c}		136.7^{cd}
PP$_{L}$ + T2$_{39}$		-		-		146.6^{ce}
PP$_{H}$ + T2$_{39}$		-		-		131.2^{bc}
PP$_{L}$ + T1$_{39}$ + T2$_{39}$		-		-		158.7^{de}
Sources of variation	df	F-value	df	F-value	df	F-value
Site-year (S)	3	35.9***	3	21.6***	3	32.2***
Treatment (T)	2	6.8**	4	5.8***	7	9.1***
S × T	6	1.62	12	1.01	21	1.03
CV, %		19.2		16.9		24.0

* Significant at P <0.05; ** Significant at P <0.01; *** Significant at P <0.001.

† PP$_{L}$, 78 kg N ha^{-1} manure at pre-plant; PP$_{H}$, 117 or 157 kg N ha^{-1} manure at pre-plant; T1$_{78}$, 78 kg N ha^{-1} topdress at tillering; T1$_{39}$, 39 kg N ha^{-1} topdress at tillering; T2$_{39}$, 39 kg N ha^{-1} topdress at pre-stem extension.

‡ Within column and site-year, treatment means with the same lower case letter are not significantly different at P<0.05.

Table 6. Mixed model ANOVA results of mean spike density, grain yield, GS30 tissue N, grain crude protein, and grain N yield for wheat as affected by pre-plant and topdress N treatments in Maine (ME) and Vermont (VT) in 2012 and 2013.

Sources of variation	Spike density		Grain yield		GS30 tissue N		Grain crude protein		Grain N yield	
	df	F-value	df	F-value	df	F-value	df	F-value	df	F-value
Site-year (S)	3	4.7*	3	67.5***	3	33.6***	3	86.2***	3	69.3***
Treatment (T)	7	6.0***	7	8.7***	4	31.5***	7	13.0***	7	14.8***
S x T	21	2.1**	21	2.8***	21	2.7**	21	1.8*	21	3.2***
CV, %		17.0		12.7		7.2		4.2		12.7

* Significant at P <0.05; ** Significant at P <0.01; *** Significant at P <0.001.

Table 7. LSD and ANOVA results for spike density, grain yield, GS30 tissue N, grain crude protein (at 120 g kg^{-1} grain moisture), and grain N yield for wheat grown with different pre-plant and topdress N treatments in Maine (ME) and Vermont (VT) in 2012 and 2013. VT-2012 GS30 tissue N and grain CP data were transformed (λ= -2 and -4, respectively). Back transformed values are in parentheses.

Site-year	Treatment	Spike density (spike m^{-2})	Grain yield (Mg ha^{-1})	GS30 tissue N (g kg^{-1})	Grain crude protein (g kg^{-1})	Grain N yield (kg ha^{-1})
ME-2012	Check	476^{c}†	3.57^{d}	24.5^{b}	99^{b}	61^{d}
	PP$_L$‡	445^{c}	4.75^{bc}	24.3^{b}	96^{b}	79^{cd}
	PP$_H$	544^{bc}	4.55^{c}	24.6^{b}	99^{b}	78^{cd}
	T1$_{78}$	657^{ab}	5.71^{a}	34.2^{a}	115^{a}	113^{a}
	PP$_L$ + T1$_{39}$	656^{ab}	5.82^{a}	32.8^{a}	104^{b}	104^{ab}
	PP$_L$ + T2$_{39}$	553^{bc}	5.35^{ab}	-	98^{b}	91^{bc}
	PP$_H$ + T2$_{39}$	629^{ab}	5.96^{a}	-	101^{b}	105^{ab}
	PP$_L$ + T1$_{39}$ + T2$_{39}$	739^{a}	6.01^{a}	-	103^{b}	107^{ab}
	Source of variation			ANOVA		
	Treatment	**	***	***	*	***
	CV, %	14.6	10.3	4.6	6.8	13.9
ME-2013	Check	333^{d}	1.79^{c}	32.5^{c}	118^{cd}	36^{b}
	PP$_L$	459^{bc}	1.96^{bc}	30.4^{c}	119^{bcd}	40^{b}
	PP$_H$	432^{cd}	2.01^{bc}	31.8^{c}	117^{d}	41^{b}
	T1$_{78}$	569^{ab}	2.77^{a}	44.9^{a}	130^{a}	62^{a}
	PP$_L$ + T1$_{39}$	484^{bc}	2.87^{a}	40.3^{b}	116^{d}	58^{a}
	PP$_L$ + T2$_{39}$	505^{abc}	2.58^{ab}	-	127^{ab}	56^{a}
	PP$_H$ + T2$_{39}$	535^{abc}	2.50^{ab}	-	125^{abc}	54^{a}
	PP$_L$ + T1$_{39}$ + T2$_{39}$	606^{a}	2.83^{a}	-	130^{a}	63^{a}
	Source of variation			ANOVA		
	Treatment	**	**	***	**	***
	CV, %	16.1	18.3	6.5	4.3	16.3
VT-2012	Check	431	2.52^{b}	0.101 (32.0)	8.04 (106)^{d}	46^{c}
	PP$_L$	558	2.96^{b}	0.106 (31.3)	7.88 (106)^{cd}	54^{bc}
	PP$_H$	336	2.89^{b}	0.098 (32.3)	7.45 (108)^{bcd}	49^{bc}
	T1$_{78}$	552	4.31^{a}	0.084 (36.0)	5.32 (118)^{a}	88^{a}
	PP$_L$ + T1$_{39}$	696	3.12^{b}	0.072 (38.0)	6.32 (112)^{abc}	60^{bc}
	PP$_L$ + T2$_{39}$	629	3.01^{b}	-	6.14 (113)^{ab}	59^{bc}
	PP$_H$ + T2$_{39}$	473	2.87^{b}	-	5.40 (118)^{a}	63^{b}
	PP$_L$ + T1$_{39}$ + T2$_{39}$	535	3.07^{b}	-	4.75 (121)^{a}	63^{b}
	Source of variation			ANOVA		
	Treatment	ns	**	ns	**	***
	CV, %	30.3	16.2	21.7	17	16.9
VT-2013	Check	612^{ab}	4.41^{bc}	40.5	114^{d}	87^{b}
	PP$_L$	604 ab	5.20^{a}	41.6	129^{abc}	116^{a}
	PP$_H$	623^{a}	4.19^{c}	42.8	125^{c}	90^{b}
	T1$_{78}$	670^{a}	4.22^{c}	45.8	130^{abc}	95^{b}
	PP$_L$ + T1$_{39}$	495^{b}	4.27^{c}	43.2	127^{bc}	94^{b}
	PP$_L$ + T2$_{39}$	725^{a}	4.26^{c}	-	129^{abc}	95^{b}
	PP$_H$ + T2$_{39}$	689^{a}	5.01^{ab}	-	132 ^{ab}	114^{a}
	PP$_L$ + T1$_{39}$ + T2$_{39}$	709^{a}	4.02^{c}	-	135^{a}	93^{b}
	Source of variation			ANOVA		
	Treatment	*	*	ns	***	**
	CV, %	12.9	10.6	6.2	3.7	11.8
df		7	7	4	7	7

* Significant at P <0.05; ** Significant at P <0.01; *** Significant at P <0.001; ns: not significant at P <0.05.

† Within column and site-year, treatment means with the same lower case letter are not significantly different at P<0.05.

‡ PP$_L$, 78 kg N ha^{-1} manure at pre-plant; PP$_H$, 117 or 157 kg N ha^{-1} manure at pre-plant; T1$_{78}$, 78 kg N ha^{-1} topdress at tillering; T1$_{39}$, 39 kg N ha^{-1} topdress at tillering; T2$_{39}$, 39 kg N ha^{-1} topdress at pre-stem extension.

Impacts of the PP-only treatments on grain yield varied by site-year. Significant increases were observed in ME-2012 and VT-2013 when site-years were analyzed individually. In ME-2012, both PP-only treatments increased yields relative to the check by an average of 30%. In VT-2013, only the PP_L treatment increased yields by 18%. The $T1_{78}$ treatment increased grain yields by 20% in ME-2012, 41% in ME-2013, and 46% in VT-2012, but reduced yields by 23% in VT-2013. The PP_L + $T1_{39}$ treatment increased grain yields in ME-2012 and 2013 versus the PP_L treatment by 23 and 46%, respectively, but reduced grain yields by 22% in VT-2013. The PP_H + $T2_{39}$ treatment increased grain yields by 31 and 20% in ME-2012 and VT-2013, respectively, over the PP_H treatment. The PP_L + $T1_{39}$ + $T2_{39}$ treatment had no influence on grain yield in any site-year compared with PP_L + $T1_{39}$ or PP_L + $T2_{39}$ treatments.

Thousand kernel weights were measured for the ME site-years but are not presented because there were no significant treatment effects. Thousand kernel weights averaged 39.5 g in 2012 and 29.5 g in 2013, and were significantly correlated with grain yields (r = 0.48, $p < 0.01$ and r= 0.59, $p < 0.001$ for 2012 and 2013, respectively).

3.5. GS30 Tissue N, Grain Crude Protein, and Grain N Yield

Treatment effects on GS30 wheat tissue N concentrations were evident only in ME and were restricted to tillering N additions; N applied at pre-plant had no significant effects (Table 7). Compared with PP_L, the $T1_{78}$ treatment increased tissue N by 44% on average and the PP_L + $T1_{39}$ produced a 34% average increase.

Grain CP averaged 102, 123, 113, and 128 g kg^{-1} in ME-2012, ME-2013, VT-2012, and VT-2013, respectively (Table 7). The PP-only treatments had no significant effect on CP except in VT-2013 where the 78 kg N ha^{-1} rate increased CP by 13% as compared with the check. The $T1_{78}$ treatment increased CP compared with the PP_L and PP_H treatments by an average of 14% at the ME sites and by 11% in VT-2012, but had no effect in VT-2013. The PP_L + $T1_{39}$ treatment produced no measurable increases in CP and the PP_L + $T2_{39}$ treatment increased CP in VT-2012 by 7% compared with the PP_L treatment. The PP_H + $T2_{39}$ treatment increased CP in three of four site-years compared with the PP_H treatment by 7, 9, and 6% in ME-2013, VT-2012, and VT-2013, respectively. The PP_L + $T1_{39}$ + $T2_{39}$ treatment increased CP only in ME-2013 by 12% compared with the PP_L + $T1_{39}$ treatment.

Grain N yield results were similar to grain yield results with two exceptions (Table 7). In ME-2012, the PP_H treatment did not increase grain N yield compared with the check, and in ME-2013, the PP_L + $T2_{39}$ treatment increased grain N yield by 40% compared with the PP_L treatment.

3.6. In-season Tests

Tiller density was a better predictor of grain yield (r = 0.52, $p < 0.001$; Figure 1) than tissue-N at GS30 (r = 0.09, p = 0.426; data not show) when compared across site-years. The residuals from the regression line in Figure 1 were not influenced by treatment (p = 0.175).

Correlations were weak when analyzed by site-year (data not shown) likely due to limited tiller range and variability within site-year. Tiller densities in ME-2012, for example, ranged from 1184 to 1668 tillers m^{-2} with a standard deviation of 149 tillers m^{-2}. Tissue N at GS30 was a good predictor of CP (r = 0.75, $p < 0.001$; Figure 2). The residuals from the regression line in Figure 2 were influenced by treatment (p = 0.005), indicating that additional variance in the model was explained by the treatments.

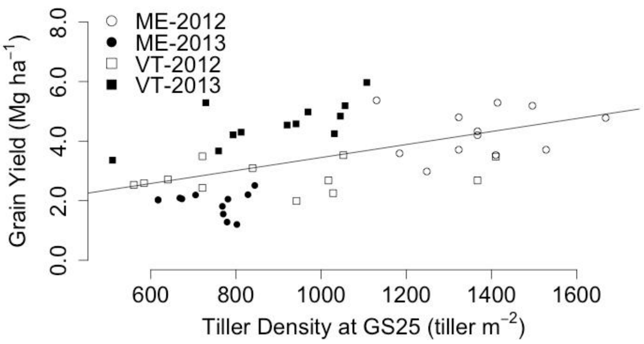

Figure 1. Correlations between tiller density at GS25 and grain yield in Maine (ME) and Vermont (VT) in 2012 and 2013 across different pre-plant N treatments (y = 0.0021x + 1.2668; r = 0.52; $p < 0.001$). Data are treatment means from each site year. The standard error of the regression coefficients was 0.540 and 0.001 for β_0 and β_1, respectively.

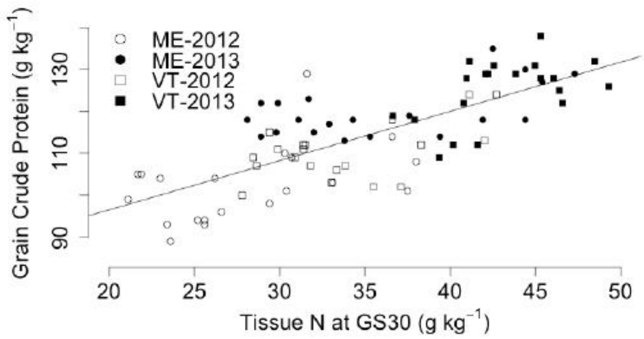

Figure 2. Correlation between tissue N and crude protein in Maine (ME) and Vermont (VT) in 2012 and 2013 across different pre-plant and topdress N treatments (y = 1.176x + 72.963; r = 0.75; $p < 0.001$). Data are treatment means from each site year. The standard error of the regression coefficients was 4.164 and 0.116 for β_0 and β_1, respectively.

4. Discussion

4.1. Site-year Effects

Differences in growing conditions among the four site-years made it difficult to draw general conclusions and recommendations based on the effects of N treatment. In both ME-2012 and VT-2013, favorable growing conditions supported high yield potentials, as evidenced by the high yields in the check, although varying levels of N availability from soil %OM may have caused different treatment responses between these two site-years. Where %OM was low (ME-2012), all N treatments produced positive and substantial increases in grain yield. The only treatment to increase CP was the highest single topdress application. When an increase in yield occurs, it is often accompanied by an increase in grain N yield but not necessarily CP as the increase in carbohydrates dilutes the N [2,41]. In contrast, where %OM was high (VT-2013), N treatments produced fewer positive yield responses but more increases in CP. While soil nitrate was not measured in this study, the relatively high %OM measured at this site suggests greater soil N supply [42,43]. This site-year also was the only one to demonstrate an increase in CP from the PP-only treatments. These results are congruent with a study by Woodward and Bly [4] showing that N must be of sufficient amount and appropriately timed to support positive responses in both yield and CP. Terman et al. [10] found that under high soil nitrate conditions ($>$67 kg ha^{-1} NO_3-N), additional N applied to hard red winter wheat produced an increase in grain protein content but low or absent responses in grain yield. Similarly, Frederick and Marshall [44] found early spring topdressing to soft red winter wheat on soils with high N reserves decreased grain yields by reducing kernel weight or productive tillers below the level necessary for optimal yield.

In ME-2013 and VT-2012, the check treatment yields suggest reduced yield potentials. In ME-2013, yield potential was likely limited by observed weed and disease pressure resulting from a lack of rotation. It was less likely the preceding mustard affected wheat yield because the mustard crop accumulated relatively little biomass before incorporation and stand counts show no effect as compared with prior years when wheat followed fallow (data not shown). Nonetheless, these factors did not limit the responsiveness of this site to N treatments. Treatment effects were observed for both grain yield and CP. As a consequence of the low yields, CP potential was relatively high, which was consistent with the tradeoff between yield and protein reported by others [9,11]. All treatments exceeded 110 g kg^{-1} CP and nearly all those receiving topdress N had CP levels above 120 g kg^{-1}. In VT-2012, it was possible that heavy rainfall, occurring 2 days after the pre-plant applications, may have been a contributing factor to the relatively low grain yield potential and limited yield response to N. Only the highest topdress N rate (T1$_{78}$) produced a yield response, whereas more treatment responses were observed for CP.

4.2. Nitrogen Treatment Effects

The PP-only treatments improved yields at three of four site-years (when ME site-years were analyzed together) but were less likely to produce an increase in CP. These results are congruent with others who have found that applications at the pre-plant timing alone do not supply an adequate amount of late-season available N to enhance CP in winter wheat [3,45]. At the majority of site-years, it was possible that the amount or timing of mineralization from the organic N fraction of manure was insufficient to support protein production. The VT-2013 site was the exception because high %OM may have impacted yield-CP dynamics as previously described. These results support findings that matching the N availability of organic N sources with the periods of high crop N demand presents a major challenge for organic bread wheat producers [14].

The T1$_{78}$ treatment produced increases in both yield and CP in the majority of site-years suggesting that the springtime application was better matched with crop N demand than the pre-plant application timing. This enhanced plant N uptake at soft dough and ANR over the PP-only treatment. Increases in both grain yield and CP also suggest available N was in excess of yield requirements and was sufficient to increase CP [11,46]. It should be noted that N application timing and source are confounded in these comparisons and the difference in N source (manure vs. CN) could also be a factor in the observed effects.

Treatments receiving a topdress application often showed a yield and CP advantage over the PP-only treatments. The PP$_L$ + T1$_{39}$ treatment produced some measurable increases in yields and plant N uptake compared with the PP-only treatments but never produced a measurable increase on CP. More frequent effects on yield and CP were found with the PP$_H$ + T2$_{39}$ treatment versus the PP$_H$ comparison possibly because greater mineralization of N from the PP$_H$ treatment may have been adequate to support CP. The timing of supplemental N applied in the PP$_L$ + T1$_{39}$ and PP$_L$ + T2$_{39}$ treatments had no influence on yield and CP results likely because the applications were too close in time to cause differences. The application at T2 was relatively early compared to other studies that showed topdress applied later, at flag leaf (GS39) and boot (GS45), were more effective at increasing CP than the T1 application [3].

Nitrogen applied at both T1 and T2 did not increase yields relative to supplemental N applied at T1 or T2. The fact that N applied at both timings produced among the highest yields in ME, indicates two topdress applications were in excess of that required to reach a yield plateau. Interestingly, the opposite effect occurred in VT; treatment yields with two topdress applications were equivalent to the check and among the lowest of all treatments. The VT data suggests this response was attributed to the aforementioned site factors. Application timing may have also been a factor in the observed effects on CP. Two N applications increased CP relative to the T1 application at two of four site-years but never increased CP relative to the T2 application. Greater differences in CP may have occurred if the second application of topdress were delayed to GS45 or later [41].

These findings indicate that when yield potential was high, treatments that included topdress N generally produced CP greater than the 100 g kg^{-1} threshold considered sufficient by local artisan bakers in our region. With this in mind, the costs of applying organic-approved sources of N must be compared against the crop value. Chilean nitrate is cheaper (US$ 229 ha^{-1}) than other organic-approved sources of N though it is not allowed in Canada and Europe and may be prohibited in the future under the US National Organic Standards Board. Other topdress sources, such as dehydrated poultry litter, are more expensive (US$ 459 ha^{-1}) and may have lower N availability compared with the soluble CN [3], which may reduce its efficacy.

4.3. In-season Test: Tiller Density

Results indicated that tiller densities can be a predictor of grain yield but a wider range of densities is needed to better understand the utility of this measurement as a decision tool. When tiller densities were below the 1000 tillers m^{-2} threshold established by Scharf and Alley [47], there was not a yield penalty for delaying supplemental topdressing from GS25 to GS30 in ME-2013, VT-2012, and VT-2013. Similarly, when average tiller densities were <1000 tillers m^{-2}, there was no penalty for supplying N earlier at GS25 [6]. In fact, only the PP_L + $T1_{39}$ and PP_L + $T1_{39}$ + $T2_{39}$ treatments in the ME site-years enhanced yields over the PP_L whereas the PP_L + $T2_{39}$ treatment did not. Nitrogen topdress rates of 39 and 78 total kg N ha^{-1} applied in this study were slightly above the range recommended of approximately 30 to 56 kg N ha^{-1} for densities <1000 tillers m^{-2} [6]. It is possible that tiller densities in this study were not low enough to produce the measurable yield differences between the PP_L + $T1_{39}$ and PP_L + $T2_{39}$ applications that others have found. For instance, in a study with non-organically managed no-till winter wheat, Weisz et al. [48] showed that when tiller densities were below 550 tillers m^{-2}, treatments with supplemental N applied at GS25 and split applied between GS25 and GS30 produced greater yields than the treatment with supplemental N at GS30. Therefore, fully evaluating topdressing timing effects at the threshold established by Scharf and Alley [47] was limited by the fact that tiller densities at most site-years were adequate but never well below the threshold (738, 906, and 890 tillers m^{-2} in ME-2013, VT-2012, and VT-2013, respectively) and exceeded 1000 tillers m^{-2} in just ME-2012 (1371 tillers m^{-2}). The PP-only treatments did not produce statistically different tiller densities and an effort to capture a wider range through seeding rates and dates may be needed. For instance, Weisz et al. [48] found that different seeding rates and dates produced a range of 162 to 1774 tillers m^{-2} in soft red winter wheat.

4.4. In-season Test: Tissue Nitrogen

Tissue N values in this study ranged from 24.3 to 45.8 g kg^{-1} and were similar to the >20.0 to <50.0 g kg^{-1} values reported by Baethgen and Alley [26] for soft winter wheat. A stronger correlation between tissue N and CP than between tissue N and yield suggests this test may be useful to guide N management for CP even though other studies do not explore this purpose. Using the slope of the regression line (Figure 2) the critical level for achieving CP of 120 g kg^{-1} was a tissue N concentration of 40.0 g N kg^{-1}. This value aligns with the critical value of 39.5 g N kg^{-1} reported by Baethgen and Alley [26] for achieving 90% relative yield without further fertilization. The critical level was met at the site-years with the highest overall CPs. Specifically, delaying topdress N until GS25 in ME-2013 and all N treatments in VT-2013 met the critical level (Table 7). In site-years with low overall CPs such as ME-2012 and VT-2012, the critical level was never met but individual cases suggest the tissue N test has the predictive power to obtain the desired CP response.

In ME-2012, low tissue N concentrations (24.4 g kg^{-1} for the PP-only treatments; 32.8 g kg^{-1} for PP_L + $T1_{39}$) implied the need for approximately 120 and 78 kg N ha^{-1}, respectively, at GS30 according to Alley et al. [6]. The rate of 39 kg N ha^{-1} applied at GS30 was possibly inadequate because the desired CP was never met. Conversely, in VT-2012, the 39 kg N ha^{-1} applied at GS30 for the same treatment (PP_L + $T2_{39}$) aligned more with the rate recommended by the tissue test (47 kg N ha^{-1}) and was adequate to meet the desired CP.

These results suggest that testing various N application rates at GS30 against the measured tissue N values would broaden understanding of the rates need to maximize CP. Beyond applying a sufficient N rate, N application timing may have been an influential factor in the aforementioned results such that the N applied at GS30 may have been too early to increase CP. Others have found later applications of N at the boot stage (GS45) were more effective at increasing CP in hard red winter wheat than applications at or prior to GS30 [3,22,49]. However, Gooding et al. [50] noted foliar urea applied at or soon after anthesis increased CP but post anthesis applications pose higher risk of N loss. For an organic producer, the threshold at which tissue N testing is relevant should be based on the producer's means to apply an N source later in the season as well as their access to an organic approved N source with rapid N availability. As discussed by Mallory and Darby [3], while topdressing could be a good strategy for organic winter bread wheat producers, further evaluation of topdress N sources is needed. Lastly, measuring tissue N concentrations beyond GS30 may reveal that late-season mineralization from organic N attributes to CP, but studies addressing this area are lacking. Brown et al. [41] and Brown and Petrie [45] reported that flag leaf total N taken at early heading or anthesis (GS50-60) was better related to CP at harvest than samples collected earlier because the majority of plant N uptake occurs by flag leaf emergence.

5. Conclusions

The primary objective of this study was to analyze split N application regimes and in-season tests to guide N applications for organic production. The PP-only treatments were unreliable for producing market quality bread wheat. The T1$_{78}$ treatment produced the highest yield and CP, except for one case, but delaying all N application until spring is challenging in terms of the feasibility of applying a cost-effective fresh animal or green manure N or the cost of easily applied pelletized organic N sources. Topdressing supplemental N was effective at increasing yield and CP when preceded by the PP$_H$ application. The PP$_L$ + T1$_{39}$ + T2$_{39}$ treatment generally did not enhance results compared with single topdress application at T1 or T2. Responses to added N were variable among site-years and influenced by yield potential and soil %OM. In-season tests hold promise as decision tools for organic winter bread wheat production but additional evaluation and calibration is needed. Future studies should include a variety of organic-approved and locally available pre-plant and topdress sources, a wider range of background tiller densities and topdress N rates, and perform tissue testing at growth stages beyond GS30, but prior to GS60.

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Ideas and Perspectives

Basic Substances under EU Pesticide Regulation: An Opportunity for Organic Production?

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Abstract: Some of the active substances allowed in organic production are now approved as basic substances under the EU plant protection products regulation. Previously, all organic farming permitted active substances were approved as conventional plant protection products. In accordance with the criteria of Article 23 of the EU regulation (EC) No 1107/2009, basic substances are granted without maximum residue limits and have a good prospect for being included in Annex II of organic farming Regulation (EC) 889/2008. In fact, most of them are already permitted in organic farming. At this stage, it seems desirable to organize applications in order to avoid duplications and to clarify strategy across Europe. This organization should be planned in order to identify corresponding knowledge and data from field experiments, and to further constitute the most crucial issues related to organic production. A work of this nature was initially supported by IFOAM-EU for lecithin, calcium hydroxide and *Quassia* extract. The Institut Technique de l'Agriculture Biologique (ITAB) was previously engaged in a large-scale approval plan motivated by the continuous demand for the regularization of compounds/substances already in use and has a mandate for testing and approving new compatible substances. Thus, the horsetail extract (*Equisetum arvense*) was the first approved basic substance and ITAB has obtained 11 of the 15 basic substances approved at the EU level.

Keywords: Article 23; basic substance; Regulation (EC) No 1107/2009; Regulation (EC) No 889/2008, Annex II

1. Introduction

Recently created [1] by Article 23 of EC Regulation No 1107/2009 [2], the *"Basic Substances"* category is now operative with 15 approvals at the EU level [3]. Basic Substances are plant protection products with specific criteria for approval. Consequently, this status specified no maximal residue limit and high potential for inclusion in the Organic Farming regulation (EC) No 889/2009 [4] Annex II. Clearly, bio-sourced and traditional botanical extracts (as decoction, herbal tea...), light supports/aids, and plant defence enhancers used as crop protection are obvious candidates. Diverse applicants were engaged for different initial reasons and have succeeded in their applications. These biorational candidates clearly targeted the organic agriculture crop protection market or were even carried out by the organic sector itself.

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2. First Implication of the Organic Sector: Implementing Regulation EU No 354/2014

Annex II of organic farming regulation for exclusively managing plant protection substances was previously focused on products of low concern, although some of them were not approved under general pesticide regulations [1]. Horizontal harmonization of pesticide regulation in organic farming was clearly needed and was achieved after a substantial change in 2014 following few years of unchanged situation. Thus, when the Implementing Regulation (EU) No 354/2014 [5] came into full force, Annex II of organic farming regulation was widely modified. Indeed, Implementing Regulation (EU) No 354/2014 suppressed quite a number of substances from Annex II and some others will or may follow in the future for a number of reasons, including: candidate substances for substitution, non–renewal of the approval following decision of the applicants, toxicological concerns, or limited economic interest.

