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Evaluating nitrogen fertilization strategies to optimize yield and grain nitrogen content in top winter wheat varieties across Switzerland

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ABSTRACT

Winter wheat (*Triticum aestivum)* is one of Switzerland's most important field crops and requires large quantities of nitrogen (N) to produce a high yielding and high-quality crop. However, N is an expensive input for producers that is prone to environmental losses, thus causing pollution and environmental degradation. Furthermore, the N use efficiency (NUE) of wheat remains low. Our goals were to test whether different N fertilizer splits could maximize grain yield and grain N content (a proxy for grain quality) while improving nitrogen use efficiency, identify key variables in determining yield and N grain content, and to determine if variety- and site-specific fertilization recommendations provide additional benefits to nutrient use efficiency. We designed a four-year study across three sites in Switzerland that incorporated five top Swiss varieties of winter wheat and six different N application regimes applied at BBCH 21, 31, and/or 39 that totaled either 0, 80, or 160 kg N ha^{-1}. In addition to mixed model analyses, we used random forest to identify key genetic, environmental, and management variables in determining wheat grain yield and grain N content. Grain N content and yield were found to be maximized at 8000 kg ha^{-1} and ≈ 2.24% N. Further, more N is required to increase grain N content compared to yield. With respect to nitrogen use efficiency, we found that the total amount of N applied has a greater effect on NUE compared to the rate of the N splits. In general, the rate of the first and second N applications were most important in determining both yield and grain N content, but the rate of the third N application was important in determining the latter. In general, our results did not show broad support for variety-specific fertilization, however site-specific fertilization holds some promise. Creating a winter wheat fertilization system that reduces negative environmental externalities while retaining high yields and quality remains challenging. However, we found that under the conditions tested here, a N regime of 40-40-80 kg N ha^{-1} may maximize both yield and grain N content, and under some circumstances, improve nitrogen utilization efficiency. This may provide environmental benefits, as well as monetary benefits for producers. Given the current focus on nitrogen use and regulation, research maximizing the utility of nitrogen applications to ensure high grain yield, grain N content, and nitrogen use efficiency is vitally important. This and future work will help producers grow high-quality crops while lessening the environmental impact.

1. Introduction

Winter wheat (*Triticum aestivum)* is an annual staple crop grown over 23 million hectares in the European Union, with an average yield of 5500 kg per ha^{-1} dry weight in 2021 (FAO, 2022). In Switzerland, wheat is a major crop and has a harvested area of 85,000 ha (3.7% of total EU wheat cropland) and an average yield of 6200 kg ha^{-1}, which has remained relatively stable since 1991 (Herrera et al., 2020; FAO, 2022). In Switzerland, about 36% of all land area is used for agricultural purposes (FDHA and FSO, 2018) and approximately 21% of all arable land in Switzerland is used to cultivate wheat (FAO, 2022), making it one of the largest crops by land area in the country (FAO, 2022). The Mittelland, or Swiss Central Plateau, is the region with the greatest agricultural production, with roughly 50% of the area in this region dedicated to agricultural production (FDFA, 2021).

Swiss winter wheat producers are not only paid based on the yield of

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grain delivered, but also partly on the protein content (a proxy for grain nitrogen (N) content), depending on the collection requirements of the grain elevator and miller. Environmental conditions have a considerable effect on protein content (Kramer, 1979) and yield, with both being managed through N application rates and timing (Mohammed et al., 2013). Additionally, winter wheat has been bred to increase protein content (Johnson et al., 1967, 1973), though the genetic contribution to protein content may be smaller than environmental considerations (Kramer, 1979). Further, protein production in the grain may be stable or unstable across differing production conditions, depending on variety (Levy Häner et al., 2016), indicating considerable environmental and management control. Generally, N fertilization rates in Switzerland are roughly 140 kg N ha^{-1} (Fossati et al., 2010) split among two to three applications throughout the spring season. Adequate N supply at critical physiological periods is directly related to high yields and protein contents (Ellen and Spiertz, 1980; Weisz et al., 2001; Bly and Woodard, 2003). While N applications made early in the season, like those prior to grain set, contribute to increasing biomass and overall yield, N applications made late in the season from flag leaf appearance (BBCH 39) onwards aide in increasing protein content and thus enhance quality (Terman et al., 1969; Giordano et al., 2023). However, the relationship between yield and quality is inversely related due to source-sink interactions (Kramer, 1979; Kibite and Evans, 1984). A successful bread wheat crop requires producers to balance the yield-quality tradeoffs.