This last category clearly corresponds to the basic substances definition in Recital 18 of Regulation (EC) No 1107/2009 [1]. Some parties believe that this decrease in the number of substances was very important, but these adjustments regarding general pesticide regulation were necessary and were taken on legal grounds. Although the existence of traditional plant protection products (PPP) is evident in Organic Farming, their EU approval under general regulations is compulsory. This action was called horizontal legislation alignment by DGAgri in Recital 6 of Implementing Regulation (EU) No 354/2014:

"As regards the horizontal legislation for plant protection products, Commission Implementing Regulation (EU) No 540/2011... it is appropriate to adapt the relevant parts of Annex II to Regulation (EC) No 889/2008 to that list. In particular, gelatine, rotenone extracted from Derris *spp. and* Lonchocarpus *spp. and* Terphrosia *spp., diammonium phosphate, copper octanoate, potassium aluminium (aluminium sulphate, kalinite), mineral oils and potassium permanganate should be deleted from that Annex"* [5].

Some other substances were maintained in Annex II as described in the Recital 7 of Implementing Regulation (EU) No 354/2014:

"As regards the active substances lecithin, quassia extracted from Quassia amara *and calcium hydroxide for which applications for approval have been already submitted to the Commission under Regulation (EC) No 1107/2009, it is appropriate at this stage to keep them exceptionally on the list in Annex II to Regulation (EC) No 889/2008 until their assessment is finalised. In view of the conclusions of the assessment the Commission will take appropriate action regarding the presence of the three substances concerned on the list in Annex II to Regulation (EC) No 889/2008".*

This was the case for lecithin, calcium hydroxide and Quassia maintained in Annex II as the application Dossiers were constituted and submitted. Accordingly, these applications permitted the approval of the first 2 of these 3 substances. Quassia dossier is currently waiting for the outcome by the European Food Safety Authority (EFSA).

While certain applications were individually launched [6] without any contact or collusion between applicants, nor coordination, some approvals were organized by the organic farming sector [4]. We believe that these applications need to be organized now, and even driven through an organizational strategy by the organic farming sector itself, especially for its needs at EU level.

2.1. Current Implications for Organic Farming

2.1.1. Interest

Initial interest was manifested by the organic sector. Furthermore, as soon as the candidate substance is identified as not being a biocide, foodstuff or from edible vegetable or animal origin, it is entitled and even preferred. Initially, the organic sector proposed a list of potential candidate substances [7], but, at the same time, some small- and medium-sized enterprises started to investigate this opportunity [8]. Moreover, some Member States also applied for basic substances [9]. Following, horsetail extract (*Equisetum arvense*) [10] approval, the French Institut Technique de l'Agriculture Biologique (ITAB) obtained approvals for 8 more basic substances [6].

2.1.2. Organic Farming: Source of Candidates Dossiers

A primary list of possible basic substances is maintained by DGSanté [7]. Although this list was informative in the beginning and is still helpful while being constantly updated, the number of items recorded is quite restrictive compared to the vast field of possible applications. Even only considering the traditional organic uses or biodynamic preparations of botanicals, the list is impressive. Envisaged botanicals at the fourth stage of the previous plant protection products regulation [11] and previous substances not approved [12] may also be good candidates.

2.1.3. Affordability of the Approval Process

Regarding cost, no fee is charged [3]. Dossiers applications are accessible to any growers' association or technical organization. These light financial charges explain the high level of applications, although these applications fit perfectly Recital 18 (page 3 of plant protection products Regulation) [1]:

"Certain substances which are not predominantly used as plant protection products may be of value for plant protection, but the economic interest of applying for approval may be limited. Therefore, specific provisions should ensure that such substances, as far as their risks are acceptable, may also be approved for plant protection use".

However, for the constitution of chapter number 3 of the application (agricultural uses or Good Agricultural Practices, utility or efficacy) field trials are at same level of cost for chemicals, bio-control agents or organic farming biopesticides; idem for ecotoxicological tests requirements (i.e. bees or earthworms trials).

3. Consideration by Organic Farming Sector: Recent Impact

Although, these applications have been spontaneous, it is clear that regulation of the approved basic substances by the organic farming sector is needed. Until now, all approved substances reached the Annex II categorization and were, according to our point of view, eligible to organic farming and most of them are candidates. Considering this emphasis of candidacy, the organic farming sector should not be alarmed by the multiplication of substance applications since most of them have no biocidal properties at all. For instance, a recent plant seed extract may be a good candidate [13] as sunflower oil is undergoing the application process. Of course, mild biochemicals or herbicides may apply and succeed, but are likely to be excluded by organic farming rules, although they may still be considered by some countries outside the EU [14]. Considering this surge in applications, after a few approvals, including the two maintained substances in Annex II by Implementing Regulation (EU) No 354/2014, another modification of this Annex was published early in 2016 corresponding to a reorganization [15].

The entry into force of the last modification of the Annex II of organic farming regulation (Implementing Regulation (EU) No 2016/673 [15]) links to an extensive change of this annex. The sub-class of “Basic substances” box was generated and corresponding criteria designated for substances only for the control of pests and diseases.

“Only those basic substances within the meaning of Article 23(1) of Regulation (EC) No 1107/2009 of the European Parliament and of the Council [2] that are covered by the definition of “foodstuff” in Article 2 of Regulation (EC) No 178/2002 of the European Parliament and of the Council [16] and have plant or animal origin”.

Thus, insecticides and fungicides were clearly included, together with plant strengtheners, repellents and lure compounds, whereas substances used as herbicides are excluded. It could therefore be concluded that direct inclusion in Annex II of approved basic substances may occur when these criteria are respected and corresponding substance may be used directly after approval in organic production.

3.1. Uncertainties

Because application does not mean approval and since the pathway is quite an obstacle course, not all applications end up with a positive vote. Admissibility is one of the deciding steps together with the outcome from EFSA [3] and the final decision from the Commission. In this journey, small Non-Governmental Organization applicants may find the application process more difficult than official European Member States’ governmental agencies. Few dossiers have already been rejected (non-approbation), or are expected to be rejected, although they are of interest for organic farming. The main question raised is the final issue of the claimed usages (good agricultural practices), especially of the so called “orphans”. If these orphan or minor uses are not fulfilled by the way of these basic substances, it is unlikely that small- and medium-sized enterprises would invest millions of Euros for such substances if they were freely available on the non-plant protection products market.

3.2. Questioning

Suspected toxicity is the main argument for the non-approval of an evaluated applied Basic Substance. Maximum residue limits arising from these considerations may be the key point. Are these substances recyclable as regular active substances (plant protection products)? Are the same substances as active substances giving rise to plant protection products with maximum residue limits good candidates and of interest from the organic farming point of view? Is this line of questioning in organic farming similar to the current H2020 *“EU Framework Programme for Research and Innovation”* [17] SFS-08 research program “Organic inputs—Contentious inputs in organic farming” [18]?

4. Conclusion

Basic Substances are effective as a new category of mild crop protection products. Some are of interest for organic farming or even driven by the organic production sector itself. Aside from this, approved Basic Substances suitable for organic farming Annex II will increase content of this annex with numerous approved basic candidates. Questions are already being raised by Member States unaware of the work of diverse applicants (organic farming Non-Governmental Organizations, Member States, small- and medium-sized enterprises) regarding officially permitted substances. Not overcoming the regulatory prerogatives of the Expert Group for Technical advice on Organic Production (EGTOP) and the Regulatory Committee on Organic Production (RCOP), the question is asked about the collection, the organization and the rule of these candidacies and applications by organic farming parties in the near future and ultimately, the acceptance or not by the organic production sector itself.

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Research Article

Changes in Knowledge Management Strategies Can Support Emerging Innovative Actors in Organic Agriculture: The Case of Participatory Plant Breeding in Europe

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Abstract: The "transfer of technology", typical of a top-down linear process of innovation cannot be used in the new contexts of sustainability, characterised by uncertainty and complexity. There is a need to redefine categories and concepts around which innovation and agricultural policies are built, as those currently in use provide only a partial representation of reality. Innovation paradigms underpinning technological development and public policies design will have a direct impact on decisions regarding which agricultural models will ultimately be supported. Looking at local learning capacity and systems of relations can help to understand the potential to develop innovation within a specific context. This work contributes to the definition of new actors who are developing innovation for sustainability in rural areas. The study focuses on the knowledge systems of farmers who are applying alternative breeding strategies: it uses a network approach to explore the knowledge system in which individual farmers are embedded in order to understand their specific relational features. Three main conclusions emerge from the study: for enhancing the agro-ecological innovation paradigm there is a need to define the 'innovation broker', to revise the evaluation system of public research and to integrate innovation and agricultural policies.

Keywords: innovation paradigms; network analysis; advisory services; research evaluation

1. Introduction: Emerging Innovative Actors in Agriculture

Various agricultural models exist in the global context, with family or peasant agriculture and industrial/corporate farming at opposing ends of the spectrum. Van der Ploeg [1] underlines how a peasant-farming model based on direct management of resources, struggle for autonomy and cooperation among rural actors is achieving success as a response to the economic, social, food and ecological crisis [2]. Based on intense knowledge exchange activities, such models would seek to establish fresh niches of autonomy within a broader economic context characterized by farmers' independence from external actors and marginalisation by the demand of the global market. Several actors are experimenting with such organizational models at a local level and supporting them would enable the realization of this transition. Rural sociological studies of the last two decades have reviewed such scenarios; however, current agricultural policies often do not meet needs of these actors [3]. Likewise, characterising the peasants as not yet possessing entrepreneurial skills or as a disappearing group is clearly deficient [1]. There is a need to redefine the categories and concepts around which agricultural policies are built, as those currently in use provide only a partial representation of reality.

The aim of this work is to contribute to the definition of new actors who are developing innovation for sustainability in rural areas, with a focus on the knowledge systems in which they are embedded. Innovation paradigms underpinning technological development and public policies have a direct impact on decisions regarding which agricultural models will ultimately be supported. This process is particularly evident in plant breeding. Conventional plant breeding strategies aimed at high crop yields and high technological quality through uniformity and wide adaptation, are partially responsible for the increased erosion of agricultural diversity [4] and for the abandonment of marginal agricultural areas such as mountainous and hilly land [5,6]. However, today such areas are frequently those where new innovative actors are developing their activities and where alternative breeding strategies are elaborated and tested.

This paper will focus on the study of knowledge systems of individual farmers applying crop breeding strategies based on local adaptation through decentralisation and participation. It uses a network approach to explore the knowledge systems in which farmers are embedded in order to understand their specific relational characteristics. Networks of four organic farmers are used as case studies. The first two sections will describe emerging divergent agricultural innovation paradigms in Europe, with a focus on knowledge management and plant breeding. The second section will present the analysis of the four innovative organic farmers knowledge networks developed within the framework of the European Framework Programme 7 research project, SOLIBAM. The structure and actors included in the knowledge networks of the four case studies will be illustrated and discussed. Finally, some policy recommendations on how agricultural research systems can support emerging innovative actors will conclude the paper.

2. How Innovation Paradigms Influence the Transition Towards Sustainability

System innovations are multi-factor, multi-actor and multi-level processes [7]. Different innovation paradigms result in different roadmaps and models towards the future of agriculture.The idea of sustainability as a normative notion that should assure justice among humans of present and future generations and among humans and nature [8,9] has several interpretations depending on the scientific approach used. Furthermore, the main paradigm chosen to underpin research policies has a direct influence on the direction of society's transition to sustainability. The Knowledge Based Bio Economy (KBBE) innovation paradigm, which predominates in the EU, strongly relies on technical innovation developed through the life sciences, following a reductionist approach and strong scientific specialisation. The analysis of behavioural and institutional changes is not directly included in the associated innovation process as it is delegated to other scientific disciplines [7]. The KBBE approach in agricultural research focuses mainly on the maximisation of productivity and economic efficiency on a global level. It could lead to incremental system changes that may support the transition to sustainability, but it could also potentially turn agriculture into a factory-like production system [7], reducing the importance of local and traditional knowledge systems [10,11]. There is a need, therefore, to take into account the long-term effects that any single specific innovation could generate. Following a vision of integral sustainability, innovation in agriculture should work to build new relationships between local actors, communities and their natural contexts [12,13]. Sustainability becomes a question of governance and the role of communities and the promotion of social and institutional learning for sustainable development become key elements of sustainability science [14]. Social innovation is needed as much as technological innovation based on life science to reach the sustainability goals. "Agroecology" [15,16] represents an innovation paradigm that is gaining importance in Europe as complementary to that of the KBBE in agricultural sciences. According to this paradigm agricultural systems are designed in such a way as to minimize the need for external inputs and improve reliance on ecological interactions [8]. The original notion of organic agriculture is one basis upon which to conceptualise this paradigm. Following this approach, one of the main requirements for sustainability is to base food production within agroecological settings [17].

3. Plant Breeding as an Example of Knowledge Management in Two Agricultural Innovation Paradigms

In the KBBE paradigm, innovation is a research-driven process based on scientific knowledge and promoted by intellectual property rights, where specific policies are defined in retrospect for dissemination of science and innovation transfer [18] following a linear model of innovation [19,20]. According to the agroecological vision, research and innovation policies should promote the combination of different types of knowledge (scientific, lay, tacit, local) and worlds (science, production, consumption, etc.) in a process of mutual learning, with the aim of finding practical solutions for complex problems [21]. Here, the associated knowledge management model is that of Agricultural Knowledge and Innovation Systems (AKIS), which considers innovation as a process of networking and iterative learning among a heterogeneous set of actors [22,23].

The KBBE model leads to expert-dominated discourses that need specific policies in order to be applied by end users. However, fixed rules and thresholds based on scientific evidence, (e.g. tools for sustainability assessment based on linear optimization models; command and control policies based on pesticide residues etc.) are more likely to hinder rather than to promote rural development, excluding rural actors and their (local, tacit) knowledge from the transition to sustainability [24]. In contrast, in the AKIS model the involvement of a variety of stakeholders in the development of innovation and research is the key to finding a suitable solution for each specific context.

Plant breeding strategies permit the identification of the knowledge management approach to which a specific farmer or researcher refers, and, as a consequence, the agricultural innovation paradigm associated with it. In the KBBE paradigm, breeding is mainly a tool to increase productivity or achieve other objectives through genetic uniformity. It is based on the idea of wide adaptation of varieties i.e. the same variety should be cultivated across as large an area as possible in order to recover the cost of research and development [25]. In the agroecological innovation paradigm and, in particular, in the research developed for organic farming, local adaptation through genetic diversity is one of the main drivers of innovation [8,26]. Crop breeding for organic agriculture is a good example to illustrate how a better understanding of technological changes should be integrated with changes in rules, behaviours of individual stakeholders, culture, institutions and science. Organic agriculture requires crop varieties adapted to different agricultural, environmental, cultural and social contexts, avoiding the need for external inputs and increasing the ecological interactions among biological components to stimulate the internal potential for soil fertility building, productivity and crop protection [27]. However, commercially available crop varieties, even if certified organic, are characterised by genetic uniformity and are mostly selected under conventional farming conditions, which traditionally use high-energy inputs such as chemicals for fertilization and plant protection. The use of such varieties in organic agriculture promotes an input substitution approach increasing the risk for conventionalisation [28]. The diffusion of selection processes across different environmental conditions incorporating the direct involvement of farmers has great potential to develop crop varieties better adapted to different organic and low input farming systems [29]. Decentralized and Participatory Plant Breeding (PPB) is a promising approach for developing innovation in plant breeding following the agroecological paradigm. PPB has been carried out traditionally with small farmers in developing countries [25,30,31]. More recently it has been proposed as an alternative breeding approach for organic and low input farming in Europe [32] with a focus on adaptation to climate change [29]. PPB is often criticized for the high investment in time and resources required to build farmer networks, but in a context where farmers are already embedded in social networks such investments can be significantly lower and may not entail additional efforts for dissemination or marketing of the varieties released [33]. We used the plant breeding approach to identify farmers that are following the agroecological innovation paradigm. To understand how to support the development of this emerging approach to innovation as complementary to the dominant one, we studied the knowledge management networks of farmers involved in Participatory Plant Breeding in Europe.

4. Analysis of Farmers' Knowledge Networks

Network analysis [34–38] has great potential to describe complex farm systems that aim to integrate the goal of productivity with those of autonomy, stability, diversity and quality. Farmers are moving in complex environments with several economic, environmental, social and cultural factors influencing their behaviour and they often tend to see their practices and the reasons they use them in terms of social relationships [39]. Van der Ploeg [1] used the term "autonomy" to refer to the need of individual farmers to reduce their dependency on external inputs and market prices. In this view, the new peasant needs to work hard on developing synergies with ecological processes and social connections. The analysis of social connections that influence knowledge management was conducted on four case studies of farmers involved in PPB experiments in the framework of the SOLIBAM project. The strategies of SOLIBAM focus on the integration of breeding approaches (such as Evolutionary or Participatory Plant Breeding, increasing stability through genetic diversity and the development of organic wheat varieties) with agronomic methods of farm management (such as intercropping and associated crops). They represent an example of technologies that promote farmers' interactions with nature [40].

4.1. Research Methodology

As the aim of this study was to contribute to the description of emerging innovative actors in organic agriculture with a focus on knowledge management strategies, we selected organic farmers involved in PPB experiments in Europe. In particular we identified four case studies of farmers that local researchers and stakeholders recognise as examples of best practice of innovative organic farmers in their region. Four organic farmers in France and Italy were studied in terms of the actors involved in their knowledge system and their respective roles. A description of the farms is given in Table 1. The focus was on person-based processes that influence the decision making of individual agricultural stakeholders [41]. The four farmers were interviewed regarding their innovation strategies, being asked to describe the actors involved in their knowledge network. A participatory approach for data collection, known as Participatory Mapping [42], was used with the aim of improving the data collection procedure for personal networks in rural contexts. Using this approach, farmers were directly involved in defining their 'relationship maps' through a facilitated workshop with researchers. The result was a directed graph (i.e. a network of nodes and directed arrows) showing the relationships that directly and indirectly influence the functioning of the farm.

The visualisation of the relationship map helps farmers to give more information about the connections between actors than the use of a questionnaire. The researcher firstly asked the farmer to identify actors influencing his innovation strategy and then to show the connections between actors by asking: who are the actors you exchange knowledge with? [38]. This process allowed a large amount of data to be collected in a short time and resulted in being a particularly suitable approach to describe innovative agricultural models through their knowledge systems. The mapping approach allowed the respondents to visualize their relational systems while describing it verbally to the researcher, increasing the potential of network analysis as an awareness-building tool [43].

Table 1. Main features of the four case study farms.

	IT1	**IT2**	**FR1**	**FR2**
Location	Tuscany	Friuli	Brittany	Brittany
Ha	300	21	70	8
Workers	9	1	2	1 (3h/week)
Household	3 people	2 people	4 people	4 people
Total revenue (2010)	Between 150.000 and 200.000	Between 100.000 and 150.000	Between 150.000 and 200.000	Between 25.000 and 30.000
Organic/mix	Organic since 1987	Mix, 6 ha organic	Organic since 1985	Organic since the start up phase
Main crops	Arable crops: cereals, legume crops.	Vegetables (6 ha organic), Arable crops: cereals (conventional)	Livestock (cows, chicken, pigs) and arable crops: cereals.	Cereals, vegetables, fruits, livestock (rabbit, chickens)
Products	Bread and pasta	Vegetables and cereals	Cheese, butter, yogurt, cereals and flour	Bread, gallettes, meat, apple juice, cider
Supply Chain	Food processing and on farm sale of bread and pasta	Fresh vegetables for direct selling and raw cereals for processing	Food processing on farm for dairy products and flour production	Food processing on farm for bread and gallettes (paysan-boulanger)
Seeds/breeding	Old varieties since 2006. Home saved and reproduced in a network of farms	Home saved, reproduced on farm.	Home saved, agrobiodiversity, seed exchange, on farm selection	Home saved, reproduced on farm
Innovations	Conversion to organic farming- Introduction of old cereal varieties in the fields -Processing plants for old cereal varieties.	Modification of the farm organizational model - Crop diversification - Reduction of cultivated land.	Swiss cow landrace, cereal selection for hay to feed animals - Cereal selection for high quality bread and flour production - On farm conservation of old varieties	Use of animal labour in the field (60%) - Bread making strategies - Bread home delivery.
Funds	EU RDP	EU CAP	Research projects (EU, private foundations etc.)	EU CAP
Market channels	On farm shop, e-commerce, consumers groups , local shops and restaurants.	Farmer's markets and consumers' groups for vegetables. Large distribution for cereals	Farmers'markets, on farm shop, consumers'groups, local bakeries and shops.	Consumers'groups, on farm shop shared with other farmers, bread home delivery, local shops.

4.2. Results

The selected farmers have a vision of agriculture as an activity that goes beyond simply food production, embedding the development of social relationships in the production process [41]. This vision is communicated through direct contact: the possibility to buy the products directly on farm guarantees a continuous exchange of knowledge between the farmer and the consumers. This exchange is deeper when other activities with consumers, such as voluntary work and on-farm visits, bring people with different backgrounds to the farm. The public research institutions contribute to the farmer's innovation development with scientific knowledge that responds to the consumers demand for sustainable production. These farmers represent practices at a local level that can become a point of reference for other local actors interested in organic and low input farming. If this approach to agriculture meets a local and regional context in which substantial changes among various stakeholder groups have taken place simultaneously, there is a high possibility for the local system to improve sustainability as a governance process. Moreover, they have a large potential in improving local sustainability thanks to their capacity to connect with other producers, rural stakeholders and wider society actors.

The choice to be organic and to follow values and principles of the original organic vision coincides with their choice to be farmers. It represents a baseline in their process of developing innovative organisational models. The farmers interviewed have quite different backgrounds. The French farmers are part of the stream of people who, in the 1970s and again in recent years, started moving back to the land as an alternative to urban industrial life. FR1 is a second generation organic farmer who wants to improve their parents' decision, while FR2 started farming ten years ago. Also ten years ago, FR1 started to experiment with cereal selection on an organic dairy farm, while FR2 bakes bread on farm and sells it to local consumers. The Italian farmers come from traditional agricultural families; both of them made the choice to change their farm structure and organisation with the aim of keeping agriculture as the main income for the family. IT1 invested in the cultivation of local and old varieties of cereals and processing the grain to produce bread and pasta. IT2 focused on reducing costs, reducing farm size and introducing vegetable production with high agrobiodiversity.

Figure 1 shows the knowledge network of the four farmers, using different colours for the economic sector of the actors involved and different node dimensions for the relevance of each actor in terms of the number of connections. FR2 and IT2 tend to have a strong role of the farmer in the knowledge networks, as they are the only nodes of a large size and all knowledge exchange flows pass by the farmer. FR1 and IT1 have a more polycentric network, where other actors, e.g. members of the farmer family or technicians working in collaboration with the farmers, have a significant role in managing the knowledge exchange process.

Networking is an important aspect in the development of a new farm or an innovative approach to farming. FR2 and IT2 had to create relationships with local actors over time that they directly manage. Both FR2 and IT2 are the main individuals responsible for the farm activity and the ones who make choices every day. The role of the workers is secondary in this type of farm due to the specific organisational model based on family work, and also to the small size of the farms (3 to 6 ha). Most of the actors in these farmers' networks are individuals; a direct connection with collective actors that would have a potential to enlarge their network is missing. FR2 listed 28 actors while IT2 described a total of 20 actors: they are looking at other actors in terms of what they can get from them to improve their system. Most of the actors in the network are directly related to the farmer without any connection to each other. The analysis of the FR2 network allows us to identify the two associated realities in which the farmer is embedded: seed networks and organic agriculture associations (see Appendix for complete list of actors). However, FR2 also described his relationships with individual farmers that are based on personal continuous knowledge exchange with a high level of reciprocity. FR2 nominated five individual farmers and the person who sold him the oven for baking his own bread as people that contribute to his knowledge exchange network on specific problems. The other farmers represent an important source of information on what is going on outside the local area and they can give important insights for the management of the farm. The knowledge network of IT2 focuses on the farmer's direct contacts. The low receptivity of the local context hampers his possibility to share his innovative vision with local actors. However, his breeding experience in horticulture attracted several researchers at national and international level, thanks to the contact with other farmers. This allows IT2 to be involved in research projects and to exchange his seeds with other farmers with similar experiences. The knowledge exchange network of both IT2 and FR2 involves the actors related to market channels such as consumers groups, consumers on farm and farmers markets.

Even if, as part of the farmers' visions, direct contact with consumers should be key to the farm model they want to develop, they have a low capacity to influence this in practice due to the short time the farmers have to spend in the shop talking to consumers. FR1 and IT1 are examples of family farms of a significant size (between 70 and 300 ha) that decided to invest in innovation to maintain farming activity as the main source of income. They have larger knowledge exchange networks than the other farmers observed in this work. Their active participation in research projects and the continuous development of PPB make FR1 and IT1 familiar with the mutual learning process between farmers and researchers.

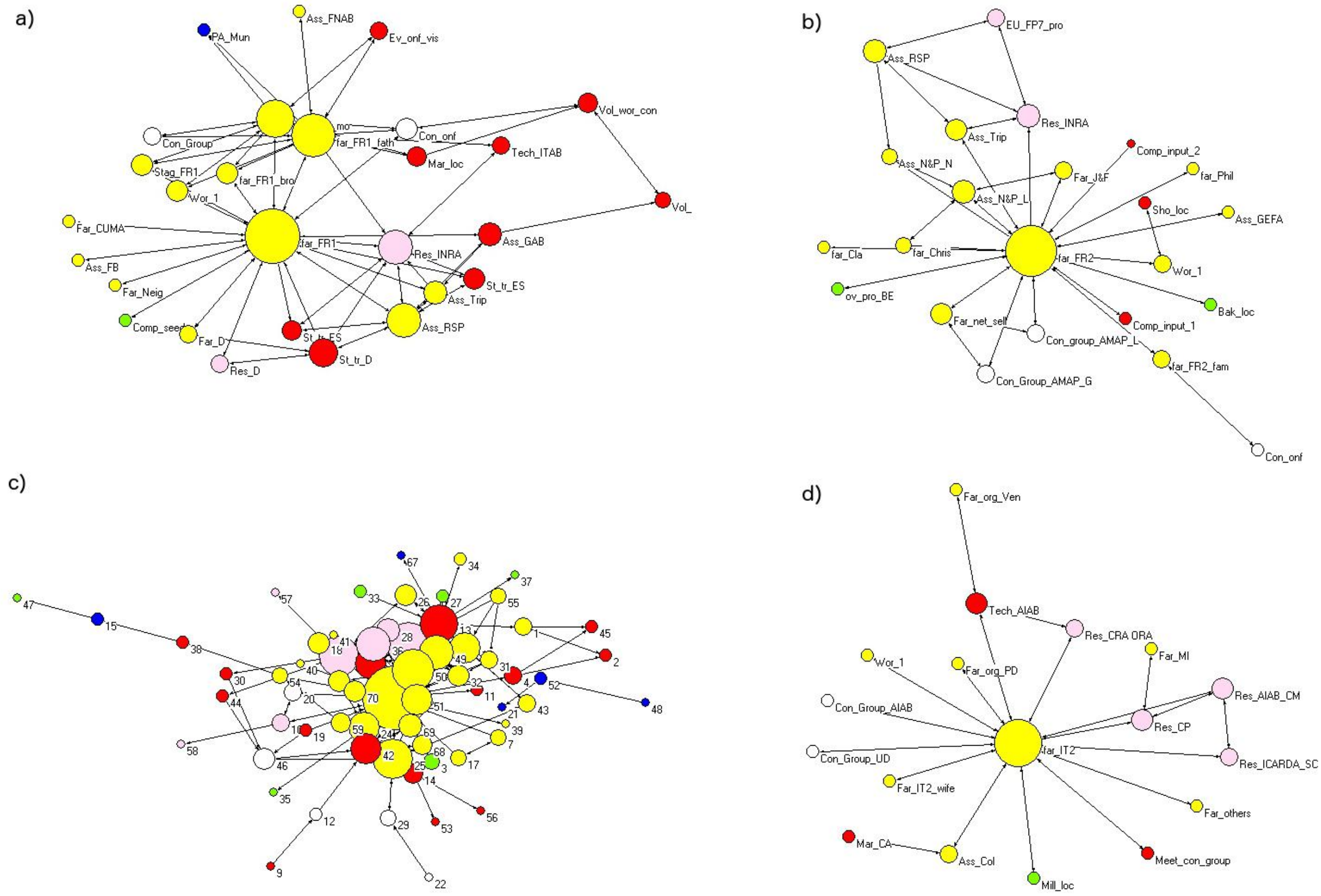

Figure 1. Farmers knowledge networks (a) FR1 b) FR2 c) IT1 d) IT2) by number of connections and economic sectors (Agriculture (yellow), Processing industry (green), Services (red) Public Administration (blue), Research (pink) Households (white)). The size of the circle is related to the number of connections that each node has with others, while the lengths of the connections do not have any significance. The graphs are developed following an ego network analysis approach and using the program "Pajek". See Appendix for a complete list of actors.

Innovation activity is managed by the farmers with the direct participation of other actors such as researchers, farmers' associations, consumers etc. This structure of "collective management" of on-farm innovation that directly includes the spread of innovation in the process of innovation development itself is one of the most important characteristics of this transition pathway. These farmers work in close collaboration with individual researchers from public research institutions. The connection to a specific researcher is often followed by an exchange of material, in general seeds, with the researchers themselves or with other relevant actors. More than one person in the family is active in the farming system. Local administrators exchange knowledge with both IT1 and FR1 for the development of local projects with different aims: educational activities with schools, projects to close the cereal supply chain at a local level, projects to reproduce seeds of old traditional varieties etc. Concerning farmers' associations, FR1 and IT1 perceive several actors as relevant. Their membership of different farmers associations is in both cases connected to a strong participation and inclusive attitude. The influence on innovation development of such organisations is often related to the opportunity to meet with other farmers who have similar interests in different contexts. The peer to peer exchange is recognised as a good strategy to develop and spread innovation. In particular FR1 participated in three study trips to other countries (Spain, Syria, Germany), the latter of which has been followed by a direct contact with farmers and researchers from Germany not just for knowledge but also for seed exchange. IT1 included the contact with the French RSP (Reseau Semences Paysannes – www.semencespaysannes.org) that he developed thanks to a study trip and an event that the Italian RSR (Rete Semi Rurali – www.semirurali.net) organized on his farm to meet with farmers and bakers from all over Europe.

At the same time, the farmers offer opportunities for exchanges among farmers on their own farms. The funding mechanisms through the EU to the regional administration and then to the farmers are well described by both subjects. IT1 is aware of the funding for Rural Development Plans because he used them to fund the building of his mill and on farm pasta processing plant. FR1 is more aware of the research funding mechanisms, because he was involved in research projects in collaboration with INRA. The vision of internal and external actors in terms of contribution to the functioning of the system is variable among the interviewed farmers. FR1 considered just himself, his family and the worker as internal to the system; FR2 defined six actors as internal and the rest as external. The internal actors could be divided into two groups, those who have more relationships with the external actors and those who have mainly internal relationships. IT2 includes consumers as actors internal to the system as "the system cannot function without them". IT1 considers all the actors and the link described as internal to the farm system, as in his vision they all contribute to its functioning.

5. Discussion: Moving Through an Agroecological Innovation Paradigm

The aim of this study was to explore an innovative approach to describe actors emerging in rural areas, following an agroecological approach to innovation in agriculture. Four case studies are not exhaustive, but the exercise shows promising results and it would be interesting to replicate it in other cases. Networks indicators such as centrality measures can be used for a more detailed analysis on the role of individual actors in the network. Looking at local learning capacity and systems of relations seems to give an effective contribution to understanding the potential to develop innovation within a specific context.

From the analysis of case studies, it emerged that the main relevant actors in knowledge exchange of farmers that represent innovation "best practice" are other farmers and farmers' associations together with consumers or citizens and individual publicly funded researchers. No role is given to extension services, which are otherwise often considered as key actors for innovation transfer by public policies. The advisors nominated by the farmers are independent with a high level of commitment to their work, and often have a key role in enlarging the individual farmers knowledge systems. The "transfer of technology" typical of a top-down linear process of innovation is not effective in the new context of sustainability [20,44], which is characterised by complexity and uncertainty [45]. The role of technicians identified by the four farmers is more similar to that of innovation brokers. Moreover, the farmers investigated in the case studies are collaborating with public research agencies, which can play a pivotal role in promoting decentralised and participatory research [46]. This result confirms the need to give a completely new role to extension services to enhance knowledge sharing. The facilitator and/or innovation broker should be considered as intermediate actors enhancing AKIS at a local level. Advisors should be part of the network together with researchers, farmers, local institutions and all other stakeholders. In this framework, agricultural and rural innovation policies should promote the dynamic exchange of knowledge among peers as a training tool in agriculture, e.g. through funding visits to others with similar experiences in different regions or countries.

Another important aspect is related to public research systems. In a perspective of integral sustainability of agriculture it is crucial to maintain and increase public funds for agricultural research on organic and low-input farming systems and do more to strengthen the participation and decentralization of public research systems. However what is also important is to revise the evaluation system of public research institutions encouraging a Result Based Management approach [47] to agricultural research.

In fact, a trend that needs to be reversed in order to promote sustainability pathways based on the agro-ecological paradigm is the disincentive for researchers and institutions to be involved in AKIS and to contribute to the collective development of new knowledge. As stated by Wolf et al. "the Impact Factor and other journal-based metrics are increasingly considered inappropriate for comparing the scientific output of individuals and institutions" [48]. Furthermore, "innovation" is becoming a synonym for "patent" and has no relationship at all with the actual uptake of a solution by end-users, especially farmers. According to the agroecological approach to innovation, an excellent piece of research which is published in a high-ranked international scientific journal, but whose knowledge is not applied by the end users is not an innovation at all.

It is also worth noting that in the world of research there is a clear idiosyncrasy. On the one hand, the trend towards evaluation of researchers and institutions based on bibliographic indicators and patents (with clear consequences on fund allocation) is being strengthened [49]. On the other hand, major funders (e.g. the European Commission through the new Horizon 2020 framework programme) are advocating a multi-disciplinary, multi-actor approach in agricultural research. This means that stakeholders (actors) should be actively involved in research projects from the very beginning much more so than in the past rather than being passive recipients of disseminated project results. In agricultural research, this applies to farmers and their organisations, companies (including breeders) and other potential contributors to and end users of new knowledge generated in research projects. Following this pathway should guarantee that collaboration between researchers and multiple actors (including farmers) will be fully exploited for the mutual benefit of all partners engaged in a project and of society at large. The current approach of agricultural research evaluation denies the recent trends in research funding fostered by the EC and hence the importance of multi-actor collaboration (e.g. EIP AGRI experience). For the time being, there is little structural incentive for researchers or institutions to become engaged in participatory research because this part of their work is not considered a valuable research output. The consequence is that inter- and trans-disciplinary research, the basis for innovation in the agroecological sense, is discouraged [46]. Here, we are not downgrading the importance of producing excellent research publications and patents: we are simply claiming that considering these as the only valuable outputs of research is narrow-minded, will increase the gap between researchers, farmers and other end-users, and will jeopardize the production of innovation.

In order to reverse this trend the approach of research evaluation agencies should promptly incorporate new indicators valorising inter- and trans-disciplinary research. There are new developments on this subject [48], but "these are still confronted by incentive systems that favour the old style of evaluation and old method of producing research: mono-disciplinary, with a focus on publication in international journals" [49].

National and EU Agricultural policies which support novelties and bottom-up innovations with subsidies and niche market development, if integrated with innovation policies that encourage knowledge exchange using a multi-actor approach, could facilitate a more coherent scaling up of such innovations, as is already happening in some contexts. The possibility of scaling up innovations developed in a knowledge system with a network structure, in which different actors work side by side, can improve the likelihood of contributing at a micro level to radical changes in the systems required by a territorial approach to sustainability. This approach sees the creation of a network of best practices at a local level as the basis to attain sustainability goals at society level [50].

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Appendix

Table A1. IT1

	Code	Label	Actor
IT1	1	far_mul	farmers
IT1	2	far_soc	farmers
IT1	3	Bak_alt	retail outlets
IT1	4	Tech_GP	consultants
IT1	5	Res_ProfSB	knowledge institutes and researchers
IT1	6	Tec_MB	consultants
IT1	7	Ass_Bdyn	NGOs
IT1	8	Res_RB	consultants
IT1	9	Doc_Onc	knowledge institutes and researchers
IT1	10	Res_ProfGB	knowledge institutes and researchers
IT1	11	Tech_C	consultants
IT1	12	Cit_health	customers
IT1	13	Tech_CP	consultants
IT1	14	Act_com	retail outlets
IT1	15	PA_Mun_PT	farmersfarmers
IT1	16	Ass_RSR	NGOs
IT1	17	far_Bdyn	farmers
IT1	18	Ass_CTPB	cooperation at local and extralocal level
IT1	19	Com_DR	suppliers
IT1	20	DES_PI	customers
IT1	21	PA_EU	political institution
IT1	22	Con_fam	customers
IT1	23	far_IT1	farmers
IT1	24	far_IT1_bro	farmers
IT1	25	far_IT1_wife	farmers
IT1	26	Far_meet	cooperation at local and extralocal level
IT1	27	Bak_flo	retail outlets
IT1	28	Res_RF	consultants
IT1	29	Con_Groups	customers
IT1	30	Agr_Nut	consultants
IT1	31	Ev_Fr_far	cooperation at local and extralocal level
IT1	32	Ev_br_08	cooperation at local and extralocal level
IT1	33	Bak_Mad	retail outlets
IT1	34	Coop_ster	cooperation at local and extralocal level
IT1	35	Comp_mach	suppliers
IT1	36	Res_PM	knowledge institutes and researchers
IT1	37	Mill_MS	processing industries
IT1	38	Onf_Sho_PT	retail outlets
IT1	39	far_N_15	farmers

Table A2: *Cont.*

	Code	Label	Actor
IT1	40	far_N_20	farmers
IT1	41	far_N_50	farmers
IT1	42	On_sho_IT1	retail outlets
IT1	43	Far_others	farmers
IT1	44	Doc_PR	knowledge institutes and researchers
IT1	45	Schools	education
IT1	46	Cons_pr	customers
IT1	47	Pro_old_mill	processing industries
IT1	48	PA_Pro_PI	political institution
IT1	49	RDP_06	processing industries
IT1	50	RDP_10_Mill	processing industries
IT1	51	RDP_11_Pasta	processing industries
IT1	52	PA_Reg_Tus	political institution
IT1	53	Cus_Rest	retail outlets
IT1	54	Far_net_sell	farmers
IT1	55	Ass_RSP	NGOs
IT1	56	Cus_Org_Sho	retail outlets
IT1	57	Res_UNIFI	knowledge institutes and researchers
IT1	58	Res_UNIPI	knowledge institutes and researchers
IT1	59	Wor_1	suppliers
IT1	60	Far_IT1_Mot	farmers
IT1	61	Loc_Bank	financial service providers
IT1	62	Website	communication
IT1	63	Comp_Pack	suppliers
IT1	64	BC_proc	processing industries
IT1	65	BC_Agr	farmers
IT1	66	Wor_5	suppliers
IT1	67	PA_Mun_Semp	political institution
IT1	68	Wor_2	suppliers
IT1	69	Wor_3	suppliers
IT1	70	Wor_4	suppliers

Table A3. IT2

	Code	Label	Actor
IT2	1	far_IT2	Farmer IT2
IT2	2	Tech_AIAB	AIAB Technician
IT2	3	Res_CP	Researcher from ER Region CP
IT2	4	Res_AIAB_CM	AIAB Researcher CM
IT2	5	Ass_Col	Association Coldiretti
IT2	6	Res_CRA ORA	Researcher Agricultural Research Council (CRA ORA)
IT2	7	Far_others	Other Farmers
IT2	8	Far_org_PD	Organic farmer in PD
IT2	9	Wor_1	Worker
IT2	10	Far_IT2_wife	Farmer IT2 wife
IT2	11	Con_Group_UD	Consumers'group in UD
IT2	12	Con_Group_AIAB	Consumers'group AIAB
IT2	13	Meet_con_group	Meetings with consumers' group
IT2	14	Mill_loc	Local miller
IT2	15	Res_ICARDA_SC	Researcher ICARDA Ceccarelli
IT2	16	Far_org_Ven	Organic farmers in Veneto
IT2	17	Far_MI	Farmer in Milan Province
IT2	18	Mar_CA	Farmers Market "Campagna Amica"
IT2	19	Comp_input_loc	Local input provider company
IT2	20	Cons	Consumers

Table A4. FR1

	Code	Label	Actor
FR1	1	far_FR1	Farmer FR1
FR1	2	far_FR1_mo	Farmer FR1 Mother
FR1	3	far_FR1_fath	Farmer FR1 Father
FR1	4	far_FR1_bro	Farmer FR1 Brother
FR1	5	Stag_FR1	Stagiers
FR1	6	Wor_1	Worker
FR1	7	Tech_ITAB	Istitut technique de l'Agriculture Biologique - ITAB
FR1	8	Ass_FNAB	Federation Nationale d'Agriculture Biologique - FNAB
FR1	9	PA_Mun	Local Municipality
FR1	10	Con_Group	Consumers Group - AMAP
FR1	11	Ev_onf_vis	Visits on farm
FR1	12	Mar_loc	Farmers Market
FR1	13	Con_onf	Consumers on farm
FR1	14	Comp_seeds	Commercial seeds company
FR1	15	Far_Neig	Neighbour Farmers
FR1	16	Ass_FB	Association Formation blè
FR1	17	Ass_GAB	Groupement des Agriculterurs Biologique - GAB
FR1	18	Ass_Trip	Association Triptoleme
FR1	19	Ass_RSP	Reseau Semences Paysannes - RSP
FR1	20	Res_INRA	Institut National de la Recherche Agronomique - INRA
FR1	21	St_tr_ES	Study trip in Spain
FR1	22	Far_D	Farmers from germany
FR1	23	Vol_wor_con	Events of voluntary work for consumers
FR1	24	Vol_wor_seed	Events of voluntary work for seeds
FR1	25	Far_CUMA	Farmers from Coopératives d'Utilisation de Matériel Agricole - CUMA
FR1	26	Cus_Org_Sho	Organic Shop
FR1	27	PA_Reg	Regional Administration
FR1	28	Pr_Found	Private Foundations
FR1	29	PA_EU	European Union
FR1	30	St_tr_D	Study trip in Germany
FR1	31	Res_D	Researchers from Germany
FR1	32	St_tr_ES	Study trip in Syria

Table A5. FR2

	Code	Label	Actor
FR2	1	far_Chris	Farmer Christopher
FR2	2	Ass_N&P_N	Association “Nature et progres” - National group
FR2	3	far_Phil	Farmer Philippe
FR2	4	Wor_1	Worker
FR2	5	Comp_input_1	Local input provider company - VEGAM
FR2	6	Ass_Trip	Association Triptoleme
FR2	7	EU_FP7_pro	EU FP7 Project SOLIBAM
FR2	8	Far_J&F	Farmers Julie et Florent
FR2	9	Res_INRA	Institut National de la Recherche Agronomique - INRA
FR2	10	Ass_GEFA	Group for collective land purchase - GEFA
FR2	11	far_FR2_fam	Farmer FR2 Family
FR2	12	Sho_loc	Local grocery shop
FR2	13	far_FR2	Farmer FR2
FR2	14	Con_onf	Consumers on farm
FR2	15	far_Cla	Farmer Claude
FR2	16	ov_pro_BE	Oven provider from Belgium
FR2	17	Bak_loc	Baker
FR2	18	Con_group_AMAP_L	Consumers group AMAP - L
FR2	19	Con_Group_AMAP_G	Consumers group AMAP - G
FR2	20	Far_net_sell	Network of 4 farmers for selling
FR2	21	Ass_RSP	Reseau Semences Paysannes - RSP
FR2	22	Ass_N&P_L	Association “Nature et progres” - local group
FR2	23	Comp_input_2	Local input provider company - PINAILT SA
FR2	24	Coop_Org_Sho	Organic shop - BIOCOOP
FR2	25	Comp_input_3	Local input provider company - APROBIO
FR2	26	Coop_CUMA	Coopératives d’Utilisation de Matériel Agricole - CUMA
FR2	27	Org_cert_body	Organic certification body
FR2	28	PA_DDTM	Agency for public funds - DDTM

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Research Article

Evolutionary Effects on Morphology and Agronomic Performance of Three Winter Wheat Composite Cross Populations Maintained for Six Years under Organic and Conventional Conditions

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Abstract: Three winter wheat (*Triticum aestivum* L.) composite cross populations (CCPs) that had been maintained in repeated parallel populations under organic and conventional conditions from the F_5 to the F_{10} were compared in a two-year replicated field trial under organic conditions. The populations were compared to each other, to a mixture of the parental varieties used to establish the CCPs, and to three winter wheat varieties currently popular in organic farming. Foot and foliar diseases, straw length, ear length, yield parameters, and baking quality parameters were assessed. The overall performance of the CCPs differed clearly from each other due to differences in their parental genetics and not because of their conventional or organic history. The CCPs with high yielding background (YCCPs) also yielded higher than the CCPs with a high baking quality background (QCCPs; in the absence of extreme winter stress). The QCCPs performed equally well in comparison to the reference varieties, which were also of high baking quality. Compared to the parental mixture the CCPs proved to be highly resilient, recovering much better from winter kill in winter 2011/12. Nevertheless, they were out yielded by the references in that year. No such differences were seen in 2013, indicating that the CCPs are comparable with modern cultivars in yielding ability under organic conditions. We conclude that—especially when focusing on traits that are not directly influenced by natural selection (e.g. quality traits)—the choice of parents to establish a CCP is crucial. In the case of the QCCPs the establishment of a reliable high-quality population worked very well and quality traits were successfully maintained over time. However, in the YCCPs lack of winter hardiness in the YCCP parents also became clearly visible under relevant winter conditions.

Keywords: baking quality; climate change; dynamic management; evolutionary breeding; heterogeneous populations; resilience; sustainable agriculture; *Triticum aestivum*; winter wheat

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Additional Abstract in German

Drei Winterweizen (*Triticum aestivum* L.) Composite Cross Populationen (CCPs), die von der F_5 bis zur F_{10} in parallelen Populationen unter ökologischen und konventionellen Anbaubedingungen erhalten worden waren, wurden in einem zweijährigen Feldversuch unter ökologischen Bedingungen verglichen. Verglichen wurden die Populationen miteinander, mit einer Mischung der Elternsorten der Populationen und mit drei Winterweizensorten, die im Ökolandbau häufig angebaut werden. Bonitiert wurden Fuß- und Blattkrankheiten, Halm- und Ährenlänge, Ertrags- und Backqualitätsparameter. Die CCPs zeigten deutliche Unterschiede von einander, was auf Unterschiede in der Genetik der Elternsorten zurückzuführen ist, und nicht auf die ökologische und konventionelle Anbaugeschichte der Populationen. Die CCPs mit Hochertragssorten im Hintergrund (YCCPs) zeigten höhere Erträge als die Populationen mit Qualitätssorten im Hintergrund (QCCPs; bei Abwesenheit von extremem Kältestress im Winter). Die QCCPs zeigten ein vergleichbares Qualitätsniveau wie die Referenzsorten, die ebenfalls Sorten mit hoher Backqualität sind. Verglichen mit der Mischung ihrer Elternsorten zeigten die CCPs große Flexibilität und erholten sich sichtlich besser von den großen Auswinterungsschäden im Winter 2011/12. Dennoch lagen die Erträge der Referenzsorten in diesem Jahr über denen der CCPs. Derartige Unterschiede waren 2013 nicht zu beobachten, was darauf hindeutet, dass die CCPs unter ökologischen Anbaubedingungen ein vergleichbares Ertragsniveau haben wie moderne Liniensorten. Wir folgern aus den Ergebnissen, dass die Wahl der Elternsorten bei der Erstellung von CCPs ausschlaggebend ist, besonders wenn der Fokus auf Merkmalen liegt, die keinem direkten Selektionsdruck unterworfen sind (z.B. Qualitätsparameter). Im Falle der QCCPs war die Erstellung einer Population mit verlässlicher hoher Backqualität erfolgreich und die Eigenschaften konnten auch im Verlaufe der Zeit erhalten werden. Die mangelnde Winterhärte der Elternsorten der YCCPs wurde unter entsprechenden Winterverhältnissen allerdings auch sehr deutlich sichtbar.

1. Introduction

The challenges of climate change, increasing demand for finite resources, and population growth are calling for a paradigm shift in resource use [1,2] combined with new, different and efficient strategies to face the challenges of climate change [3,4]. Diverse farming systems have shown to be more resilient in the face of perturbations and buffer extreme climatic events and adverse growing conditions to a wider extent than large monocultures do [3,5,6]. Beneficial effects of crop genetic diversity on productivity, population recovery from disturbance, and other ecological processes have been reviewed by Finckh and Wolfe [7] and Dawson and Goldringer [8] and agrobiodiversity has been placed very high in the list of potential solutions to the growing demand for food. Since the early 20^{th} century trends in agriculture, plant breeding and breeding legislation have tended towards an increased use of genetically uniform varieties [9–12]. As a consequence most crop varieties have been selected to cope well in monocultural high-input growing systems [13,14]. This disregards the fact that genotypes selected for high performance under high-input conditions do not necessarily perform very well in marginal environments or in farming systems with lower inputs [15]. It is also argued that such uniform and genetically 'stable' cultivars are inappropriate for dealing with unpredictable environmental changes because their response to environmental fluctuations is not buffered by genetic diversity and they have no capacity to react to novel stress factors [5,16,17].

Responding to the continuous restriction of genetic variability in plant breeding, Simmonds [18] and Allard and Hansche [19] called for mass reservoirs of genetic variability as supplements to conventional breeding that help broaden the genetic base of crops and are well suited for dynamic conservation of genes and genotypes.

For the self-pollinating cereals, evolutionary breeding based on the composite cross approach was developed. In evolutionary breeding, heterogeneous, segregating crop populations (composite cross populations, CCPs) [20] are subjected to natural selection. It is expected that the high level of genetic diversity allows adaptation to the prevailing growing conditions because plants with good adaptation to the local growing conditions will contribute more seed to the next generation than plants with lower fitness [16,20].

While genetic variability is expected to decrease in each population over time under the combined effects of drift and selection, overall diversity is supposed to be maintained through the differentiation among populations [21]. Over time the populations adapt to the conditions under which they are grown and their resilience to stressful and variable growing conditions is seen as a major advantage under the predicted threats of climate change [16,17]. This simple and efficient way of managing genetic resources *in situ* is a potent tool for the sustainable use of plant genetic resources on the one hand and can be a potent solution, especially under low-input growing conditions, on the other hand.

In 2001, three winter wheat CCPs suitable for European growing conditions were created in the UK by the John Innes Centre (JIC, Norwich, UK) in cooperation with the Organic Research Centre (Newbury, UK) [22]. The parental varieties were successful European varieties, released between 1934 and 2000, with a focus on varieties of British origin, approximately representing the breeding progress at the beginning of the twenty-first century. Key criteria for selection were a diverse genetic base and potential for stable performance under low-input growing conditions. The parental varieties were grouped into three groups: one group containing twelve varieties with high baking quality (group Q), one group containing nine high yielding varieties (group Y), and the third group containing all 20 varieties (group YQ).

The variety 'Bezostaya', known as high yielding as well as high quality in Russia, was included in both groups Y and Q. A comprehensive analysis of the performance of the individual parental varieties was published by Jones et al. [23]. The half diallels of the Q parents and the Y parents resulted in the QCCP and the YCCP, respectively. The intercross of the Y by Q parents in the YQCCP. The initial setting up and maintenance of the European composite cross populations established at the JIC in 2002 has been described by Döring et al. [24] in detail.

After two years of multiplication at two organic and two conventional sites in the south and east of the UK, F_4 seed of the four sites was bulked, and about 2 kg each was sent to the Department of Ecological Plant Protection, Faculty of Organic Agricultural Sciences, University of Kassel, Germany in autumn 2005, where they have been maintained since under contrasting agronomic conditions. Each F_4 population was divided into two and sown into an organically managed trial site and into a conventional trial site (resulting in three CCP_{org} and three CCP_{conv}).

In autumn 2006, enough seeds were available to split the populations one more time. Since then, within each system two Y, two Q, and two YQ populations have been maintained as two parallel populations. This has enabled the comparison of changes in the populations over time within and between systems. Random changes and changes in the populations that occurred due to effects of the environment (e.g. organic vs. conventional growing conditions) can be distinguished. The populations are maintained in separated plots of minimum 100 m^2 to ensure that at least 5000 individual plants are grown, which is the effective population size (*Ne*) that should be sufficient to avoid genetic drift in the populations [21,25].

Thus, since the F_6, a total of twelve CCPs (six CCP_{org} and six CCP_{conv}) have been maintained at the two trial sites in the absence of fungicides and insecticides with no artificial selection applied apart from the removal of the tallest plants ($>$ 130 cm) in the early generations to prevent the populations from gaining too much in plant height. Results from France show a disproportional advantage of tall plants in the populations due to competition for light and an overall increase in height over time [26,27].

In 2011/12 and 2012/13 a field trial was carried out at the University of Kassel comparing the total of twelve winter wheat CCPs in an organically managed field a) to each other and b) to three modern pure line varieties well suited for the local growing conditions. The main questions addressed in the field trial were:

1. What are the effects of organic versus conventional selection environments on population performance?
2. What are the effects of genetic background on population performance?
3. How do the populations perform compared to modern pure line wheat varieties currently popular in organic farming?

To assess morphology and the agronomic performance of the CCPs, straw height, ear length, foot and foliar diseases, yield parameters and baking quality parameters were assessed. The results give an insight into the agronomic performance of CCPs that were shaped over several years in contrasting environments.

2. Materials and Methods

2.1. Field Site and Experimental Design

2.1.1. Field Site

The trial was carried out at the Research Station of the University of Kassel in Neu-Eichenberg, located 51°22' N and 9°54' E at an altitude of 247 m above sea level. Mean annual precipitation (2000-2013) is 684 mm, and mean annual temperature (2000-2013) 9.3 °C. The fields have been managed organically since 1984; no mineral fertilizers, fungicides, insecticides or herbicides were applied, and weeds were controlled mechanically through harrowing and/or hoeing at the tillering stage. The soil is a deep Haplic Luvisol with 76 soil points [28].

2.1.2. Experimental Design

In 2011, enough seed of the F_{10} of all 12 CCPs was saved to allow for a two-year field trial. Therefore, in 2011/12 and in 2012/13, the F_{11} of the six CCP_{org} and the six CCP_{conv} were compared to each other, to three reference varieties ('Achat', 'Akteur', 'Capo') and to an equal mixture of the 20 parental varieties (referred to as 'mixture' from now on) in a randomized complete block design with four replications.

The trials were carried out in an organic field, the precrop in 2011 was canola, in 2012 it was two years of grass-clover. The mean availability of mineral nitrogen (kg N/ha) measured in early spring (BBCH 20) in three layers of soil (0–30, 30–60 and 60–90 cm) was 83.7 kg/ha in total in spring 2012 and 84.0 kg/ha in total in spring 2013. At the flowering stage (BBCH 65) the soil could only be sampled down to a depth of 60 cm, due to very dry soil conditions. Mean availability of mineral nitrogen in total of both depths was 21.6 kg/ha in 2012 and 27.1 kg/ha in 2013. Soil samples were taken and analysed according to the standards of VDLUFA [29].

The sowing date in 2011 was the 31st of October, in 2012 it was the 10^{th} of October; plots were 11 m $\times$ 3 m which is the double width of a standard trial plot, allowing assessments and sampling on one side and leaving the other half for yield survey. Seed rate was 350 germinable seeds/m^2 and rows were spaced 30 cm to allow for hoeing.

2.2. Assessments

Growth stages were assessed regularly throughout the season. Straw height and ear length (cm) were measured in 50 randomly chosen stems per plot (BBCH 90) in order to evaluate morphological variation. Straw height was measured from the ground to the start of the ear, ear length was measured from the first full spikelet to the tip without awns.

Foliar diseases caused by fungal pathogens were assessed at BBCH stage 73/75. Non-green leaf area was estimated in % (1–100%). The three leaf levels of flag leaf (F), leaf below flag leaf (F-1) and leaf below F-1 (F-2) were assessed separately at six locations per plot.

To assess foot diseases (*Fusarium* spp., *Pseudocercosporella herpotrichoides, Rhizoctonia cerealis*), plant samples were taken at five to six points per plot (minimum 30 stems) with roots at BBCH 75. The lower stems were freed of soil and leaf sheaths and scored for foot rot symptoms based on the key of Bockmann [30] where 0 is healthy, 1 is symptoms on <50% of the stem perimeter, 2 is symptoms on 50–100% of the stem perimeter, 3 is stem brittle/rotten (*P. herpotrichoides* only). Based on a pictorial key of symptoms [31] *Fusarium* root rot, *P. herpotrichoides* and *R. cerealis* were assessed separately.

Grain yield on a plot basis was measured in t/ha at 14% moisture content, additionally the thousand kernel weight (TKW) was measured in g at 14% moisture. Ear bearing tillers/m^2 were calculated from three rows of 1 m length. Plants were cut shortly before harvest in order to assess morphological traits.

Protein content (%) was calculated from the nitrogen content of the seeds (N [%] $\times$ 5.7), which was analysed in ripe seeds that were dried for 72 h at 60°C, milled (ultracentrifugal mill, Retsch, Type ZM 2) and analysed in the elemental analyzer vario MAX CHN (Elementar Analysesysteme GmbH, Hanau, DE).

Hagberg falling number (HFN; sec.; ICC Method no. 107), sedimentation value (Zeleny; ml; ICC Method no. 116), and wet gluten (%; ICC Method no. 106/2) were analysed in the Aberham Laboratories, Großaitingen, DE. HFN was assessed in pooled samples in the first year of the trial and per plot in the second year. Sedimentation value and wet gluten were assessed in pooled samples from the four replications in both years.

Baking volume of test loaves (ml) was assessed using an internal method credited to Aberham Laboratories: test loaves were baked from wholemeal, no ascorbic acid was added but due to very high HFN of some samples the addition of malt flour was necessary to prevent the bread crust form liquefying. Baking volume was assessed per plot in the second trial year only. For a detailed rating system and its translation into a color code of the respective values see Table A1 in Appendix.

2.3. Data Processing and Statistical Analysis

Foliar disease severity per plot was calculated as the means per leaf level. Means were weighed 4:3:3 for the flag (F) leaves, the F-1 and F-2 leaves, respectively to account for the greater contribution of the flag leaf to the total dry matter of ripe seeds compared to the lower leaves [32].

A foot disease severity index (DI) was calculated based on the severity classes as:

$$DI = \frac{x_1 + 2x_2 + 4x_3}{n} 25 \quad (1)$$

where $x_1 \ldots x_3$ are the number of stems with disease scores 1 to 3, respectively, and n is the total number of stems assessed. The resulting index values fall between 0 and 100 and can be calculated for each of the three foot diseases separately or as an index of all three together.

The statistical calculations were performed using IBM SPSS Statistics (Version 22). Data were tested for normal distribution of residuals (Shapiro-Wilk-Test and Q-Q-plots) and for homogeneity of variance (Levene test) and transformed if required. When data were normally distributed and variance was homogeneous, a univariate ANOVA with subsequent Tukey-B-Test was calculated where appropriate to find significant differences between group means at $p < 0.05$.

Where normal distribution was the case but not homogeneity of variance, the Games-Howell post hoc test was used (foliar diseases in both trial years, total incidence of foot diseases in 2011/12, and ear length in both trial years). Linear contrasts were calculated to compare

$i)$ the three groups of populations (YQCCP, QCCP and YCCP),
$ii)$ populations and the reference varieties 'Achat', 'Akteur', and 'Capo',
$iii)$ populations and the mixture, and
$iv)$ CCP$_{org}$ and CCP$_{conv}$.

3. Results

3.1. Weather Data

Average temperature during the wheat growing season 2011/12 was 9.7°C, which is higher than the long-term average (2000–2013) of 9.3°C and the long-term average (1977–1994) of 7.9°C. During the growing season 2012/13 average temperature was between the two known long-term averages (8.5°C). Apart from two divergences and extremes in February/March 2012 and in February/March 2013, temperatures measured during the two growing seasons of the experiment from September 2011 to August 2013 roughly followed the 14-year trend from 2000 to 2013 (Figure 1).

The distribution pattern of the monthly precipitation, however, showed strong deviations from the long-term average. The average total annual precipitation from 1977 to 1994 was 619 mm, from 2000 to 2013 it was 684 and in 2012 and 2013 it was 792 and 657 mm, respectively. There were very dry periods in November 2011, February and March 2012 and in spring 2013, and some extremely wet months in winter 2011, summer 2012 and May 2013 (Figure 1).

The combination of extremes in winter 2011/12 exposed the plots to a severe winter. After two unusually mild and wet winter months temperatures suddenly dropped at the end of January 2012. Three weeks of black frost with minimum temperatures reaching down to -15°C resulted in soil frozen to a depth of about 50cm. Although the number of frost days (= daily minimum temperature below 0°C) in February 2012 was not different than in other years, the number of days with daily maximum temperature below 0°C was higher in 2012 than it was in 2011 or 2013. Also av-

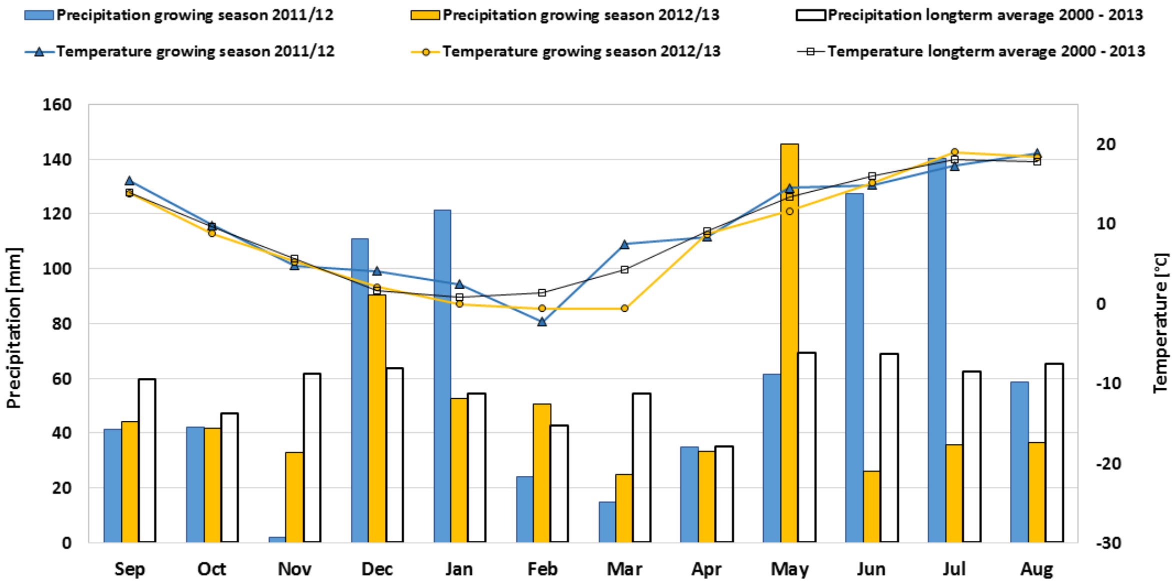

Figure 1. Monthly mean temperatures (°C) and monthly total precipitation (mm) in the wheat growing season 2011/12 and 2012/13 compared to the long-term average (2000–2013).

erage minimum and maximum temperatures (−9.9°C and −5.7°C) were considerably lower in February 2012 than in the years before and after (Table 1). The lack of snow left the plants unprotected from these extremes.

In mid-February, temperatures increased again and March was warm (average monthly temperature 7.5 °C which is 3.3 °C above the 14-year trend of 4.2 °C) and dry (precipitation was 15 mm, which is only 27% of the 14-year trend). These six relatively warm weeks of drought following the extreme cold worsened the effect of the cold and put surviving plants in the frozen soil under severe water stress. The CCP plots were noticeably damaged, but they recovered. However, most of the 20 parent varieties grown in 2011/12 next to the trial plots in two times replicated plots for seed multiplication, could not cope with the extreme climatic conditions and the severe winter resulted in winterkill in 16 out of the 20 varieties. On average only 33 plants/m^2 were left in the plots in April 2012 and only the four varieties 'Bezostaya', 'Monopol', 'Renan' and 'Hereward' survived with an average of more than 50 plants per m^2 (Figure 2). For winter wheat a density of 80 plants/m^2 or less is seen as an indicator for plowing the whole stand [33] and all plots of the parental varieties were abandoned.

Table 1. Number of frost days in February and average minimum and maximum temperatures.

Year	No. of frost days with daily minimum temperature below 0 °C	No. of frost days with daily maximum temperature below 0 °C	Average minimum temperature(°C)	Average maximum temperature (°C)
2011	20	7	−3.8	−2.1
2012	19	13	−9.9	−5.7
2013	16	9	−2.3	−1

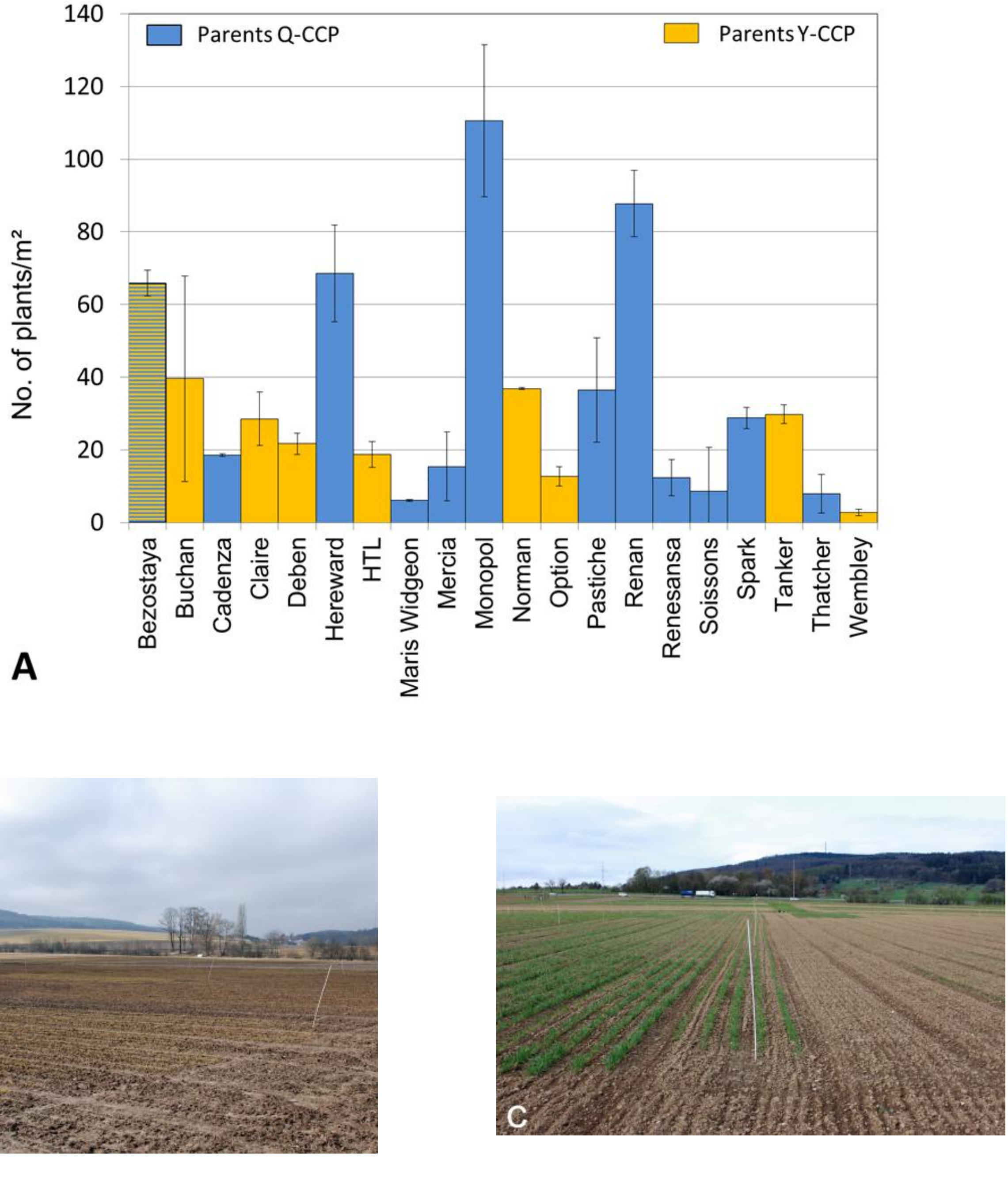

Figure 2. A: Top: Number of plants/m^2 in 20 winter wheat varieties (parent varieties of the CCPs, replicated twice in plots for seed multiplication) counted on the 19th of April 2012. Error bars denote the standard deviation for each variety (n = 2). B: CCPs straight after the frost, photo taken on March 1st 2012. C: CCPs (left) and parent varieties (right) six weeks later (photo taken on April 16th 2012).

3.2. Foliar and Foot Diseases

Disease pressure in both years was low. In both years the dominant disease was *Septoria tritici*. In 2012, the average infestation of plants on the three top leaf levels was 14% (BBCH stage 73/75), in 2013 it was even lower (10%). In 2012, infestation rates ranged from 12% (CY I) to 17% (OY II), in 2013 disease ranged from 7% ('Achat') to 10% (CA I). There were no relevant differences among treatments in both years (data not shown).

For **foot diseases**, total incidence and disease severity indices (DI) were slightly higher in 2013 (2012: 13; 2013: 20). The contribution of the two high infection severity classes to DI was, however, low in both years (data not shown) and therefore, overall the plants could be considered almost healthy. In both years *Fusarium* spp. was the dominating foot disease (DI 11in 2012; DI 16 in 2013), followed by *Pseudocercosporella herpotrichoides* (DI 2 in 2012; DI 4 in 2013), and *Rhizoctonia cerealis* ranged last in both years (DI 1 in 2012; DI 0.4 in 2013). There were only small differences among populations and references. A statistically significant difference in overall DI and *Fusarium* infestation between the CCP_{org} and CCP_{conv} is considered biologically not relevant and was disregarded (data not shown).

3.3. Morphological Traits—Straw and Ear Length

In 2012, overall straw length was considerably lower than in 2013 (77.2 cm vs. 90.5 cm, respectively). Overall, the CCPs were significantly shorter than the reference varieties in 2012 but not in 2013 and significantly taller than the mixture of the parental varieties in both years. The QCCPs were always significantly taller than the YCCPs (Table 2).

As expected, within-plot variation of **straw length** was in both years less for the references than for the CCPs and the mixture. As the references are pure line varieties, within-plot variation of plant height is very limited. The CCPs in contrast, originating from the intercrossing of several parental varieties of different height, show considerable variation in plant height. In 2012, the population CYQ II was tallest (85.0 cm), CY I was the shortest CCP (69.7 cm), and the mixture was even shorter (64.6 cm). CY I and CY II, although significantly taller than the mix of parents, were shorter than the other CCPs and references. All four YCCPs were shorter than the mean height of plants in the trial while all YQCCPs, QCCPs and the references were taller than the mean (Figure 3).

In 2013, 'Capo' was significantly tallest (99 cm), the mix of parental varieties was shortest (65 cm). The two other references were also very short ('Achat' and 'Akteur' with 86 and 87 cm respectively). While 'Capo' was tall or tallest in both years, 'Achat' and 'Akteur' changed in terms of their ranges in straw length values. While 'Achat' and 'Capo' were considerably shorter in 2012 than in the year after, absolute height of 'Akteur' changed only very little (83 cm in 2012 vs. 87 in 2013) and its change of position in the range of varieties and CCPs is only due to the overall taller plants in 2013.

In the group of CCPs, CY I was the shortest in 2013 (88 cm) as it was in 2012, followed by the three other YCCPs. Again, all YCCPs were shorter than the mean height of plants in the trial, forming a subgroup that was statistically distinguishable from the group of the taller YQCCPs and QCCPs (Figure 3, Table 2).

Variation in **ear length** of the references was similar to the variation in the CCPs. In 2012, ears varied between 8.1 cm (CQ II) and 9.9 cm ('Akteur'), with a mean of 8.8 cm in the trial and no statistically significant differences (data not shown). In 2013, ear length varied between 8.7 cm ('Capo') and 10.2 cm ('Akteur'), with a mean of 9.1 cm. In this year 'Achat' with 10.1 cm and less variance than 'Akteur' had the statistically longest ears. Overall, ear length of the references was significantly greater than that of the CCPs in both years (Table 2).

Table 2. Straw and ear length. Within the years means of a-priori defined groups were compared using linear contrasts.

Year	Comparison group		Straw length [cm]			Ear length [cm]		
	1	2	1	2	p-value	1	2	p-value
2012	CCPs	References	77	81	0.012*	8.8	9.2	0.046*
	CCPs	Mixture	77	65	<0.01*	8.8	9.1	0.294
	YQCCPs	QCCPs	89	80	0.589	8.8	8.6	0.432
	QCCPs	YCCPs	80	72	<0.01*	8.6	9	0.12
	YQCCPs	YCCPs	80	72	<0.01*	8.8	9	0.415
	CCP_{org}	CCP_{conv}	77	77	0.71	8.9	8.6	0.228
2013	CCPs	References	93	91	0.13	8.9	9.6	0.001*
	CCPs	Mixture	93	65	<0.01*	8.9	9.3	0.463
	YQCCPs	QCCPs	94	95	0.269	8.9	8.9	0.977
	QCCPs	YCCPs	95	93	<0.01*	8.9	9	0.376
	YQCCPs	YCCPs	94	93	<0.01*	8.9	9	443
	CCP_{org}	CCP_{conv}	93	92	0.331	9	8.8	0.133

* Groups differ at $p \leq 0.05$ or $p \leq 0.01$ (linear contrasts).

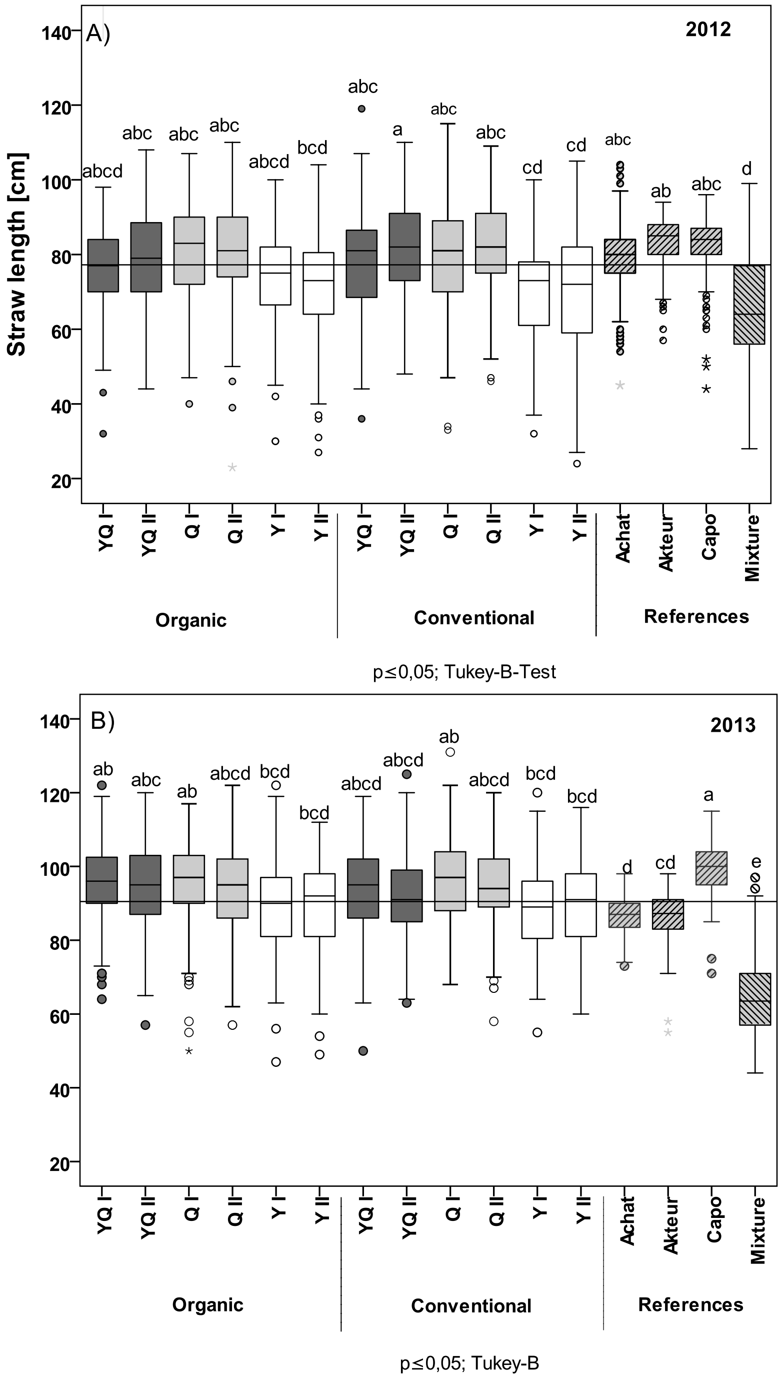

Figure 3. Straw length, 1st and 2nd trial year. n = 200. Shown are median, box signifying upper and lower quartiles, minimum and maximum, and, where required, outliner (o = outliner between 1.5 between 1.5× interquartile range and 3× interquartile range; * = extreme value >3× interquartile range). Horizontal line indicates the mean length in the trials. Populations/varieties with the same letter do not differ at p ≤ 0.05 according to Tukey-B test.

3.4. Grain Yield and Yield Components

3.4.1. Ear-Bearing Tillers/m^2

The average number of ear-bearing tillers/m^2 was 130 in 2012, with the fewest tillers found in the mixture plots (107) followed by OQ I plots (121). Most tillers were growing in CQ I plots (140; Figure 4). In 2013, the average number of ear-bearing tillers/m^2 was higher (202), fewest tillers were counted in the 'Achat'-plots (172) and most tillers in OY I plots (229; Figure 4).

While in the first experimental year no differences between groups could be found apart from a significant difference between CCPs and the mixture, some groups varied considerably in the second year. References formed significantly fewer ears than CCPs. The YCCPs (223 ears/m^2) produced significantly more ears than QCCPs and YQCCPs (202 and 197ears/m^2 respectively). There were no differences between CCP$_{org}$ and CCP$_{conv}$ (Table 3).

3.4.2. Total Grain Yield

In 2012, average yield in the trial was 4.2 t/ha with 'Akteur' yielding significantly highest (5.5 t/ha) and the mixture yielding lowest (2.9 t/ha). For all four YCCPs yield was less than the average. In 2013, average yield in the trial was 6.1 t/ha, which was 1.9 t/ha more than in 2012, with CY I (C = conventional) yielding highest (6.7 t/ha) and CYQ II yielding lowest (5.4 t/ha). In this year, the YCCPs yielded above average or just about average while QCCPs and YQCCPs yielded lower or just about average (with the exception of OYQ II (O = organic) which also yielded above average). Differences in yield were, however, not statistically significant in 2013 (Figure 4).

In 2012, the reference varieties yielded significantly higher than the CCPs while in 2013 there was no difference. The mixture yielded significantly less than the CCPs in both years and in 2012 the YCCPs yields were significantly lower than the QCCPs and the YQCCPs. The six CCP$_{org}$ did not differ significantly from the six CCP$_{conv}$ (Table 3).

3.4.3. TKW

The average TKW was 49.6 g in 2012 (Figure 4) and 48.6 g in 2013 (Figure 4). In 2012, TKW of OYQ I was highest (52.0 g) and of CY II lowest (47.9 g), in 2013 'Achat' had the highest TKW (51.2 g) and the mixture the lowest (44.2 g). In both years, TKW of the CCP$_{conv}$ was 0.8 g lower than for the CCP$_{org}$. In 2012, but not in 2013, the difference was statistically significant. Also, in 2012 TKW of the yield-group was significantly lower than the QCCPs and YQCCPs. TKW of references and populations did not differ (Table 3). In both years the TKW of the mix was significantly lower than that of the CCPs.

3.5. Baking Quality

For the **Hagberg falling number (HFN)** values $<$180 and $>$280 are considered poor with values in between 240–280 good and 180–239 moderate. The other quality parameters (protein content, sedimentation value, wet gluten, baking volume) are usually assigned to three to six class values. Where the rating is done in three classes, values are grouped into the classes good, moderate and poor; based on these classes the cells in the overview table (Table 4) are color coded, with green indicating good, yellow indicating moderate and red indicating poor, in addition to listing the measured values. More detailed ratings can be done for some parameters with classes ranging from very good to inacceptable, these classes are described in Table A1 in Appendix.

Table 3. Ear-bearing tiller/m^2, grain yield [t/ha], and TKW [g] of populations and reference varieties in both trial years.

Year	Comparison group		Ear-bearing tillers/m^2			Yield [t/ha]			TWK [g]		
	1	2	1	2	p-value	1	2	p-value	1	2	p-value
2012	CCPs	References	132	130	0.852	4	4.9	<0.01*	49.4	50.2	0.148
	CCPs	Mixture	132	107	0.013*	4	2.9	<0.01*	49.4	46.9	<0.01*
	YQCCPs	QCCPs	132	131	0.915	4.1	4.2	0.565	49.9	50.3	0.312
	QCCPs	YCCPs	131	132	0.892	4.2	3.8	<0.01*	50.3	48	<0.01*
	YQCCPs	YCCPs	132	132	0.977	4.1	3.8	0.011*	49.9	48	<0.01*
	CCP$_{org}$	CCP$_{conv}$	129	134	0.401	4	4	0.532	49.8	49	0.013*
2013	CCPs	References	207	180	0.002*	6.1	6.1	0.824	48.5	49.1	0.333
	CCPs	Mixture	207	181	0.049*	6.1	5.3	0.015*	48.5	44.2	<0.01*
	YQCCPs	QCCPs	197	202	0.607	6	6	0.895	48.8	48.5	0.513
	QCCPs	YCCPs	202	223	0.026*	6	6.3	0.148	48.5	48	0.59
	YQCCPs	YCCPs	197	223	0.007*	6	6.3	0.116	48.8	48	0.245
	CCP$_{org}$	CCP$_{conv}$	207	207	0.918	6.1	6.1	0.818	48.9	48	0.135

* Groups differ at $p \leq 0.05$ or $p \leq 0.01$ (linear contrasts).

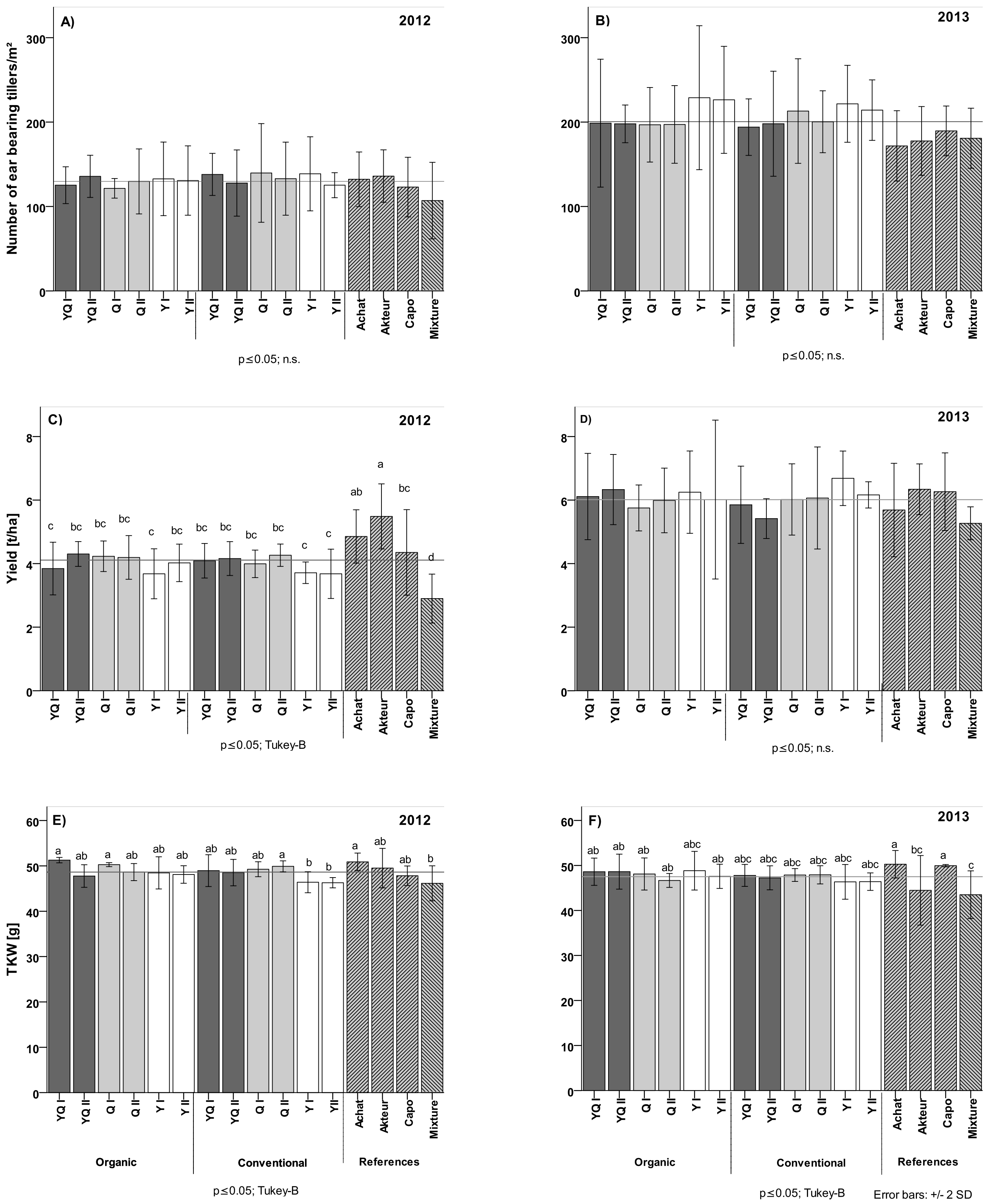

Figure 4. Number of ear-bearing tillers, grain yield, and TKW in both trial years (n = 4). Horizontal lines indicating average values in the trial, populations/varieties with the same letter do not differ at $p \leq 0.05$.

HFN, which was done for pooled samples in 2012 and by replicate in 2013, was rather high in both years, with an average of 292 sec. in 2012 and 282 sec. in 2013. **Sedimentation values** were extremely good in 2012 (41 ml on average) and 32 ml in 2013, which is still good, although sedimentation values for several samples were lower (Table 4). **Wet gluten** was higher in 2012 (average: 28.5%; good) than in 2013 (average: 26.3%; satisfactory). The mean **protein content** [%] in the trial was medium in both years (12.1% in 2012 and 11.3% in 2013). **Baking volume** assessed in the second year of the trial was 383 ml on average, which is a good result for wholemeal test loaves. Volume ranged between 344 ml (OY II; satisfactory) and 428 ml (OQ I; very good; Table 4).

In general, it could be observed that in both years YCCPs were clearly separate from the other populations and varieties with the YCCPs ranging lowest for all baking quality parameters tested. The QCCPs were in both similar to the reference varieties, which is also consistent for all parameters except protein content in 2013, where QCCPs had a significantly higher protein content than the references. The YQCCPs ranged in both years between the other groups of populations and varieties regarding all values tested and also the finding that CCP_{org} and CCP_{conv} did not differ is generally true for both years and all parameters tested (Table 5). The mixture of parents, which yielded very low in both years, showed much better results regarding baking quality parameters.

Values for protein content, HFN, baking volume, wet gluten as well as sedimentation value were close to the average of the trial in both years (Table 4).

When comparing groups (Table 5) the significantly higher **baking volume** of QCCPs was confirmed. YQCCPs ranged in the middle and YCCPs had the lowest baking volumes. Comparing the CCPs with the references, volume of references was significantly higher. A comparison of CCP_{org} and CCP_{conv} yielded no relevant differences, also the difference between QCCPs and references is not significant.

For **HFN** in 2013, the comparison of CCP groups showed the statistically significant highest HFN for the group of QCCPs (average HFN of group 310 sec.) followed by YQCCPs (average HFN 262 sec.), followed by the significantly lowest group of YCCPs (average HFN 205 sec.). While an average HFN of 310 sec. is considered poor (too high), 262 sec. is good, and 205 sec. is moderate. The references had a significantly higher HFN (average HFN 370 sec.) than the CCPs, which is extremely high and thus poor.

For **protein content** the comparison of groups showed in 2012 a significantly higher protein content of the CCPs vs. references and higher protein content of QCCPs compared to YCCCPs, YCCPs, and references. In 2013, protein content of QCCPs was higher than the group of YCCPs and the group of references (Table 5).

Table 4. HFN, protein content, wet gluten, sedimentation value, baking volume (data from pooled samples). Populations/varieties with the same letter do not differ at p $\leq$ 0.05 (Tukey-B test). Green = good, yellow = moderate, red = poor.

Year	2012				2013				
	HFN [sec.]	Sedimentation value [ml]*	Wet gluten [%]*	Protein content [%]	HFN [sec.]†	Sedimentation value [ml]*	Wet gluten [%]*	Protein content [%]†	Baking volume [ml]†
OYQ I	275	41	29.4	12.2^{abc}	246^{abc}	30	26.7	11.2	373^{abc}
OYQ II	293	38	28.4	11.8^{bc}	236^{abc}	31	28.4	11.7	379^{abc}
OQ I	309	51	28.5	12.5^{ab}	308^{abc}	39	27.5	11.9	428^{a}
OQ II	349	49	28.4	12.3^{abc}	325^{abc}	37	27.3	11.7	418^{ab}
OY I	207	29	28.5	12.2^{abc}	181^{c}	19	25.4	11.3	350^{bc}
OY II	206	27	27.8	11.9^{bc}	237^{abc}	20	25.8	11.1	344^{c}
CYQ I	274	39	29	12.1^{abc}	284^{abc}	29	25.4	11.1	361^{abc}
CYQ II	256	40	29.5	12.1^{abc}	291^{abc}	29	25.6	11.4	367^{abc}
CQ I	307	49	28.8	12.5^{ab}	295^{abc}	37	27.6	11.7	401^{abc}
CQ II	296	47	28.6	12.5^{ab}	313^{abc}	41	28.2	11.9	413^{abc}
CY I	204	30	29	12.2^{abc}	203^{bc}	20	24.7	11	359^{abc}
CY II	219	29	27.7	11.7^{bc}	208^{bc}	22	25.4	11.1	361^{abc}
Achat	396	46	26.9	11.5^{c}	370^{a}	41	27.5	11.3	408^{abc}
Akteur	424	38	24.8	10.8^{d}	347^{ab}	34	21.6	10.1	403^{abc}
Capo	371	66	31.6	12.9^{a}	392^{a}	46	27.1	11.5	385^{abc}
Mixture	302	55	29.2	12.6^{abc}	242^{abc}	30	26.7	11.3	363^{abc}
mean	292	41	28.5	12.1	282	32	26.3	11.3	383

* Data from pooled samples.
† Data from replicated samples (n = 4; protein contents 2013 not significant.

Table 5. Baking quality (baking volume, HFN, protein content), comparison of groups.

Comparison group		Protein content [%] 2012			Protein content [%] 2013			Baking volume [ml] 2013			HFN [sec.] 2013		
1	2	1	2	p-value	1	2	p-value	1	1	p-value	1	2	p-value
CCPs	References	12.2	11.7	<0.01*	11.4	11	0.118	380	399	0.033*	260	370	<0.01*
CCPs	Mixture	12.2	12.3	0.282	11.4	11.3	0.816	380	363	0.246	260	243	0.574
QCCPs	References	12.4	11.7	<0.01*	11.8	11	0.016*	415	399	0.127	310	370	0.014*
YQCCPs	QCCPs	12	12.4	<0.01*	11.3	11.8	0.144	370	415	<0.01*	262	310	0.042*
QCCPs	YCCPs	12.4	12	<0.01*	11.8	11.1	0.033*	415	354	<0.01*	310	205	<0.01*
YQCCPs	YCCPs	12	12	0.938	11.3	11.1	0.489	370	354	0.104	262	205	0.014*
CCP_{org}	CCP_{conv}	12.1	12.2	0.812	11.5	11.4	0.629	384	377	0.548	256	265	0.577

* Groups differ at $p \leq 0.05$ or $p \leq 0.01$ (linear contrasts).

4. Discussion

Overall, differences due to the parental background of the CCPs and not due to their conventional or organic history were clearly evident in the trials. Compared to the parental mixtures, the CCPs proved to be highly resilient, recovering much better from winter kill in 2012. Nevertheless, they were outyielded by the references in 2012 but not in 2013. In contrast, baking quality of the QCCPs was not different from that of the high baking quality reference varieties.

4.1. Foliar and Foot Diseases

Disease pressure was low and thus did not play a role for the performance of the CCPs or the references during the two experimental years. Overall, there was neither an influence of the choice of parents nor of the growing system visible. Parents were chosen with the focus on yield and baking quality and not in order to represent different disease resistances, therefore it is unlikely that the CCPs initially differed very much regarding their resistances. Disease pressure in the growing environment where the populations evolved was moderate and did not differ much between the organic and conventional growing area, this meant a strong differentiation of populations was not expected.

Higher disease pressure might have resulted in a different picture as the results of other experiments indicate. Observations of powdery mildew (*Blumeria graminis* f. sp. *tritici*) in wheat CCPs revealed that the frequency of B. *graminis*-resistance genes evolved differently according to the respective disease pressure [34–36] and Webster *et al*. [37] found that frequencies of *Rhynchosporium secalis*-resistance genes in a composite cross of barley changed between F_5 and F_{45} in accordance with the respective disease pressure. In years when high pressure was recorded the frequency of the resistance genes rose, in years with low pressure, it fell.

Observations in stripe rust (*Puccinia striiformis*) in a wheat experimental population in France documented that the resistance gene Yr17, which provided complete resistance to stripe rust until 1997 and was thus suspected to be under strong selection, was indeed selected between generations 5 and 10 [38].

Since 2011, new races of stripe rust have made a dramatic appearance throughout Europe [39] and the main foliar pathogen observed since 2014 in the trial site is stripe rust. In comparison to the susceptible varieties 'Akteur' and 'Naturastar', disease severity on the CCPs has been very low [40].

4.2. Morphology

The CCPs as well as the references could not reach their full height potential in the first year due to the extreme weather conditions. The same was reported from regional variety trials, where the average plant height of winter wheat grown without growth regulators in 2012 was reported to be only 87 cm [41].

The parents were equally short in both years as they were mostly dwarf types. In contrast, the CCPs were much taller indicating that the dwarfing genes have decreased in frequency. They might not have been eliminated completely though, as variation for this trait is still quite large. Nevertheless, the CCPs were within the normal height range; they were shorter than the references in the first experimental year and about the same height in the second year.

Findings of Goldringer *et al*. [27] and Le Boulc'h *et al.* [26] observing an increase in plant height cannot be confirmed. This could be due to the fact that the tallest plants (>130 cm) were removed from the populations in several successive years to limit their selective advantage. We conclude that the "good practice" of removing the tallest plants in an evolutionary population may improve their agronomic value. It might, however, have obscured any effects of natural selection on plant height.

Morphological characteristics of the parental varieties were documented in 2007 [42]. In that year, height of the yield parents was 87.5 cm while the quality parents were 97.1 cm tall on average. Thus, the significantly shorter straw length of the YCCPs compared to the other CCPs are founded in the original composition of the CCPs and should not be understood as divergent developments of the populations over time.

Measurements in the F_5 - F_9 also showed these differences in plant height of the CCPs [43]. Ear length has not previously been measured in the parental varieties. However, as the results show only marginal differences between ear length of references and populations, an influence of the parental varieties is unlikely. An influence of the two growing systems on straw height and ear length was not found.

4.3. Yield and Yield Components

Ear-bearing tillers were at the same low level for all CCPs, the mixture and the references without large variation in summer 2012, which shows that the winter conditions influenced all plots in a similar way resulting in overall low yields. Nevertheless, the resilience of the CCPs and reference varieties was remarkably higher than for most of the parents (Figure 2). Considering the poor survival of the parents in pure stands, the performance of the mixture in 2012 was impressive, demonstrating the general positive effects of mixtures over pure lines as has been shown on many occasions before [7,44].

Based on previous year's results [45] and because they were composed from high-yielding varieties, the YCCPs were expected to yield better that the other CCPs. However, in 2012 they yielded lowest of all CCPs. To explain this, the parental varieties used to create the CCPs have to be taken into account. Of the 20 parent varieties only the four varieties 'Bezostaya', 'Monopol', 'Renan' and 'Hereward' survived the winter reasonably well (Figure 2). As the CCPs were composed in the UK, 14 out of 20 parent varieties were of English origin and thus bred for a maritime climate. 'Bezostaya', however, is of Ukrainian origin, has high grain yield and quality, good frost resistance and is often used in crossing where winter hardiness is a desired trait[46]. 'Monopol' comes from Germany and 'Renan' is French [47], only 'Hereward' is an English variety.

A closer look at the pedigree reveals also here a German winter wheat variety—'Disponent'—as a crossing partner [23] which most likely provided 'Hereward' with a certain degree of winter hardiness. Of these four varieties with good winterhardiness, only 'Bezostaya' was intercrossed into the YCCP, which most likely explains why the winter conditions affected the YCCPs more than the other populations. While it is possible that selection for greater winter hardiness occurred at the German site, this cannot be concretely concluded without direct comparison of early and late generations for this trait, or of populations that have undergone evolution in different climatic conditions.

The comparably good yield of 'Achat', 'Akteur', and 'Capo' in 2012 is most likely owed to their relatively good winter hardiness and to the fact that good winter hardiness was not one of the main traits in focus when establishing the CCPs. It remains to be seen if the CCPs respond better to freezing after having survived one especially cold winter. As we used the same seed in both years the winter effects did not affect the performance in the second year. Results from experiments investigating the effect of natural selection on the winter survival of barley CCPs indicate that natural selection did increase winter survival although not uniformly over different generations [48]. In bulk populations of winter oats an improvement in winter hardiness could only be found in populations with low initial survival levels [49,50]. Also, apparent advances made in winter survival in one year can reverse in later generations due to a lack of competitive ability of the hardy types later in the growing season [49], when non-hardy types that were not eliminated resurface and restore themselves as major components in the population[48]. This shows that complex traits such as winter hardiness, that were not a main focus when establishing CCPs, are hard to achieve through natural selection only.

In 2013, yield of the YCCPs corresponded with expectations being 0.3 t/ha higher than the QCCPs and YQCCPs. These differences were, however, not statistically significant. Yield of the CCP$_{org}$ and CCP$_{conv}$ varied minimally with no indication that their maintenance in different growing systems has led to strong variation between the two groups of populations regarding yield performance. Higher numbers of ears of the YCCPs was related to the higher yielding capacities of these populations. In contrast, the high yields of the references 'Capo' and 'Akteur' were due to high TKW and high number of seeds per head, respectively. This is in contrast to what was previously published by the seed producing industry. 'Capo' is known as a density type realizing yields through many tillers and 'Akteur' as a single ear type, forming many seeds per ear with high TKW [51].

A higher TKW was the only parameter that separated the CCP$_{org}$ from the CCP$_{conv}$ in 2012. In the second year, absolute differences where at the same—low—-level, the difference was, however, not statistically significant. Apart from this observation there was no field evidence that the differing environments of an organic and a conventional farming system could have shaped the CCPs in different ways. However, a study using hydroponics and bioassays to investigate early vigour and allelopathy in the F$_6$ and F$_{11}$ of the CCP$_{org}$ and CCP$_{conv}$, documented systems' effects on the CCPs.

Characteristics for early vigour were improved after five years in the organically managed CCPs in comparison to the conventionally managed CCPs. The changes towards early vigour in the organic CCPs are thought to be due to the combined effects of selection for higher nitrogen uptake under low-input conditions, and increased competition for light and larger seeds, rather than a direct adaptation to higher weed pressure [52].

4.4. Baking Quality

As baking tests are rather costly and time-consuming, various indirect parameters such as sedimentation value, wet gluten, protein content and falling number are often used to predict the baking properties of wheat flour. It has been assumed that protein and wet gluten content strongly correlate with the baking volume determined in the RMT. This is, however, not always the case [53]. In whole-meal-baking tests protein content, sedimentation value and wet gluten content often only have a very limited influence on the baking volume [54].

In our study indirect baking quality parameters were analyzed in both years while baking tests could only be conducted in 2013. The results for protein content and HFN in 2013 were in accordance with the outcomes of the baking tests while wet gluten and sedimentation value were less suitable to predict the baking test outcome. The results show a clear differentiation of groups based on the original composition of the populations for all parameters, except wet gluten.

Baking tests are usually done with the rapid mix test (RMT), which is the usual procedure when testing superfine flour. The RMT is, however, not optimized for the processing of organically produced wheat [55] and considering this, the baking test done in 2013 to assess baking volume of the CCPs was done with wholemeal test loaves.

For a wholemeal baking test the average volume of loaves of 383 ml is a good result. Baking with wholemeal flour, lower volumes are the norm and a volume of 400 ml or above is considered very good, 350 to 400 ml is good, below 350 ml is moderate and 330 ml and below is poor (pers. comm. Dr. R. Aberham).

In the test, all CCPs and references except OY II ranged above 350 ml. The strong differences between varieties that can be observed with white flour are less pronounced when testing with wholemeal flour [56]. In this way the results are more likely to correlate with results bakers producing organic bakery products would achieve. The high volumes of the QCCPs compared with YCCPs or YQCCPs indicate that the original choice of parental varieties still has an effect, while adaptation to the farming systems seems to have had no effect on baking volume. The same was true for protein content, falling number, and sedimentation values. In contrast, for **wet gluten** the influence of parents is not as clearly visible as for the other baking quality parameters. Overall, the QCCPs that were specifically created for good baking quality, are as good (baking volume) or better (protein content, HFN) than modern elite wheat varieties.

While yield is a trait that is subject to natural selection [15,57], quality traits are not directly influenced by natural selection [15]. Without the genetic base of high-quality parents the breeding objective of high baking quality cannot be reached [58]. Including a parent with low baking quality in the setting up of a high quality CCP can be enough to counteract the high quality parents as some individuals with low quality will prevent the population as a whole from sustaining high quality [15]. Results from trials with variety mixtures show other patterns, however. In a mixture of two wheat varieties a higher total aerial biomass was achieved than was produced by each variety grown in a pure stand. This increase resulted in a grain yield similar to that one of the higher-yielding variety and an improved protein content was measured [59].

The crossing design of the CCPs developed by the John Innes Centre and Elm Farm Research Centre took it into account that quality traits are not subject to natural selection. As opposed to the early composite cross populations of wheat and barley [60,61], which were established with the aim of representing the major wheat or barley growing areas of the world in order to assemble genotypes appropriate for each cultural practice in the respective agro-climatic zone [15], the focus was narrowed to yield or quality as key characteristics of the CCPs. The results show that the quality traits were successfully inherited and maintained over time and that acceptable yield levels were also achieved not only in the populations designed to be high-yielding, but also in the high-quality populations which were not much different in yield from the high-yielding populations in the second experimental year. By using seed of the same generation in both years, these genetic effects could be clearly separated from the lack of winter hardiness in the YCCP parentage.

Looking at the yield and quality achieved by the mixture of parents a contrast of low yield in both years, but good quality becomes visible. The CCPs out yielded the parental variety mixture in both years. Here the populations seem to have a clear advantage over the mixture. The overall higher diversity and/or natural selection and adaptation over time may be responsible for this. For the quality aspect natural selection played – as mentioned above – a minor role and QCCPs and parents continued to perform similarly after a decade of selection.

5. Conlusions

The concept of evolutionary breeding can be one of the new, different and efficient strategies urgently required to face the challenges of climate change, population growth and use of finite resources. The overall question if the growing conditions on either organic or conventional fields influence the agronomic performance of the populations, cannot be answered conclusively. The two years were very different, especially regarding the climatic conditions, and many differences were not consistent over both years of the trial.

The parental selection for the CCPs has a much greater influence on their performance than the growing and management conditions to which the populations are subjected. This can be observed with regards to baking quality traits, as well as with morphological parameters, grain yield and yield parameters.

The choice of parents to establish a CCP is crucial, especially when focusing on traits which are not directly influenced by natural selection (for example, quality traits). In the case of the QCCPs the establishment of a reliable high-quality population worked very well and quality traits were successfully maintained over time.

The results clearly indicate that the intercrossing of several pure line varieties does not strongly disconnect their carefully selected traits and much of the originally exhibited characteristics remain (including lack of winter hardiness, for example). The traits present in the parental varieties determine the performance of the CCPs to a considerable degree, even after several years of adaptation to specific growing conditions, so the initial choice of parents suitable for the intended growing conditions should not be underestimated.

As the populations only evolve slowly or not at all in the absence of high selection pressure, which was illustrated by the reactions to foot and foliar diseases, they might be in danger of being outperformed by newly bred wheat varieties after a decade of maintenance and evolution. The frequent integration of well adapted, modern breeding lines into existing CCPs might help to overcome this constraint. Another strategy could be to apply additional human selection such as mass selection for vigour or disease resistance in the context of participatory breeding approaches.

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Appendix

Table A1. Interpretation of baking quality parameters HFN, sedimentation value, protein content, wet gluten and baking volume [62–65].

	Value	Rating	Further differentiation of rating where possible
HFN [sec.]	<180	poor	
	180-239	moderate	
	240-280	good	
	>280	poor	
Sedimentation value [ml]	<22	poor	
	23-29	moderate	
	30-34	good	good
	35-40	good	very good
	>40	good	Aufmischqualität
Wet gluten [%]	<20	poor	inacceptable
	20-23	poor	poor
	24-25	moderate	poor to moderate
	26-27	moderate	moderate
	28-30	good	good
	<30	good	very good
Protein content [%]	<10,5	poor	
	10,5-12,5	moderate	
	>12,5	good	
Baking volume [ml] (wholemeal)	<330	poor	
	330-349	moderate	
	350-400	good	good
	>400	good	very good

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Research Article

Weeds in Organic Fertility-Building Leys: Aspects of Species Richness and Weed Management

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Abstract: Legume-based leys (perennial sod crops) are an important component of fertility management in organic rotations in many parts of Europe. Despite their importance, however, relatively little is known about how these leys affect weed communities or how the specific composition of leys may contribute to weed management. To determine whether the choice of plant species in the ley affects weeds, we conducted replicated field trials at six locations in the UK over 24 months, measuring weed cover and biomass in plots sown with monocultures of 12 legume and 4 grass species, and in plots sown with a mixture of 10 legume species and 4 grass species. Additionally, we monitored weed communities in leys on 21 organic farms across the UK either sown with a mixture of the project species or the farmers' own species mix. In total, 63 weed species were found on the farms, with the annuals *Stellaria media*, *Sonchus arvensis*, and *Veronica persica* being the most frequent species in the first year after establishment of the ley, while *Stellaria media* and the two perennials *Ranunculus repens* and *Taraxacum officinale* dominated the weed spectrum in the second year. Our study shows that organic leys constitute an important element of farm biodiversity. In both replicated and on-farm trials, weed cover and species richness were significantly lower in the second year than in the first, owing to lower presence of annual weeds in year two. In monocultures, meadow pea (*Lathyrus pratensis*) was a poor competitor against weeds, and a significant increase in the proportion of weed biomass was observed over time, due to poor recovery of meadow pea after mowing. For red clover (*Trifolium pratense*), we observed the lowest proportion of weed biomass in total biomass among the tested legume species. Crop biomass and weed biomass were negatively correlated across species. Residuals from the linear regression between crop biomass and weed biomass indicated that at similar levels of crop biomass, grasses had lower weed levels than legumes. We conclude that choice of crop species is an important tool for weed management in leys.

Keywords: clover; conservation; grass; legume; rotation; soil fertility; species richness; weed community

1. Introduction

In agricultural production, nitrogen is a key nutrient for achieving acceptable yields and crop quality [1]. Due to globally rising costs of mineral nitrogen fertilizer and concerns over the negative environmental impact of anthropogenic nitrogen [2,3], agricultural policy makers, farmers and scientists are increasingly paying attention to the use of leguminous plants as an alternative source of nitrogen [4,5]. Through their symbiosis with rhizobacteria, legumes are able to fix atmospheric nitrogen [6] and convert it to a form that is readily available to plants [7]. After incorporating (e.g. ploughing) legumes into the soil, nitrogen accumulated in the plants' above-ground and below-ground residues is broken down by microbial activity and released for uptake by the following crop [8]. This use of legumes for fertility-building in the rotation is common in a variety of farming systems, e.g. where the use of mineral nitrogen fertilizer is considered to be too expensive, or, as in organic agriculture, where it is not permitted [9,10]. Both grain legumes and forage legumes are used for fertility building. Because of its function as the main nutrient provider, the use of forage legumes in the rotation, which in Europe is frequently referred to as the ley phase, is of central importance for certain organic (and also increasingly non-organic) farming systems.

In Western and Central Europe, organic farmers most frequently use grass-clover mixes for their leys, with white clover (*Trifolium repens*) and red clover (*T. pratense*) being popular legume species, and perennial ryegrass (*Lolium perenne*) and Italian ryegrass (*L. multiflorum*) as commonly chosen grass species [10]. Frequently, these leys are grazed or cut for silage or hay and incorporated into the soil by ploughing before sowing the next crop [11]. Depending on various factors such as climate and soil conditions, the suitability of the land for arable production and the presence of livestock on the farm, the ley phase on organic farms can vary in duration from short term (1-1.5 years) to longer term (around 5 years), but typically the ley is maintained for about 1.5 to 3 years [10,12].

A key requirement for high ley performance (e.g. as measured by above-ground biomass cumulated over time), and the subsequent provision of nitrogen to the following crops is successful establishment of the ley [13]. Ideally, plants need to cover the ground quickly and establish well in a range of environmental conditions. However, according to a consultation of UK organic farmers conducted before the start of this study, white and red clover can be difficult to establish, especially under dry conditions [14]. During the establishment period, weeds can play an important antagonistic role by competing with the sown legumes for light, nutrients and water [15,16]. Also, annual weeds that exploit the space left by poor ley establishment are more likely to contribute to the weed seed bank in the soil and may therefore become a problem later, in the crop following the ley. For these reasons, the ability to outcompete weeds, either through a high competitive ability and vigour or through allelopathy, is a desirable trait in legume species for use in leys.

At the same time, the lack of tillage during the ley phase means that an important tool for weed control in organic farming, namely the mechanical destruction and burying of weeds [17], is not available. Also, lack of tillage means that weeds are not stimulated to germinate, so that weed seeds remain in the seed bank. On the other hand, leys can be repeatedly mown or grazed during the ley phase, which provides an alternative tool for weed management [18]. Using multiple species with complementary growth habits in a ley has the potential to further enhance weed suppression by exploiting differences in functional traits [19,20]. For example, a fast growing early species that covers the ground quickly would complement a species that is taller and more competitive later in the season. Interestingly, leys appear to have the potential to increase weed seed numbers in the seed bank while simultaneously reducing weed emergence in the following crop; in a study on weeds in a wheat (*Triticum aestivum*) crop after lucerne (*Medicago sativa*)-grass leys or after potatoes (*Solanum tuberosum*) in Southern Germany, higher numbers of weed seeds in the seed bank were found after the ley than after potatoes, but a lower number of weeds emerged in the wheat following the ley [21]. However, careful management is necessary to prevent the build-up of perennial weeds such as docks (*Rumex spp.*) and creeping thistle (*Cirsium arvense*) in leys [22–24]. Such species pose a potential problem not only for ley performance but also for subsequent crops and can pose a serious threat to productivity of organic crops [23,25].

Despite the potentially negative effects of annual and perennial weeds in leys, the weed flora may simultaneously contribute to the farm's biodiversity [26,27]. Weeds provide vital resources for invertebrates and other wildlife [28–31], thereby also helping to regulate pest populations in agroecosystems [32]. In addition, some weed species in leys can be a source of mineral nutrients for livestock [33]. Thus, weeds can be seen to provide a range of ecosystem services. However, these same services may also be provided by the crop, especially if multiple crop species in a ley are used. For example, including species with a variety of flowering times would extend the period of nectar and pollen provision [34].

Ecological research on the function and diversity of weeds in organic farming systems has so far mainly concentrated on weeds occurring in arable crops [35,36]. Where research has investigated the weed suppression by various small-seeded legume species, the focus has mostly been on the use of these legumes as short term cover crops [37,38]. In contrast, current knowledge about weed diversity and weed control in organic rotational leys is limited. As part of a larger study on optimizing ley composition and management [39] we monitored the dynamics of weed communities in replicated and on-farm trials at multiple locations throughout the UK.

Specifically, we asked: (1) Which legume and grass species typically used in legume-based leys show the highest competitive ability against weeds? (2) Which are the dominant weed species in typical organically managed leys in the UK? (3) What is the typical species richness of weeds (as measured by species richness) in organically managed leys? (4) Does crop species richness in the ley affect weed cover and weed species richness?

2. Material and Methods

2.1. Overview

The study was conducted over two years, starting in spring 2009 and consisted of two main experimental series. In series I, we set up replicated field trials at six sites across the UK, evaluating various legume and grass species in monocultures and in a multi-species mixture of legumes and grasses (Tables 1 and 2).

Table 1. Legume and grass species included in the trials: Latin and common name, variety, seeding rate (kg/ha), seed weight (Thousand Kernel Weight, TKW in g) and seeding rate in the monoculture plots and in the All Species Mix (ASM).

					Seeding rate (kg/ha)		
Abbreviation	Latin name	Common name	Variety	Inoculum*	Monoculture	ASM	TKW
AC	*Trifolium hybridum* L.	Alsike clover	Dawn	C	10	1.25	0.7
BT	*Lotus corniculatus* L.	Birdsfoot trefoil	San Gabrielle	-	12	2.5	1.2
BM	*Medicago lupulina* L.	Black medic	Virgo Pajberg	L	15	2.5	1.6
CC	*Trifolium incarnatum* L.	Crimson clover	Coutea	-	18	2.25	3.1
IR	*Lolium multiflorum* Lam.	Italian ryegrass	Teana	-	33	1	2.9
LT	*Lotus pedunculatus* Cav.	Large birdsfoot trefoil	Maku	-	12	2.5	1
LU	*Medicago sativa* L.	Lucerne	La Bella de Campagnola	L	20	2.5	2.4
MF	*Festuca pratensis* Huds.	Meadow fescue	Rossa	-	25	1.25	2.14
MP	*Lathyrus pratensis* L.	Meadow Pea	no specified variety	V	75	3.25	153
PR	*Lolium perenne* L.	Perennial ryegrass	Orion	-	33	2.5	2
RC	*Trifolium pratense* L.	Red clover	Merviot	C	18	2.5	1.8
SF	*Onobrychis viciifolia* Scop.	Sainfoin	Esparsette	-	80	5	19.2
TY	*Phleum pratense* L.	Timothy	Dolina	-	10	0.5	0.32
WC	*Trifolium repens* L.	White clover	Riesling	C	10	1.5	0.5
SC	*Melilotus alba* Medik.	White sweet clover	no specified variety	L	18	-	2.3
WV	*Vicia sativa* L.	Winter vetch	English Vetch	V	100	-	41

* Inoc. Inoculation prior to sowing with Clover inoculum (C), Lucerne inoculum (L) and Vetch inoculum (V).

Table 2. Details of replicated trials: locations, plot sizes, sowing dates and pre-crops; * taken from one quadrat (50 × 50 cm) per plot; ** taken from three quadrats (each 50 × 50 cm) per plot.

Site	Barrington Park	Duchy (Rosewarne)	IBERS Aberystwyth	Rothamsted	SAC Aberdeen	Wakelyns Agroforestry
Abbrevation	B	D	I	R	S	W
North coordinate	51°49'52.2"	50°13'38.2"	52°25'48.1"	51°48'38.6"	57°11'05.6"	52°21'36.7"
West coordinate	1°40'12.3"	5°18'23.0"	4°01'22.1"	0°22'02.4"	2°12'45.1"	-1°21'09.2"
Altitude (m)	150	42	29	114	109	51
Plot width (m)	1.5	1.5	2	2	1.5	1.2
Plot length (m)	10	5	8	5	12	10
Sowing (date 2009)	20-Apr	24-Apr	23-Apr	15-Apr	13 May	29-Apr
First mowing (date 2009)	24-Jun	14-Jul	20-Jul	5-Aug	23-Jul	27-Jul
Previous crop	winter barley	fallow	winter oats	fallow	spring barley	potatoes
Biomass sampling dates						
2009*	-	18-Aug	1-Sep	5 Oct	20-Aug	24-Aug
2010* (1)	-	20-Apr	-	15-Apr	13 May	28-Apr
2010* (2)	-	18 May	21-Sep	13 May	11-Jun	28 May
2011**	-	13-18 Apr	Mar	-	Apr	Apr

Table 3. Details of on-farm trials: Geographic coordinates and soil properties.

Farm Nr.	Coord. North	Coord. West	Elevation (m)	Soil Texture*	Sand (%)	Silt (%)	Clay (%)	Soil pH	P (mg/L)	K (mg/L)	Mg (mg/L)	OM (%)
1	52°21'36.71"	−1°21'9.24"	51	C	22	20	58	7.4	31.6	122	58	ND
2	52°37'50.17"	−0°20'42.67"	1	C	28	31	41	7.6	38.2	441	424	ND
3	52°8'28.18"	0°2'57.15"	45	CL	43	22	35	8.2	16.8	247	61	ND
4	52°31'17.36"	0°9'46.39"	0	CL	39	33	28	6.7	34.2	201	103	ND
5	51°29'47.91"	1°3'30.22"	52	CL	41	40	19	6	33.6	77	63	2.6
6	51°27'1.65"	1°9'39.6"	99	CL	46	33	21	7.2	31.4	185	51	3.3
7	52°22'1.61"	1°24'47.37"	73	C	42	21	37	6.6	30.4	336	108	ND
8	51°31'5.7"	1°27'25.92"	162	CL	32	42	26	8	21	110	35	8.2
9	51°18'56.26"	1°31'9.32"	170	CL	29	42	29	7.6	28.4	123	42	3.8
10	51°22'49.14"	1°32'3.67"	125	CL	43	38	19	7.4	47.4	134	44	3.4
11	51°26'28.01"	1°54'5.71"	164	SL	16	61	23	7.1	20.4	95	53	2.6
12	51°43'56.32"	1°56'21.42"	135	SC	18	36	46	7.7	17.2	224	71	3.6
13	57°16'52.58"	2°7'56.92"	97	SaL	45	39	16	5.5	34	213	77	8
14	57°11'5.6"	2°12'45.13"	109	SaL	58	29	13	5.8	94.2	179	171	7.8
15	57°33'3.04"	2°18'0.48"	120	CL	38	41	21	5.7	18	103	80	8.3
16	57°18'38.38"	2°18'29.9"	194	CL	43	38	19	6.2	30.4	212	90	9.2
17	57°40'16.47"	3°16'30.66"	20	LSa	77	16	6	6.3	34	110	73	2.4
18	53°0'38.65"	3°38'48.06"	309	SL	7	58	35	4.9	21.2	131	65	ND
19	52°37'45.57"	4°5'1.99"	56	SaL	77	12	11	6.2	16.2	89	161	ND
20	52°2'44.28"	4°35'59.37"	70	SC	7	47	46	4.9	19.2	67	62	ND
21	51°48'22.52"	5°4'5.39"	85	CL	32	41	27	5.9	18.4	170	121	6.5

*C: Clay; CL: Clay Loam; SC: Silty Clay; SL: Silty Loam; SaL: Sandy Loam; LSa: Loamy Sand; OM: Soil organic matter; ND: Not determined

Figure 1. Photograph of an on-farm trial at Wakelyns Agroforestry, Suffolk, taken in the summer of 2010. On the left, slightly paler, the control ley (white clover-chicory-black medic mix), on the right the All Species Mix (ASM). A different site on the same farm was also used for replicated experiments.

In series II, the same multi-species mixture was sown on 21 organic farms in the UK as non-replicated 0.5 ha strips alongside farmer-chosen control leys (Table 3, Figure 1). In the following text we call the series I trials "replicated trials" and the series II trials "on-farm trials". In both series, trials were performed only once per site. Therefore, effects of year-to-year variation (e.g. effects of yearly differences in weather on weed emergence in the establishment phase of the ley) cannot be analysed. However, although effects of the age of the ley and the study year cannot be separated, this was at least partly compensated for by including a large number of trial sites in the study.

2.2. Species Selection and Composition of Species Mixture for Use in Field Trials

Leys can be sown with mixtures of different plant species, which may provide insurance against the failure of individual species. In addition, mixing species is a way to combine desirable species-specific traits. To compose optimal species mixtures, a useful criterion for species selection is the functional complementarity of the different species [32,40–42], with the aim of minimizing functional redundancy.

According to this idea, we collected data on the ecological and agronomic traits of 22 legume species and five grass species from the literature [for details see [43]]. To assess complementarity, a principal component analysis (PCA) was conducted on traits of the 22 legume species (maximum height, flowering time, seed size, rooting depth, productivity, establishment and competitive ability, [see supplementary material of reference [43]]). The distance of individual species from each other in the PCA bi-plot was considered to be an indicator of functional divergence and potential for complementarity, in terms of coexistence and delivering multiple ecosystem functions when grown together in a mixture. Additional selection criteria included agronomic and practical aspects such as frost tolerance, resistance to grazing and seed availability of the species in the UK.

As a result of this selection process we chose a subset of four grass species and twelve legume species with functionally complementary properties for the replicated and on-farm trials (Table 1). Further details of the selection process, as well as the identity of the non-selected species are given elsewhere [39]. All four selected grass species, as well as ten of the twelve tested legume species, were combined in an 'All Species Mixture' (ASM) (Table 1), which was tested in both the replicated and on-farm trials. Two species (*M. albus* and *V. sativa*) were not included in the ASM because of concerns by the participating farmers about potential detrimental effects of these species on animal health or agronomic management. Seed densities of the monocultures were chosen according to general recommendations for the UK [44]. The average plant density in the monocultures was 1180.5 plants m^{-2}, whereas the total plant density in the ASM was 1811.1 plants m^{-2}. The different densities mean that diversity or species richness effects cannot be separated from density effects in this study. In farming practice, however, density in species mixtures often exceeds the densities of their components [45,46], but see [47]]. This is because on the one hand, an additive mixture is frequently considered to be impracticable as its density is too high and causes too much competition among plants, especially when including a large number of species in the mixture. On the other hand, a substitutive mixture may not make full use of the larger resource space available to the mixture. The relative seed rates of species in the mixture were chosen on a number of criteria including expected productivity, seed cost, and seed availability.

2.3. Replicated Field Trials

In the replicated field trials we evaluated 18 treatments. In total, twelve legume species and four grass species were each grown singly as monocultures. In addition, two treatments were reserved for the ASM, which was grown both with and without *Rhizobium* inoculation (see below). At all six trial locations, the experiments were sown in spring 2009 (Table 2). All trials were laid out as single-factor randomized complete block designs with three replications.

Following common practice, and to remove the possibility of any differences being due to lack of natural inoculum at sites, seed lots of the four clover species, *V. sativa, M. sativa* and one of the ASM treatments were inoculated with rhizobial preparations before sowing (Table 1), with 1 % (w/w) substrate per total seed weight. No suitable commercial inoculants could be obtained for the other legume species prior to sowing. The locations, plot sizes and sowing dates are listed in Table 2. Trial sites were distributed over a large geographical area within the UK. All trial sites were mown twice per year at 5-10 cm height, with the first mowing date after establishment in 2009 being between late June and early August (Table 1).

2.4. On-Farm Trials

In addition to the replicated trials, the inoculated ASM was sown by 21 organic farmers across the UK, including sites in East England, South England, North East Scotland and Wales. A further 13 sites were also included in the study, but data could not be included in the analysis because of incompleteness (e.g. sampling undertaken only in one of the two study years).

Seed of the ASM was provided for a 0.5 ha strip which was sown by the farmers next to or within a control ley (Figure 1). Most of the 21 farmers sowed the leys in spring 2009, while some delayed sowing until later in 2009 for reasons of rotational planning (Table 3). On each farm, the management for the ASM and the control ley were identical (Table 4), but ley management differed among farms. The species composition and seed rates of the control ley were chosen by each farmer individually and differed greatly in the species richness of the sown mixtures (Table 4). On 16 of the 21 farms white clover was included in the control ley.

2.5. Weed Cover Assessments

Weed and crop species were assessed for percentage cover several times during the trial duration, using 0.25 m^2 sectioned quadrats. Within the replicated trials (series I), visual cover assessments were carried out at one of the sites only (Barrington Park), by estimating percentage ground cover five times over the trial period in two quadrats per plot.

In the on-farm trials (series II), all weed and crop cover assessments were carried out with a 0.25 m^2 sectioned quadrat. On each farm, cover was assessed in four locations within each treatment, i.e. both in the ASM strip and in an adjacent strip of the control ley, resulting in eight assessment points per farm and date. Sampling locations were chosen randomly but at least 10 m were left between any two assessment points. Assessments were performed twice per farm: in 2009 several weeks after sowing (i.e. late spring in most cases) and in the following year at a similar time in the growing season. Although this method, with a relatively small total sampling area per farm and low temporal sampling frequency, did not allow us to build complete species lists for each trial area it did provide information about the most frequent weed species.

Table 4. Management details for on-farm trials.

Farm Nr.	Sowing month	Mowing*	Grazing**	Sown species in control ley***
1	April	yes	none	AC,BM,CH,WC
2	April	yes	none	RC
3	April	yes	none	AC,LU,PR,RC,WC
4	May	yes	none	AC,BM,WC
5	July	yes	none	CC,LU,RC,WC
6	April	no	S	BT,CF,MF,RG,WC
7	April	yes	none	LU
8	April	NA	S	RG,RC,WC
9	April	no	S	RG,WC
10	April	no	S	BM,BT,CF,PR,RC,WC
11	April	yes	C	AC,BM,BT,CC,CF,CH,MF, PR,RC,RG,SB,SF, WC,YW****
12	June	NA	S	BT,IR/PR,WC
13	April	no	C	PR,RC,TY,WC
14	April	no	S	PR,RC,WC
15	April	no	C	PR,TY,WC
16	May	no	S	RC,PR
17	April	yes	none	RC,PR
18	May	yes	S	WC
19	April	yes	C&S	RC,WC
20	April	yes	C	IR,RC
21	May	yes	C& S	WC

* NA: No information available

** S: Sheep, C: Cattle

*** CH: Chicory (*Cichorium intybus* L.); SB: Salad burnet (S*anguisorba minor* Scop.). RG: Ryegrass (*Lolium spec.* L.) YW: Yarrow (*Achillea millefolium* L.); other species abbreviations are the same as Table 1

**** This complex mix contained two additional species that could not be identified

2.6. Weed Identification

In most cases, weeds were identified to species level. Where this was not possible, individual plants were assigned to a species group. For example, docks (*Rumex spp.*), could not always be assigned to *R. crispus* L., *R. obtusifolius* L. or the hybrid *R. crispus* x *obtusifolius*. Therefore, all docks were summarized under *Rumex spp.* However, where differentiation was possible, *R. obtusifolius* was the most dominant taxon. Volunteer crops, such as potato (*Solanum tuberosum* L.), wheat (*Triticum aestivum* L.) and oats (*Avena sativa* L.), which were encountered in weed assessments were excluded from further data analysis.

2.7. Weed and Crop Biomass Measurements

In the replicated trials, above-ground biomass samples were taken in 2009, 2010 and 2011 on five of the six trial sites (Table 2). Quadrats for sampling biomass had a size of 0.50 x 0.50 cm and were randomly placed within plots; along the length of the plots, the outermost 1 m was avoided for sampling to minimize edge effects. Sampling quadrats were aligned diagonally in the plot. Sampling was performed on one sampling quadrat per plot (2009, 2010) or three quadrats per plot (2011). While the samples were still fresh, weeds were manually separated from crops and the weed and crop fractions were separately dried at 80 °C until sample weights were constant. The timing of sampling in 2011 was chosen to reflect the situation directly prior to incorporation of the ley into the soil.

2.8. Soil Sampling and Other Environmental Variables

Immediately prior to sowing in 2009, soil samples were taken on all trial sites, including the on-farm trials. Soil samples were collected across the field with a soil corer to a depth of 15 to 20 cm (i.e. the typical depth of ploughing in the study area) and then bulked into a single composite sample. Individual corer samples were obtained on each trial field when walking the field in a W-shape with sampling points 2 to 4 m apart.

The samples (>300 g) were air-dried and analysed at Natural Resource Management Ltd (Bracknell, UK) analytical laboratories. Samples were analysed for soil texture (percentage sand, silt and clay) using pipette sedimentation. Textural classes followed the UK Classification (Sand 2.00–0.063 mm, Silt 0.063–0.002 mm, Clay < 0.002 mm). Soil organic matter was determined using the wet oxidation Walkley Black colorimetric method. Plant available P was determined according to Olsen at 20 °C; plant available K was extracted using 1 M NH_4NO_3 and K concentration was determined by flame photometry. Available Mg was extracted using 1 M NH_4NO_3 and Mg concentration was determined using AAS.

Geographic coordinates (latitude, longitude and altitude; Table 3) of all sites were obtained from publicly available digital maps. Management data such as sowing and cutting dates were requested from the participating farmers.

2.9. Statistical Analysis

All statistical analyses were performed with the program R, version 2.14.1 [48].

2.9.1. Weed Cover in All Species Mix and in Monocultures

We compared the cover in the ASM with the average cover from all component monocultures, either weighted or not weighted by the respective seed density in the ASM. The weighted average of weed cover was calculated as follows. If s_i is the seed rate of species i (in g m^{-2}) in the ASM; and w_i is the weight per seed for species i (in g); then $n_i = s_i/w_i$ is the number of sown plants per m^2 of species i within the ASM. The relative proportion p_i of the species i in the ASM can then be defined as $p_i = n_i/\sum_i n_i$. If c_i is the weed cover in plots of crop species i (in %), the average weed cover c_w across the monocultures of all species that constitute the ASM, weighted by the proportion of species within the ASM is $c_w = \sum_i c_i p_i$, whereas the unweighted average of the weed cover is $c_u = (\sum_i c_i)/m$, where m is the total number of species in the ASM.

Proportions of individual species within the ASM (measured by the relative number of sown plants) were relatively high for white clover (0.166), large birdsfoot trefoil (0.138) and birdsfoot trefoil (0.115), and relatively low for meadow pea (0.001), sainfoin (0.014) and Italian ryegrass (0.019). The weighting by the relative seed density in the ASM was performed to account for the unequal proportions of individual species in the mixture. Specifically, assuming that the effects of individual species on weeds increases with their proportion in the mixture, the expected weed cover in the ASM (in the absence of any effects of diversity or absolute seed density) would be equal to the proportional weed cover values in all constituent monocultures, i.e. c_w. Differences in weed cover between ASM and the unweighted or weighted average of the monoculture were tested with linear mixed effects models using days after sowing as continuous random effect. Because this analysis revealed significant time x treatment interactions, treatment effects were analysed for each time separately with one-factorial analyses of variance. Block effects were non-significant in all cases of this analysis and were removed from the model. Normality of model residuals was checked with the Shapiro-Wilks test. No significant deviations from normality occurred in the weed cover data in the replicate trial.

2.9.2. Weed Cover in All Species Mix Compared to Control Ley on Farms

In the on-farm trials, weed cover data were analysed with analysis of variance to test differences between ASM and control ley. However, weed cover data from 2009 and 2010 was found to be significantly non-normal ($P < 0.001$). Since non-normality of the 2009 data could not be removed by (logarithmic) data transformation, a non-parametric sign test was applied to data of both years. This test assesses the significance of the direction of the difference between ASM and control ley. In addition, the 2010 weed cover data was log-transformed and the transformed data subjected to an analysis of variance.

2.9.3. Weed Biomass and Crop Biomass in Different Legume and Grass Monocultures

Weed biomass and crop biomass in the replicated trials was analysed in the following way. To account for strong site effects in weed and crop biomass, we first calculated for each plot the relative differences (in weed biomass and crop biomass) between individual plot data and site means, i.e. for weeds $W^{\star}_{s,b,i} = (W_{s,b.i}–W_s)/W_s 100\%$, where $W^{\star}$ is the relative difference in weed biomass from the site mean for species i at site s in block b; $W_{s,b.i}$ is the absolute weed biomass for species i at site s in block b; and W_s is the site mean of absolute weed biomass across all species and blocks.

Analogous calculations were performed for crop biomass to determine relative crop biomass as $C^{\star}_{s,b,i} = (C_{s,b.i}–C_s)/C_s 100\%$. Further, to determine the relationship between relative weed biomass $W^{\star}$ and relative crop biomass $C^{\star}$, we performed a linear regression of $W^{\star}_i$ against $C^{\star}_i$ across species; in order to avoid inflation of degrees of freedom and to account for non-independence of data within sites, values of $C^{\star}_{s,b,i}$ and $W^{\star}_{s,b,i}$ were averaged across sites and blocks for each species prior to the analysis of linear regression. In a subsequent analysis, residuals of individual species values from the linear regression function of $W^{\star}$ against $C^{\star}$ were tested for significance based on a mixed-effects model with site as a random factor, using the *lme* function in R.

To compare the various species with regard to, $K_i = W_i/(C_i + W_i)$, i.e. the proportion of weed biomass in total above-ground biomass, the data from all sites was analysed with a linear mixed-effects model with site as a random factor followed by Dunnett's test to separate means of individual species from the means of a set control species; these control species were chosen as white clover for the legume species and perennial ryegrass for the grass species, because these species had been found to be most commonly used by the organic farmers participating in the study (Table 4).

2.9.4. Change in the Proportion of Weed Biomass Over Time

The temporal change of the proportion K_{is} of weed biomass in total biomass was analysed by comparing K_{is} from the last biomass sampling date against the first date (2011 vs. 2009). For each species, the absolute difference in K_{is} between the two dates was tested for the direction and significance of change by a two-tailed t-test against zero, based on a mixed-effects model with site and block within site as random factors, using the *lme* function in R. To make comparisons among legume species, white clover was considered as a control and the difference between this species and all other legume species was tested with a multiple (many-to-one) comparisons test after Dunnett; the same test was employed to test the difference between perennial ryegrass and the other grass species.

2.9.5. Weed Floristic Similarity Between Study Years

Weed floristic similarity between the two study years, based on presence versus absence of individual species in each of the two years, was compared using Jaccard's index with confidence intervals given by Real [49]; Jaccard's index ranges from 0 (no similarity) to 1 (maximal similarity). For individual species, the change from the first to the second study year in the number of farms or quadrats on which the species was found to be present was tested for significance with χ^2 tests protected with a Bonferroni correction for multiple testing.

3. Results

3.1. Weed Cover in All Species Mix and in Monocultures

Weed cover at the Barrington Park site rose sharply in the first two months of the trial and then declined gradually over the remaining duration of the trial (Figure 2). At the two later assessments, weed cover in the ASM (c_{ASM}) was significantly lower than in the weighted average c_w of the component species. The comparison between weed cover in the ASM and the unweighted average c_u of the weed cover in the monoculture yielded similar results, with c_{ASM} being significantly lower than c_u at the last three assessment dates.

However, it was not possible to separate the weed-reducing effect of increased plant density in the ASM from effects of species richness, e.g. through increased weed suppression due to complementarity of growth habits of the component species.

In the on-farm trials, average weed cover was 10.6 % in year 1 and 5.1% in year 2. Weed cover was not significantly different between ASM and Control ley in either of the two trial years following a sign test; also, no significant difference between ASM and control ley was found for log-transformed weed cover data from 2010, following analysis of variance.

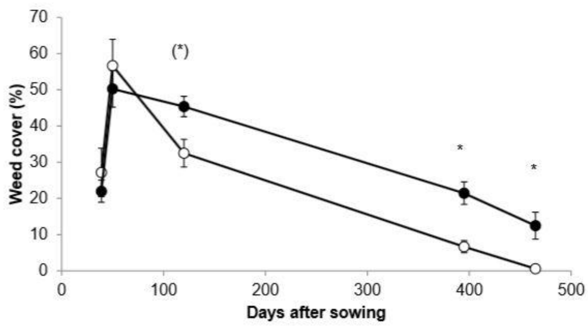

Figure 2. Development of estimated weed cover (%) over time in a complex species mixture of grasses and legumes (All Species Mix, ASM, open circles); and in the average of the ASM's component species when grown in monocultures (weighted by relative plant density in the ASM, filled circles); average over three replicates and standard errors (error bars); (*): $P < 0.1$; *: $P < 0.05$ (t-test).

3.2. Weed Suppression by Different Crop Species

The legume species with the strongest weed suppression was red clover (Figure 3). For this species, the proportion of weeds in total biomass at the first sampling was 28.3 % $\pm$ 9.9 % across sites. Averaged across all legumes, the weed proportion in total biomass at the first sampling was 56.0 % $\pm$ 7.6 %; for the grasses, this value was at 33.2% $\pm$ 7.0 %. There was a strong and highly significant negative relationship between above ground crop biomass and weed biomass across species (Figure 3; Adjusted R^2 = 0.78, $P < 0.001$, df = 16).

Interestingly, all four grass species were left of the regression line, i.e. their weed-reducing effect was higher than would be expected from their above ground crop biomass. To test the significance of deviations from the regression, a mixed-effects model with site as random factor was run, followed by a t-test on the difference between observed values and values estimated from regression line shown in Figure 3. According to this analysis, there was a significantly higher weed suppression ability in grasses than in legumes ($P < 0.001$). When individual grass species were tested, the deviation from the regression line was only significant for *F. pratensis*, ($P < 0.01$), but overall, *L. multiflorum* had the highest crop biomass and lowest weed biomass (Figure 4).

This indicates that the characteristics of species shown in Figure 3 (relative crop biomass and weed suppression) were mostly consistent over the two years of the study, since the proportions of weeds in total biomass remained largely constant over time (with the exception of Timothy grass). We observed a nearly significant ($0.05 < P < 0.1$) increase in the proportion of weed biomass over time in only two of the legume monocultures, meadow pea and white sweet clover (Figure 3). Among the grass species, the proportion of weeds in the biomass significantly decreased in Timothy grass from autumn 2009 to spring 2011 ($P < 0.01$). In most species, the proportion of weed biomass within the total above-ground biomass did not significantly change over time, i.e. the absolute temporal change in the weed proportion, over the period of autumn 2009 to spring 2011 was not significantly different from zero (Figure 3).

3.3. Weed Community Composition in On-Farm Trials

In total, 63 weed species were recorded in the leys. With a total of 56 weed species found in the first year of the ley, the species richness was twice as large as in the second year, when only 28 species were recorded. Similarly, the number of weed species per farm was higher in the first than in the second year, with 11.9 $\pm$ 1.6 and 3.8 $\pm$ 0.7 weed species per farm, respectively (average $\pm$ standard error). Floristic similarity between the two study years (2009 and 2010), as measured by Jaccard's index on species presence in either of the two years, was found to be 0.344; this was not significantly different from random similarity or dissimilarity according to confidence intervals given by Real [49]. The total number of weed species found on each farm, in both years together, ranged from 3 to 27. Weed species numbers between the first and the second years of the study were uncorrelated across farms (linear model, adjusted $R^2 = 0.08$, $P = 0.14$, $df = 16$), i.e. farms with a higher number of weed species in the first year did not necessarily tend to have a higher species number in the second year as well.

Weed species richness did not correlate with the crop species richness sampled in the ley (Adjusted $R^2 = 0.007$, $P = 0.247$), indicating that increasing the number of species within in a ley mixture does not compromise the conservation of wild farmland plants. Similarly, for both 2009 and 2010, the number of weed species was not significantly different between the ASM and the Control leys.

In the first year of the ley (2009), the most frequently encountered weed species were chickweed (*Stellaria media*), sow thistle (*Sonchus arvensis*) and field speedwell (*Veronica persica*) (Table 5). In the second year of the ley, almost all annual species decreased in frequency, i.e. the proportion of farms and of quadrats on which they were present decreased over time. Conversely, some perennial species such as dandelion (*Taraxacum officinale agg.*) and creeping thistle (*Cirsium arvense*) increased slightly but non-significantly in frequency. However, *C. arvense*, as well as the other weed species *Rumex spp.* with recognized economic relevance in organic agriculture, were relatively infrequent, being recorded in only 9 to 16 out of 168 sampling quadrats (Table 5).

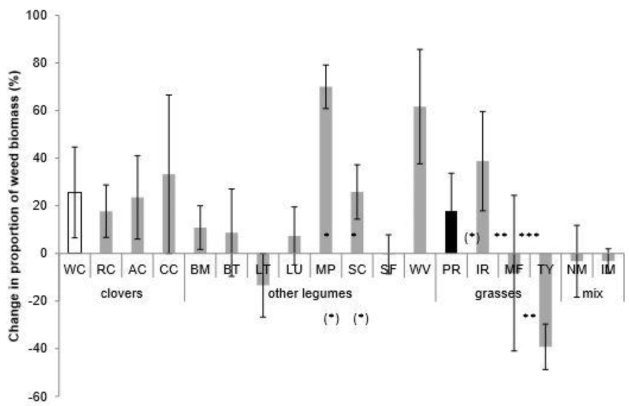

Figure 3. Proportion (in %) of weed biomass in total biomass (above ground): Absolute change from autumn 2009 to spring 2011; means and standard errors across 4 sites. Positive values mean an increase in the proportion of weed biomass in the total above ground biomass over time. Significance stars below the zero-line indicate whether this temporal change was significantly different from zero (t-test); stars above the zero-line refer to the difference between white clover (white bar) and the other legume species and or the difference between perennial ryegrass (black bar) and the other grass species (Dunnett-test). (*): P <0.1; *: $P < 0.05$; **: $P < 0.01$; ***: $P < 0.001$. For abbreviations see Table 1.

Table 5. Weed species found in year 1 and 2 of the ley on 21 organic farms: Number of farms and number of quadrats in which the weed species were present, sorted in descending order by the number of quadrats in 2009 on which the species was present; (a) species with presence on a total 10 or more sampling quadrats; (b) species with presence on a total of fewer than 10 quadrats. For individual species, the change from the first to the second study year in the number of farms or quadrats on which the species was present was tested for significance with χ^2 tests protected with a Bonferroni correction for multiple testing (***: $P <$ 0.001; **: $P <$ 0.01; *: $P <$ 0.05). No significant effect of sampling year was found for species listed in (b).

		No. farms (out of 21)		No. quadrats(out of 168)	
Presence	Species	2009	2010	2009	2010
10 or more quadrats	*Stellaria media* (L.) Vill.	15	7	82	15 ***
	Sonchus arvensis L.	10	0	38	0 ***
	Veronica persica Poiret	8	0	35	0 ***
	Persicaria maculosa L.	11	0*	33	0 ***
	Ranunculus repens L.	9	5	32	13
	Viola arvensis Murray	9	2	32	7 **
	Spergula arvensis L.	6	0	26	0 ***
	Veronica spec. L.	5	4	26	6 *
	Chenopodium album L.	8	1	23	2 **
	Poa annua L.	7	0	21	0 ***
	Lamium purpureum L.	6	2	20	3 *
	Myosotis arvensis (L.) Hill	7	1	20	1 **
	Sinapis arvensis L.	5	4	20	5
	Anagallis arvensis L.	5	0	19	0 **
	Tripleurospermum maritimum (L.) Koch	4	0	19	0 **
	Capsella bursa-pastoris (L.) Medik.	5	0	18	0 **
	Galeopsis tetrahit L.	4	0	17	0 **
	Polygonum spec. L.	4	0	16	0 **
	Rumex spec. L.	8	5	16	11
	Anthemis arvensis L.	4	2	15	7
	Convolvulus arvensis L.	5	1	15	1
	Fallopia convolvulus (L.) Löve	5	0	15	0 *
	Papaver rhoeas L.	4	2	14	4
	Polygonum aviculare L.	3	0	14	0 *
	Taraxacum officinale F.H. Wigg	5	8	13	22
	Galium aparine L.	3	1	12	1
	Cirsium arvense (L.) Scop.	5	2	9	12
	Cerastium fontanum Baumg.	2	3	8	5
	Elymus repens (L.) Gould	1	1	8	3
	Achillea millefolium L.	2	1	4	6
	Aphanes arvensis L.	1	2	3	11
Fewer than 10 quadrats	*Senecio vulgaris* L.	4	0	9	0
	Alopecurus myosuroides Huds.	3	0	9	0
	Vicia hirsuta (L.) Gray	1	0	8	0
	Geranium spec. L.	4	1	5	1
	Avena fatua L.	2	0	5	0
	Kickxia elatine (L.) Dumort.	2	0	5	0
	Plantago major L.	2	0	5	0
	Sisymbrium officinale (L.) Scop.	2	0	4	0
	Matricaria recutita L.	1	0	4	0

Table 5: *Cont.*

Presence	Species	No. farms (out of 21) 2009	2010	No. quadrats(out of 168) 2009	2010
	Brassica napus L.	1	0	3	0
	Legousia hybrida (L.) Delarbre	1	0	3	0
	Veronica arvensis L.	2	1	2	6
	Mentha arvensis L.	2	0	2	0
	Matricaria discoidea DC	2	0	2	0
	Glebionis segetum (L.) Fourr.	1	0	2	0
	Kickxia spuria (L.) Dumort.	1	0	2	0
	Aethusa cynapium L.	1	0	1	0
	Fumaria officinalis L.	1	0	1	0
	Lactuca serriola L.	1	0	1	0
	Lapsana communis L.	1	0	1	0
	Odontites vernus Dumort.	1	0	1	0
	Poa trivialis L.	1	0	1	0
	Senecio jacobaea L.	1	0	1	0
	Urtica urens L.	1	0	1	0
	Poa spec. L.	0	1	0	8
	Cirsium vulgare (Savi) Ten.	0	2	0	5
	Cichorium intybus L.	0	1	0	4
	Arabidopsis thaliana (L.) Heynh.	0	1	0	2
	Daucus carota L.	0	1	0	1
	Sherardia arvensis L.	0	1	0	1

4. Discussion

Within the context of organic rotations in Europe, this study addresses two contrasting aspects of weeds in agricultural rotations, namely weed control and weeds as constituents of farm biodiversity. It highlights, therefore, the potential conflict between agronomic and biodiversity aspects of agricultural production.

4.1. General Observations

Overall, we found total weed cover in the range of 5.1-10.6 % in the on-farm trials, which is comparable to values of total weed cover in grass/clover leys reported in a study on weeds in organic rotations in the North of England [18]. In the replicated trial at Barrington Park however, we observed much higher weed cover. It is likely that differences between these observations are due to different sampling times, since there is a large time effect on weed cover (Figure 2).

In the replicated trials, crop biomass and weed biomass were inversely related (Figure 3), confirming earlier findings [e.g. [50,51]]. Only one species deviated significantly from the regression between the two parameters relative weed biomass and relative crop biomass; meadow fescue had a lower weed biomass than would be predicted given its crop biomass (Figure 3).

This result indicates that crop productivity, measured as above-ground biomass per unit area, is an excellent indicator of competitiveness against weeds. At the same time, this relationship may to some degree suggest functional complementarity between crops and weeds. In monocultures with relatively low crop biomass, weeds filled the gap, thus resulting in relatively high weed biomass. In arable cash crops there is (almost) no complementarity between crops and weeds. In terms of yield as the primary function of the cash crop, weeds make no direct positive contribution; on the contrary, weeds limit yields through competition. Leys with their associated weeds are different in this respect. Many functions are fulfilled by both the ley crop and weed species, e.g. covering the soil and thereby protecting it from erosion, providing plant residues for building up to soil organic matter or supporting pollinators and other beneficial insects. Although some central functions of the sown ley species such as nitrogen fixation are not fulfilled by the majority of weed species, there is at least some degree of functional complementarity between crops and weeds in rotational leys.

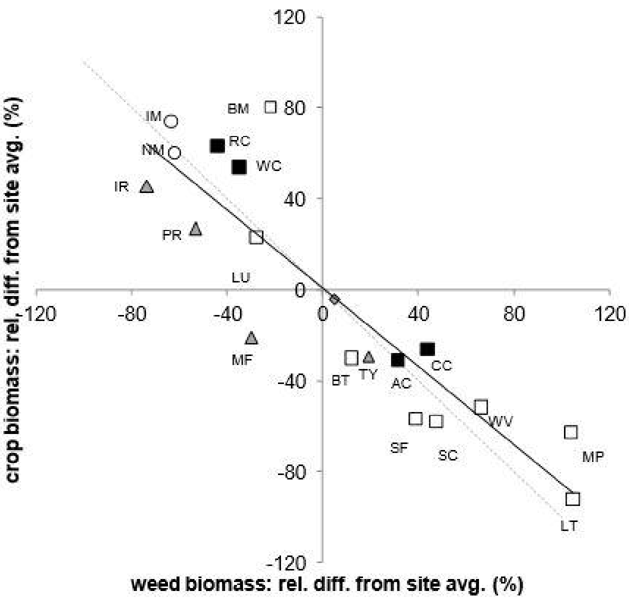

Figure 4. Relationship between weed biomass and crop biomass in early autumn 2009, both expressed as relative difference (in %) of species values from respective site averages. Filled squares: Clover species (Trifolium spec.); open squares: other legume species; grey triangles: grass species; open circles: All Species Mixtures (ASM); Grey diamond: average of monocultures (only ASM components); Black line: linear regression through all points; broken line: y = –x. Mean of five sites (all except Barrington Park). IM: Inoculated All Species Mixture; NM: Non-inoculated All Species Mixture; other abbreviations see Table 1.

Apart from this, there is a further important difference between weeds in leys and weeds in arable cash crops. In leys, the time between emergence of weeds and their destruction through mowing is typically shorter than between weed emergence and harvest of arable crops. Therefore, many annual weed species may not have completed their life cycle and set seed before the ley is cut. In fact, the first cut of organic leys is often timed before weeds have produced seed. For these reasons, we suggest that weeds can be tolerated in organic leys to a higher degree than in organic cash crops. However, it is currently unclear where the balance lies between functional complementarity and functional antagonism of sown ley species vs. weeds.

4.2. Characterisation of Individual Legume and Grass Species

In this study, we found that the proportion of weeds and crops in total above-ground biomass did not significantly change between the first and the last sampling time for most species (Figure 3); this result is unexpected because of the asymmetry of competition typically observed in plant communities [52]. With asymmetric competition it would be predicted that proportions of crops or weeds change over time, as the competition dynamics lead to shifts in the proportion of species towards the dominating species.

There may be several reasons why our observations do not support the expectations arising from asymmetric competition. First, the sampling effort may have been insufficient to detect significant effects over time. Similarly, the study period may not have been long enough for asymmetric competition to become apparent. Also, in leys, competition between crops and weeds may be reset to a certain degree with each cut and with the break in vegetative growth over winter. While spring-germinating annual weed species form a new generation each spring, most legumes tested here are perennials, but they also need to re-grow after winter, or after cutting.

In contrast to most of the ley species assessed in this study, in three species we found significant shifts over time in the proportion of weeds, namely white sweet clover and meadow pea (towards an increasing proportion of weeds), as well as timothy grass (towards an increasing proportion of the crop). In the cases of meadow pea and sweet clover, the observed increase in the proportion of weeds was likely due to poor recovery of plant growth following mowing. Large variation across sites (indicated by large standard errors) was observed for crimson clover with respect to the change of weed proportion over time (Figure 3). This species is annual but is able to re-grow from seed; here, shifts over time in the proportion of crops and weeds may reflect variation in the ability of the crop to produce a second generation.

Differences observed among species in their competitiveness against weeds may to some extent reflect the intensity of plant breeding efforts. It is indeed reasonable to assume that there is a positive feedback relationship between a species' productivity and the breeding efforts dedicated to it. For instance, both red clover and white clover, in this study found to be the two species with the strongest weed suppression (Figure 4), have received much more attention from breeders than the other legume species trialled here, which can be interpreted both as a reason for and a consequence of the relatively high productivity of white and red clover. Further, this study found that grasses outperformed legumes in terms of weed suppression, which is in line with earlier findings on the smaller weed suppression abilities of legumes in comparison to grasses [e.g. [13,15].

The analysis of the individual legume species also shows that there is a degree of redundancy in the ASM, where some species (such as meadow pea) perform too poorly to warrant an inclusion in ley mixtures. Thus, mixtures with fewer species, but with complementary functions, may optimise weed management (and crop performance) in leys. This has been supported by analyses of potential mixtures with different numbers of the species trialled in this study [43].

4.3. Weed Communities in On-Farm Trials

This study suggests that several weed species are dominant in organically managed leys typical in the UK and that weed species richness may be higher than previously reported

[16]. With the dominating *Stellaria media*, *Sonchus arvensis* and *Veronica persica* we found species that are common and typical annual weeds of arable fields in the UK and throughout Western Europe. With their short life cycles they are adapted to high-disturbance regimes. With an average value of 11.9, the number of weed species encountered per farm was slightly greater than in a single-site study investigating the effects of rotations on weeds, where 9 weed species were recorded from a grass/clover ley [18].

Further, our results showed that annual weed species typical for arable fields were dominant in the year of establishment of the ley. In terms of weed communities the start of the ley phase is thus similar to those found in arable crops. On some sites, the ley was, in fact, undersown into cereals. Further, the weed community changed considerably in the second year, towards perennial and grassland species, most probably owing to the cessation of tillage and the repeated cutting, mulching or grazing. This change in community composition from annual to perennial species following the changes in land managed is typical and has been observed in several other studies [e.g. [13,53]].

However, as pointed out in the Methods section, the sampling strategy for the weed species in the on-farm trials was not designed to generate an exhaustive picture of the weed flora in organic fertility building leys. In particular, because of spatial aggregation in weeds [54], the number of quadrats for sampling in on-farm trials was likely too small to reliably detect all species present on the leys. Therefore, it is likely that the data obtained for species richness on the organic leys underestimate the actual weed species richness [cf. [21]]. Similarly, the actual frequency of species on the farms, i.e. the proportion of farms on which a given species is present, is likely to be higher than measured with our sampling method. Further, the methods applied here do not allow us to build a picture of the weed species present in the seed bank. Finally, it is not known to which degree the ley management, e.g. cutting vs. grazing, had an impact on weed communities but this aspect was outside the scope of this study.

4.4. Ley Species Mixtures and Weeds

Compared to the average of monocultures, the ASM was found to have significantly lower weed cover (Figure 2), and ranked among the best performers with regard to both crop biomass and weed biomass (Figure 3). However, these effects cannot be ascribed to the mixing of species, since diversity effects and density were confounded in this study. Seed density in the ASM was 53.4 % higher than the average seed density of all component monocultures. In the on-farm trials, ASM was not significantly better at controlling weeds than the control leys. However, sowing rates for the control leys were not recorded. It remains therefore speculative whether differences in seed densities between ASM and control leys might be a reason for the observed results.

Generally, there is evidence that mixing species does help to control weeds, especially when crops are functionally diverse [55]. A study on weeds in short-term grassland showed weed suppression to be higher in mixtures than in monocultures [56]. Weed suppression in annual species mixtures has also been found to be better than in monocultures [46,57,58]. Further, because of functional complementarity among different sown species, seed densities in multi-species mixtures may generally be increased above the sowing rates used in respective monocultures or simpler mixtures with a lesser degree of complementarity. Thus, higher plant densities – made possible by mixing multiple species – may then be used as a tool to suppress weeds [59]. At the same time, further research is necessary to separate species richness effects on weeds from the impact of plant density in leys.

Our on-farm trials show that weed species richness as a component of farm biodiversity is not significantly reduced when including more crop species in the ley, in contrast to earlier findings [60]. Weed species richness in the ley is more likely to be influenced by the history and landscape features [61] of any particular site. In the first year of establishment, leys may be seen to provide a suitable habitat for arable weeds. For the later stages of the ley, whilst annual weed species decline, the challenge remains to control perennial weeds such as creeping thistle (*Cirsium arvense*) and docks (*Rumex spp.*). However, we speculate that these species are again likely to be mostly influenced by site history (e.g. tillage [62]) and to be relatively unaffected by the choice of species in a ley mixture.

5. Conclusions

In the past, the question of what organic agriculture contributes to the conservation of farmland biodiversity has been researched extensively [63], showing biodiversity benefits of organic farming in comparison with conventional farming [64,65]. In this debate, little attention has so far been paid to organic leys, despite legume based leys being an essential feature of many organic systems, in particular in Europe. No direct comparison is therefore possible with conventional agriculture, because typically there is no ley phase in current conventional rotations [e.g. [66,67]].

Organic leys add to the diversity on farms by including a range of crop species that are otherwise not cultivated. This study has shown that organic leys harbour a range of wild plant species that further contribute to species richness on the farm. Recent evidence shows that young leys (< 1.5 years old) provide a better habitat for spiders than cereal fields [68]. Leys therefore constitute an important element of farm biodiversity.

As we have demonstrated, the choice of species in organic leys can be used to optimise weed control. It remains open to determine to which degree the ecological functions provided by weeds may be fulfilled by designing targeted crop mixtures, i.e. by replacing weeds with crops while maintaining their ecological functions. However, it is unlikely that effective protection of rare weed species can be achieved through ley design only. Further research is needed to show how leys can be optimized for multifunctional performance.

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Research Article

The Use of Copper Pesticides in Germany and the Search for Minimization and Replacement Strategies

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Abstract: Copper pesticides used to control fungal and bacterial diseases such as grapes downy mildew (*Plasmopara viticola*), downy mildew of hops (*Pseudoperonospora humili*), apple scab (*Venturia* spp.), fireblight (*Erwinia amylovora*) and potato late blight (*Phytophthora infestans*), play an important role in plant protection. In a 2013 survey of copper application in Germany we found, that while the amounts of copper used per hectare in conventional grape (0.8 kg ha^{-1}), hop (1.7 kg ha^{-1}) and potato-farming (0.8 kg ha^{-1}) were well below those used in organic farming (2.3, 2.6 and 1.4 kg ha^{-1}, respectively), they were nearly identical to those used in apple growing (1.4 kg ha^{-1}). Due to the smaller farming area, only 24% (26.5 tonnes) of the total amount of copper was applied in organic farming compared to 76% (84.8 tonnes) in conventional farming. Since 2001, the Federal Agency for Agriculture and Food (BLE) promoted a copper research and minimization strategy which was funded with a total of €10.2 million. Our status quo analysis of research in this field shows that some progress is being made concerning alternative compounds, resistant varieties and decision support systems. However, it also shows that new approaches are not yet able to replace copper pesticides completely, especially in organic farming. In integrated pest management, copper preparations are important for the necessary active substance rotation and successful resistance management. The availability of such products is often essential for organic grapes, hops and fruit production and for extending the organic farming of these crops. We conclude that the complete elimination of copper pesticides is not yet practicable in organic farming as the production of several organic crops would become unprofitable and may lead to organic farmers reverting to conventional production. Several existing copper reduction strategies were, however, identified, and some, like modified forecast models adapted to organic farming, varieties more resistant to fungal diseases and new alternative products, already contribute to copper minimization in German agriculture.

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Keywords: application rate; copper; research program; sales volumes

1. Introduction

Copper pesticides have been used in Germany for almost 150 years, controlling plant diseases such as downy mildew of grapes (*Plasmopara viticola*) and hops (*Pseudoperonospora humuli*), apple scab (*Venturia spp.*), fire blight (*Erwinia amylovora*) and potato blight (*Phytophthora infestans*). This makes them some of the oldest plant protection products (PPPs) relevant today. Until well into the last century, application rates of 20 to 30 kilograms per hectare per year (kg ha^{-1} yr^{-1}), and occasionally even 80 or more kg ha^{-1} yr^{-1}, of copper pesticides were used in conventional farming in Germany [1].

Soil persistence and the effects on soil organisms are discussed, nationally and internationally, as possible impacts from years of copper pesticide use. Strumpf et al. [2,3] conducted extensive surveys on copper pollution of soils in organic and conventional grapes, hops and tree fruit-growing in Germany. They later performed a risk assessment of soil copper levels based on bioavailable copper instead of total copper, the previous standard [4]. Research has shown that less than 10% of the total copper in soil is easily mobilized [5], and that not only the total copper content, but also the texture [% sand content] and pH of the soil, are significant factors influencing copper mobilization.

The European Commission extended its approval for the use of copper compounds as fungicides/bactericides until January 31, 2018. However, this was done on the condition that appropriate measures are taken to reduce usage. In as early as 2009, Germany and some other EU Member States already passed resolutions to substantially reduce the maximum limits permitted for pure copper pesticides. Instead of the 6 kg ha^{-1} year limit permitted by EU regulations, Germany has a limit of 3 kg ha^{-1} yr^{-1} and 4 kg ha^{-1} year in hops. Under the aegis of the German Federation of the Organic Food Industry (BÖLW), German organic farming and integrated pest management associations, in coordination with the competent authorities, developed a targeted copper minimization strategy that aims to reduce the annual net amount of copper used in crop protection per hectare and year, even further [6].

This article provides a review of the use of copper pesticides in crop protection in Germany since 2010 by crop and farming method (conventional or organic). Previous studies on the reduction of the use of copper as a pesticide in Germany are also examined. The tested strategies are analyzed in terms of their efficacy and success, and limitations of the previous copper reduction strategies are elucidated.

2. Materials and Methods

2.1. Status Quo Analysis of the Use of Copper Pesticides in German Agriculture and Horticulture

Article 64 of the German Plant Protection Act requires that manufacturers, distributors and importers of plant protection products report to the BVL (Federal Office of Consumer Protection and Food Safety) with their annual domestic sales of such products and the active substances contained in them by amount. The BVL kindly provided the statistics on the sales of copper pesticides in Germany from 2010 to 2014.

Julius Kühn Institute (JKI) has regularly conducted surveys on the use of chemical plant protection products in the main agricultural and horticultural crops in Germany since 2000. They have been continued as “PAPA surveys”, categorized under a different legislative framework since 2011 [7]. PAPA is an acronym for Panel Pesticide Applications. Networks of crop-specific survey farms that gather and report detailed annual data on pesticide use were established under the PAPA program. The selected crops (winter wheat, winter barley, winter rye, corn, potatoes, sugar beet, dessert apples, hops and grapes) are those considered most relevant for the National Action Plan on Sustainable Use of pesticides [8]. Data on the use of copper pesticides can also be gathered from these surveys. It should be noted that the PAPA surveys only collect data from conventional farms; German organic farming associations collect the corresponding data on organic farming. This is a component of the JKI’s copper pesticide reduction strategy paper with specific consideration of organic farming [6].

2.2. Assessment of the Status Quo of Research on the Minimization and Replacement of Copper Pesticides in Germany

Since 2001, the Federal Program for Organic Farming and Other Forms of Sustainable Agriculture (BÖLN) has funded research projects aiming to contribute to the reduction of copper used in plant protection. The results and resource needs of these projects are summarized below. These measures were supported by the European CO-FREE Project (Innovative strategies for copper-free, low-input and organic farming systems, 2012-2016; funding budget: 3 million euros), in which eleven European partners collaborated to find alternatives to copper. The results of the CO-FREE project were not included in this analysis because the final reports were not yet available. The individual projects funded by BÖLN were examined prior to our literature search. Relevant projects were filter-searched on the Federal Program website (https://www.bundesprogramm.de/index.php?id=916, Accessed on 14 September 2016). A total of 67 projects involving research on copper reduction were identified using the keyword "copper" to search the list of "Research and Development Projects" under the heading "Crop". A comparative analysis of these projects was then performed (e.g. aim of the projects, effective ingredients, efficacy, costs).

3. Results

3.1. Status Quo of the Use of Copper Pesticides in German Agriculture and Horticulture

The estimated amounts [kg ha^{-1}] of copper used in conventional farming in Germany in 2003 are shown in Table 1. The amounts used for conventional farming of potatoes, hops and grapes were significantly lower than those used in organic farming. The copper application rates were less than 1 kg ha^{-1} yr^{-1} in potato and grape-growing, and approximately 1.7 kg ha^{-1} yr^{-1} in hop-growing. Conversely, the amounts of copper used for apple-growing were almost equal in organic farming (average of 1.41 kg ha^{-1} yr^{-1} in 2010 to 2013) and conventional farming (1.4 kg ha^{-1} yr^{-1} in 2013). However, comparison of the total amounts of copper used in both farming systems (Tables 1 and 2) showed that, when adjusted for differences in the sizes of application areas, only 24% (26.5 metric ton, t) of the total amount of copper was used in organic farming compared to 76% (84.8 t) in conventional farming.

Table 1. Estimated amounts of copper used [pure copper in kg ha^{-1}] in conventional farming in Germany in 2013 relative to the application area.

	Potatoes	Apple	Grapes	Hops	Total
Application area [ha]	2,500	25,500	36,800	10,400	75,200
Copper spray rate [kg ha^{-1}]	0.8	1.4	0.8	1.7	-
Pure copper total [t]	2	35.7	29.4	17.7	**84.8**

Table 2. Estimated amounts of copper used [pure copper in kg ha^{-1}] in organic farming in Germany in 2013 relative to the application area.

	Potatoes	Apple	Grapes	Hops	Vege- tables	Total
Application area [ha]	3,500	2,100	7,700	84	400	13,784
Copper spray rate [kg ha^{-1}]	1.38	1.5	2.29	2.6	2	-
Pure copper total [t]	4.8	3.1	17.6	0.2	0.8	**26.5**

Table 4 lists only those crops in which copper pesticides were used in integrated pest management. They were not used in any arable crops except potato. Table 4 clearly demonstrates how copper oxychloride (trade name: Funguran), which was initially the most prevalent active ingredient, was replaced by copper hydroxide (trade names: Cuprozin Liquid, Cuprozin Progress, Funguran Progress, and Kocide OPTI) over the analyzed time period. The applied quantities of copper oxychloride decreased from 163.7 t in 2011 to 1.8 t in 2014 (Table 5). The reverse was observed for copper hydroxide. The application rate of this active substance increased approximately three-fold, from 45.6 t (2011) to 132.6 t (2014). Use of the other two copper- containing active ingredients (copper sulfate and copper octanoate) was marginal. Because basic copper sulfate (trade name: Cuproxat) is only allowed in grape- growing and does not play a significant role, at least in conventional agriculture, it was not included in the table. Copper octanoate (trade name: Cueva) is allowed in potatoes, apples, grapes and ornamental plants, but is only used in viticulture in conventional farming.

In organic farming, the application of copper-containing pesticides is based on forecast model predictions and not always on the total cultivated area (except in hop- growing). This comprises over 90% of the total organic farming area. Table 3 shows the amounts of copper used in organic viticulture in recent years. The differences in copper application rates between the different growing regions are sometimes substantial. This can be attributed to regional differences in climatic conditions and weather profiles between the different growing regions. A regional analysis was performed on part of the collected data (Figure 1). Comparison showed that the lowest amounts of copper are used in the Ahr grape-growing region [9].

Table 3. Average copper application rates [pure copper kg ha^{-1}] in organic grape-growing in 2010 to 2013 based on the size of the treated vine area. Copper pesticides were only applied to approximately 90% of the total cultivated area.

Grapes	2010	2011	2012	2013
Total area [ha]	5,200	6,900	7,400	7,800
Analyzed area [ha]	1,894	2,260	2,408	2,868
Copper application rate [kg ha^{-1}]	2.23	1.98	2.34	2.29

The highest amounts of copper were used in organic hop-growing due to the high amount of foliage per unit area. Application rates in hops exceeded 3 kg ha^{-1} yr^{-1} in four out of six years (Table 6). The small scale of organic cultivation of this crop (max.85 ha in 2015) should be noted.

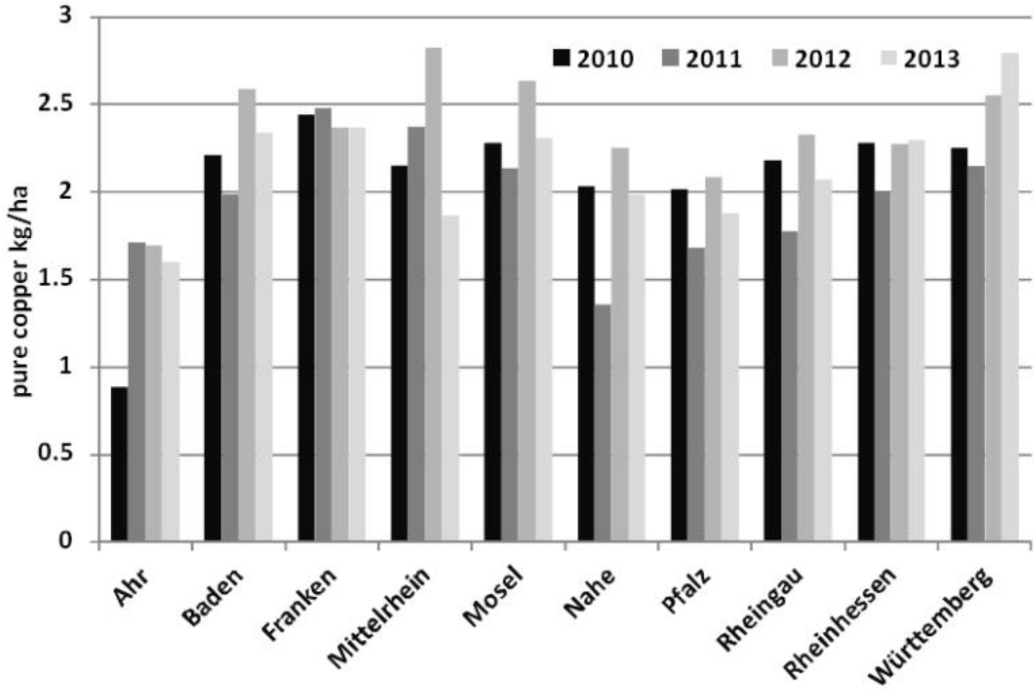

Figure 1. Average copper application rates [pure copper kg ha^{-1}] in organic viticulture by growing region in Germany from 2010 to 2013.

Table 4. Size of the area treated with copper-containing active substances in conventional farming [% cultivated land]

Active substance	Copper oxychloride			Copper hydroxide				Copper octanoate
Year	Apple	Wine	Hops	Potato	Apple	Wine	Hops	Wine
2011	61.6	16.7	77.0	1.8	37.4	21.9	0.0	1.0
2012	51.0	21.3	42.3	0.0	44.4	28.9	28.9	1.1
2013	32.3	9.8	18.6	7.0	67.1	39.0	35.5	1.1
2014	2.9	0.0	0.0	2.6	90.8	48.1	68.2	0.2

Table 5. Estimated crop-specific amounts [t] of copper-containing active substances used in conventional farming from 2011 to 2014.

Year	Copper oxychloride			Copper hydroxide				Copper octanoate	Total
Year	Apple	Wine	Hops	Potato	Apple	Wine	Hops	Wine	
2011	57.0	35.0	71.7	0.4	35.9	9.3		1.6	210.9
2012	45.7	50.3	33.5		36.9	20.4	8.5	1.1	196.4
2013	25.1	15.8	13.6	1.2	42.1	36.0	10.6	1.1	145.5
2014	1.8	n.s.	n.s.	2.9	52.8	48.9	28.0	0.1	134.5

n.s.: not specified

Table 6. Average copper application rates [pure copper kg ha^{-1}] in organic hop-growing in 2010 to 2015. Copper pesticides were applied to 100% of the hops area.

Hops	2010	2011	2012	2013	2014	2015
Total area [ha]	76	81	84	84	80	85
Copper application rate [kg ha^{-1}]	3.9	3.7	3.6	2.6	3.3	1.5

The copper application rates in organic fruit-growing on the other hand, were less than 2 kg ha^{-1} yr^{-1}. A general decrease in the amount of copper used in apples, peaches, pears and stone fruit occurred over the observation period (Table 7).

Regarding potatoes, farms associated with Demeter Association do not use copper in potato and vegetable-growing and sometimes accept the risk of considerable yield losses, alternatively they switch to low-infestation regions to protect critical crops [10]. The copper application rates in organic potato-growing were below 2 kg ha^{-1} yr^{-1} (Table 8).

In organic vegetable-growing, a substantial percentage of farming area is managed in accordance with the minimum limits of the EU organic farming regulations alone, and the German farming associations have not yet recorded the copper application rates there. The average copper application rates reported in Table 9 are, therefore, based on reports from only two organic farming associations, Bioland and Naturland. Pumpkin was the sole crop in which copper products were used in all four years studied. In cucumber, fennel, leek and ornamentals, copper was used in only one out of 4 years.

3.2. Projects Funded with Public Funds (Federal Organic Farming Scheme and Other Forms of Sustainable Agriculture – BÖLN)

Since its establishment in 2001 to 2015, BÖLN has funded a total of 67 projects for research on copper replacement and minimization. The BÖLN Federal Organic Farming Scheme has awarded a total of 10.2 million euros in grants (Table 10).

Table 7. Average copper application rates [pure copper kg ha^{-1}] in organic fruit-growing in 2010 to 2013.

	2010	2011	2012	2013
Total area [ha]	3400	3700	3900	3900
Apple	1.59	1.3	1.31	1.47
Pear	1.49	1.1	1.26	1.07
Peach	2.21	1.9	2.0	1.7
Stone fruit	1.28	0.94	0.99	0.83

Table 8. Average copper application rates [kg ha^{-1}] in organic potato-growing in 2010 to 2013 based on the size of the application area. Copper pesticides were applied to only about 40 to 50% of the cultivated area in organic farming. Demeter Association member farms are not permitted to use copper products.

Potatoes	2010	2011	2012	2013
Total area [ha]	8200	8300	8000	8100
Copper application rate [kg ha^{-1}] on treated areas	1.36	1.60	1.87	1.38

Table 9. Average copper application rates [pure copper kg ha^{-1}] in organic vegetable-growing in 2010 to 2013 based on the size of the application area. Copper products are used in only about 2 to 4% of the total cultivated area of the Bioland and Naturland farms.

Crop	2010	2011	2012	2013
Total area [ha]	10,590	10,890	10,470	10,470
Celery	2.32	1.1	0.85	-
Pumpkin	1.80	2.1	1.4	0.36
Cucumber	1.43	-	-	-
Fennel	-	0.9	-	-
Leek	-	3.0	-	-
Asparagus	-	2.0	1.2	1.09
Greenhouse crops	-	1.4	1.3	-
Ornamental crops	-	-	0.1	-
Other vegetables*	-	1.8	1.1	-

* Outdoor vegetable crops produced on small scale

The number of projects supported varies by crop type, in some cases greatly. Most of the projects carried out involved crops that are highly dependent on the use of copper, e.g., fruit and grapes. Although relatively large amounts of copper pesticide are regularly used in hop-growing, only two projects funded so far involved this crop. Projects that were not unique to a given crop type or which contributed to the minimization of copper indirectly were included in the subcategory "Others".

3.2.1. Number of Alternative Products Tested and Their Effects in the Field

At least 278 alternative products were field-tested alone or combined (Figure 2). At least 56 and 182 of these were tested in the crops "grapes" and "fruit", respectively. In other crops, a smaller number of projects and a larger number of variety and field tests were conducted.

At least 90 of the tested products showed a significant effect on the comparison variant in these trials. In some cases, however, data was from only one-year trials, thus requiring further investigation.

Table 10. Amount of funding from the "Federal Organic Farming Scheme and other forms of sustainable agriculture" (BÖLN) for research on the minimization and replacement of copper pesticides in Germany (from 2001 to 2015) by crop type.

Project Status	Grapes	Fruit	Vegetables	Hops	Potatoes	Others	Total
Finished	11	12	10	2	8	6	48
Ongoing	3	14	-	-	-	1	18
Total	14	26	10	2	8	7	67
Public funds in €1000	2,394	2,937	1,923	255	1,593	1,122	10,225

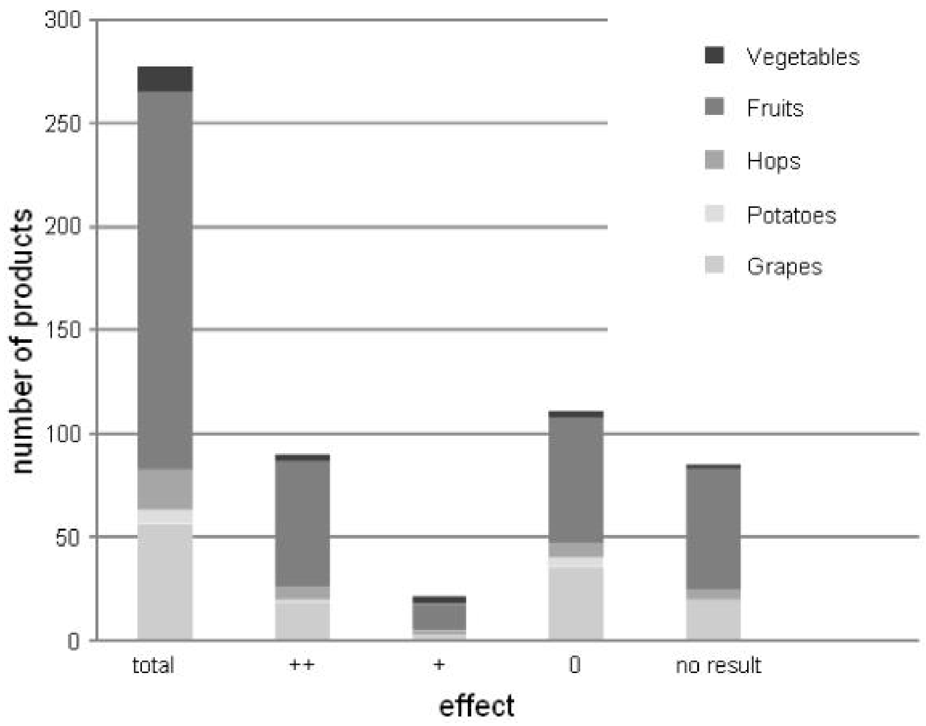

Figure 2. Number of copper alternative/replacement products field-tested under the BÖLN program and their effects in different crops. Symbols: ++ (significant effect), + (effective in the laboratory/greenhouse but not in the field), 0 (no effect).

In most cases, the copper alternatives were first tested in the laboratory and/or greenhouse (especially in fruit-growing) prior to field testing. They were only tested in the field if they showed good efficacy in the indoor setting. However, some substances were employed based on practical experience or data from literature. Consequently, some of the products demonstrated efficacy only under certain conditions, e.g., very good effects in the laboratory or good effects in the greenhouse but not in the field. A lack of rain resistance or UV resistance was frequently the presumed reason for these discrepancies. This was the case for 21 products.

At least 106 of the tested products showed no effect in the field. At least 61 of these alternative products had been tested in fruit-growing. No data could be collected on 85 products for various reasons; for example, in one case, a product was to be tested on fungal diseases, which did not develop during the observation period. In other cases, the trials have not yet been completed, so no conclusions regarding the efficacy of the products can be made.

3.2.2. Copper products tested at reduced application rates

In addition to alternative substances, 139 copper compounds were tested under field conditions in reduced application rates, new formulations or combinations (Figure 3).

Potato was the crop on which most (n = 60) of these copper compounds were tested. Improved spray technology and forecasting models were developed in these projects. In total, 21 and 38 products, respectively, were tested in the crops "grapes" and "fruit".

105 field trials showed significant effects of products tested at reduced copper application rates (Figure 3), including all products tested in hops, grapes and vegetable-

growing. Only two products tested in fruit-growing showed no effect. Problems such as difficulty producing a homogeneous spray mixture or clogging of the spray nozzles also led to the failure of sprayed products.

3.2.3. Other Project Funding

As well as finding alternative products to replace copper, other elements of plant protection were investigated, e.g., the development of more resistant crop varieties, better spraying techniques, and improved forecasting models (Table 11).

Brief descriptions of the projects by crop are presented below.

Potato: Several projects were funded in potato-growing: Two dealt with development and implementation of the ECO SIMPHYT forecast model, which contributes directly to copper minimization because it can, among other things, give farmers precise spraying date recommendations. Another two projects dealt with the use of preventative measures to control late blight (*P. infestans*) so that less copper can be applied. Two more projects investigated new resistant varieties and one, an improved spraying technique (lower leaf spraying) which may contribute to reducing the copper application rate.

Grapes: In contrast to potato-growing, the breeding of resistant varieties was a main focus of research in viticulture (Table 11). A total of five projects were funded. Two projects investigated the resistance of old grape varieties or the combination of different fungus-resistant grape varieties (PIWI), among other things. Three projects were performed for additional research into the biology of grapevine downy mildew (*Plasmopara viticola*). A reduced copper application rate of 2 kg ha^{-1} yr^{-1} is sufficient when Peronospora infestation levels are low. The efficacy of algae extracts and clays can be quite satisfactory under these conditions.

Fruit: Projects in fruit-growing involved research on fall foliage reduction [11–14]. This is important because over winter, leaves with apple scab fall and re-infect trees in spring. As in viticulture, research into the biology of various pathogens (mainly apple scab) in fruit-growing was also funded [15]. All the results from these trials aim to feed into forecasting models and software for application intensity and risk assessment of copper pesticides used by farmers [16,17]. Variety trials and two improved spraying technique trials were also conducted [18].

Vegetables: Variety selection trials were performed and cultivation methods were tested in all six projects performed in vegetable-growing. Hot water treatment of seeds successfully reduced carrot leaf blight (*Alternaria dauci*) in one study [19]. Moreover, a promising licorice-based product (*Glycyrrhiza glabra*) was developed to control fungal diseases in cucumber, tomato and potatoes [20].

Hops: The research projects performed in hops tested the ability of alternatives to copper and sulfur-containing pesticides as well as copper combinations to reduce the copper application rate. Unfortunately, none of the investigated products satisfied the requirements.

Inability to satisfactorily control primary infection of hop downy mildew (*Pseudoperonospora humuli*) was the fundamental problem. Preventive application was only able to prevent secondary infection. The use of quassia to control the hop aphid achieved reasonable results [21].

Table 11. Strategies for reducing copper application assessed within German research projects since 2001.

Strategies	**Number of Projects**						
	Grapes	Hops	Fruit	Vegetables	Potatoes	Others	Total
Alternative compounds	5	1	7 (+4*)	4	1	2	20 (+4)
Varieties	5	-	1	6	2	-	14
Decision support systems	-	-	4	-	2	-	6
Pest biology	3	-	2	-	-	-	5
Application technique	-	-	2	-	1	-	3
Others: Communication, prevention (e.g., fall foliage)	-	-	5	-	2	3	10

* Ended in 12/2016

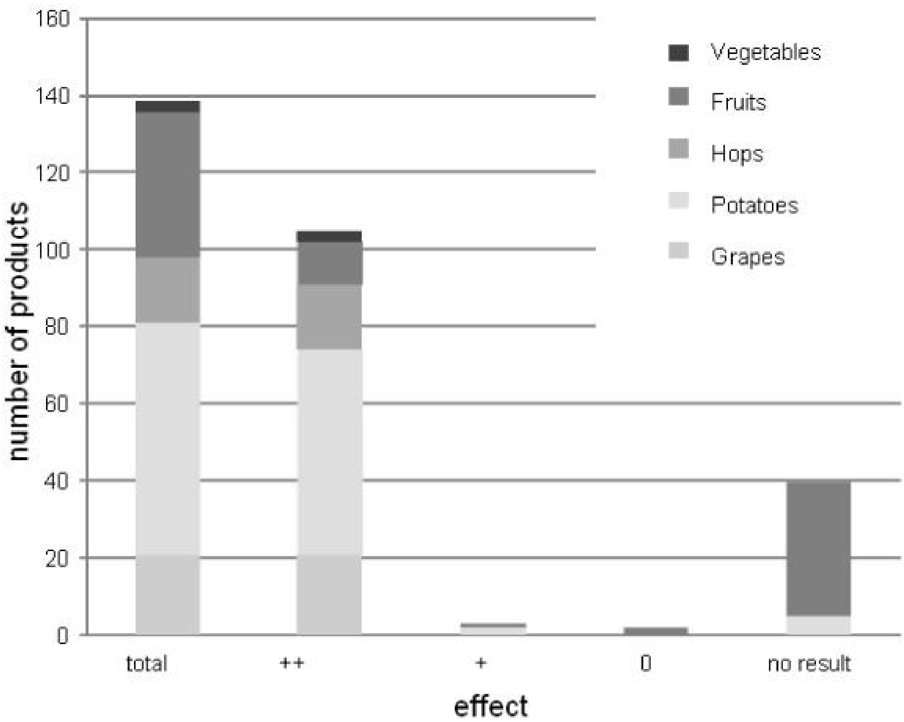

Figure 3. Number of copper products field-tested under the BÖLN program and their effects in different crops. Symbols: ++ (significant effect), + (effective in the laboratory/greenhouse but not in the field), 0 (no effect).

4. Discussion

4.1. Use of Copper Pesticides in Integrated Pest Management

The use of copper-containing pesticides in German grapes and hop growing is focused on the last application of the year (in August) in order to control downy mildew of grapes and hops. This application, therefore, has an important key function in terms of the necessary active ingredient rotation and successful resistance management. It should be noted that the copper application rates used for the final treatment in integrated viticulture are substantially higher than those normally used in organic viticulture. To date, no cases of resistance to copper-containing pesticides have been reported- since the first use of copper-containing substances in plant protection about 150 years ago. This underscores the importance of copper pesticides for crop protection as, until now, no other plant protection product has shown such a long duration of effect. In fruit-growing, copper pesticides are mainly used in the winter months (December to March) to control apple canker (*Nectria galligena*), and in March to control bark scab. The current use of copper-containing pesticides in conventional potato-growing is negligible.

5. Use of Copper Pesticides in Organic Farming

The collaboration between organic farms, researchers and medium-sized pesticide companies to promote copper reduction in recent years has led to further reduction of the amounts of copper used in various crops. Key factors that have contributed to successful copper reduction include the development of forecasting models that accurately determine the need for and timing of pesticide application [22], the implementation of agronomic and technical measures [11] and the selection of less susceptible varieties [23]. The use of alternative natural pesticides and plant strengtheners [20,24] must be incorporated in an overall strategy. Then, less effective products may also be useful components of copper minimization under certain conditions (e.g., weather, timing of application). Despite these advances, it is still neither possible nor advisable to completely refrain from using copper pesticides in organic farming.

Organic viticulture and hop-growing are particularly dependent on the availability of copper pesticides. After failure to get potassium phosphonate included in Annex II of Regulation 889/2008, no effective alternative products for these crops appear to be on the horizon any time soon. Furthermore,the willingness of conventional grape growers to change to organic farming depends largely on the availability of options for effective control of downy mildew of grapes (*Plasmopara viticola*) and grape black rot (*Guignardia bidwellii*).

In organic hops production, the application rates of copper pesticides used to reduce hop downy mildew (*Pseudoperonospora humuli*) can even be reduced to less than 3 kg ha^{-1} yr^{-1} (pure copper) during years of low disease pressure. However, rates of up to 4 kg ha^{-1} yr^{-1} may still be needed in years with high disease pressure, meaning that a high flexibility of copper application is necessary in this crop [25]. If copper-containing pesticides were banned, it is feared that organic hop farms would go out of business because equivalent alternatives are still lacking [25].

Regarding the control of apple scab (*Venturia inaequalis*) in organic orchards, improvements have already been developed and tested in the context of copper minimization strategy in the following areas: cultivation methods [12], fall foliage management [13], the use of resistant varieties [15], and the possible use of alternative products [14]. The search for new products for organic fruit production is focused on modular strategies designed to ensure that copper use is reduced while achieving the same or even better plant health [12].

Potato late blight (*Phytophthora infestans*) can cause severe economic losses in organic potato production [25]. In times of high infestation pressure and adverse weather conditions, the reliability of effect of the alternative products tested so far is still too low for them to be equivalent substitutes for copper-containing pesticides [26]. Forecasting models for late blight were adjusted to the conditions of organic farming and to the comparatively lower nutrient levels in the soil. This led to a reduction of copper applications [21,27]. Wilbois et al. [28] predict that the trend towards copper reduction will continue in the future, and that the level of copper application reached in about 10 to 15 years will correspond to the natural uptake of copper as a plant nutrient.

In vegetable-growing, copper pesticides are only relevant in a few crops and are usually applied at rates of less than 2 kg ha^{-1} yr^{-1}. The control of downy mildew in lettuce and cucumber is a major focus. Resistant varieties [29] and new licorice preparations were successfully tested [19,30,31]. Effective alternative pesticides for these applications can be expected in the future.

5.1. Research Funding to Promote Copper Reduction in Crop Protection

Research funding to promote copper reduction in German agriculture has focused on the following fields of study:

- Further development of forecast models
- Development of resistant varieties
- Improved spraying techniques
- Improved cultivation techniques
- Introduction of new copper products with low copper concentrations
- Development and introduction of copper-free alternatives
- Implementation and optimization of overall plant protection strategies
- Improvement of copper pesticide impact assessment

Our status quo analysis clearly showed that German research on copper reduction and replacement is based on close collaboration between research, practice, advisory services and industry, and needs to be continued and further developed.

At present, it is still not possible to completely refrain from using copper pesticides in organic farming. Emerging diseases, such as grape black rot (*Guignardia bidwellii*), can result in partial to complete yield loss [32]. The combination of copper- containing pesticides with sulfur-based products is currently the only effective way to combat grape black rot in organic viticulture [32]. It should be noted that research funding in Germany is accompanied by other measures that contribute to successful copper minimization. This includes the advancement of Strategy Paper on Copper Reduction in Plant Protection [6] and the annual conferences on the theme of “Copper as a Pesticide”, which are jointly organized by the German Federation of the Organic Food Industry (BÖLW) and the Julius Kühn Institute. These are important contributions to continuously documenting the progress made, and measures needed, to achieve copper reduction.

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