Accentuating the need for a careful balance between yield and protein is the Swiss system of supplementary payments for top class winter wheat based on protein content. In Switzerland, wheat is categorized into five classes depending on their use. Top class wheat is highly valued for its high quality (protein content and baking qualities), earning it a price premium at grain elevators (Brabant and Levy Häner, 2016; swiss granum, 2022a). In 2021, producers were paid 520 Swiss francs (CHF; approximately 540 €) per ton for top class wheat (swiss granum, 2022a). Additionally, sliding scale supplementary protein payments may be made if protein content is above 13.9%. However, payment deductions are made if protein content falls below 12.7%. Neither reward nor penalty is given between 12.8% and 13.8% (swiss granum, 2020). Thus, a producer must not simply focus on increasing yield, but also protein.

Adequate N is required for producing high yielding and high quality wheat crop, but N use efficiency remains low in wheat at high levels of fertilization (Salim and Raza, 2020) and N not utilized by plants can have negative environmental impacts through runoff, leaching, and volatilization (WHO, 1993; Houghton et al., 2001; Diaz and Rosenberg, 2008; Snyder et al., 2009). The damage is not limited to the environment. Nitrogen pollution from all sectors is estimated to result in between 70 to 320 billion € worth of damage annually, and in agriculture, the resulting monetary loss stemming from N application pollution is estimated to outstrip any monetary gains (European Commission, 2013). In recent years, synthetic N fertilizers sustained large price increases, mostly from an increase in natural gas prices (USDA FAS, 2022), with 2021 experiencing some of the greatest increases due to production disruptions (Baffes and Koh, 2021). Because of these environmental and economic impacts, the Swiss government has set standards through agency goals, parliamentary initiatives, and constitutional amendments aimed at reducing the environmental impacts of agriculture while increasing long-term food security (Das Schweizer Parlament, 2019; FOAG, 2021; Fedlex, 2022). Identifying mechanisms to achieve sustainability goals while simultaneously producing high yielding and high-quality wheat is crucial.

Controlling the N application splits can be used as a method to increase N utilization, yield, and quality of winter wheat. In many European countries, winter wheat is planted in the fall and all the mineral N applications are made in the spring. When soil moisture or precipitation is adequate, split N applications with a sufficiently large amount added at a late stage (e.g., after the appearance of the flag leaf; BBCH 39), is beneficial to increase protein and other baking qualities (Velasco et al., 2012; Brabant and Levy Häner, 2016). Additionally, adding some quantity of N later in the season can increase the NUE compared to applications made only early in the season (Velasco et al., 2012). However, N uptake and utilization is partly controlled by the total amount of N applied where greater N application rates lead to lower N recovery rates (Guarda et al., 2004). Further, wheat has a relatively low N recovery rate from the soil—around 50%—meaning it must have higher amounts of fertilization to obtain optimal yields (Delogu et al., 1998; Ladha et al., 2005). Reducing the amount of N available to the plant and avoiding overfertilization can increase the NUE and reduce environmental losses (Sowers et al., 1994). The use of split nitrogen applications, where smaller amounts of N are placed during critical periods of plant growth and uptake, also aide in increasing efficiency (Sowers et al., 1994). Increasing the N use efficiency can also have a positive impact on the environment (less N lost through leaching or volatilization) and on farmer profits (more complete use of N applied).

We conducted field experiments with five top winter wheat varieties at three sites across Switzerland from 2019–2022. We aimed to assess the yield and quality of Swiss top varieties of winter wheat based on variety and N application regime. Our goals were threefold. First, to assess whether winter wheat variety choice should be optimized based on location and local environmental conditions. Second, to identify a N fertilization regime that would balance both yield and protein and assess whether there is a need for genotype-specific fertilization. The ideal rates for each timed N application may ultimately be dependent on producer goals—either maximizing crop yield or protein content. Third, to assess the efficiency of N in systems with different N split applications, since current Swiss goals include reducing N losses and their environmental impact.

2. Materials and methods

2.1. Study site

Field experiments were conducted at Changins (CH; 46.40°N, 6.23°W), Goumoëns (GO; 46.66°N, 6.60°W), and Reckenholz (RE; 47.43°N, 8.52°W) between 2019–2022 (harvest years). Soils at all locations were classified as cambisol/luvisols and had a percent sand-silt-clay content of 37-36-27, 41-35-24, and 36-38-26, respectively (International Soil Reference and Information Centre, 2020). The experiment was set up in a complete randomized block design (each block equals a repetition) with two repetitions in 2019–2020 and three repetitions in 2021–2022, except for Reckenholz, which was a split-plot design according to N treatment (N_{treat}). Trials were held in Changins and Reckenholz for four years (2019–2022). Due to poor stand emergence in 2021, trials were held in Goumoëns for three years (2019, 2020, and 2022). In 2019 and 2020, plots measured 12 m long by 12 m wide. Due to better available equipment related to remote sensing (not addressed in this paper), plot size was reduced in 2021–2022 and measured 6 m long by 3 m wide (2 m wide in Reckenholz). Over the three field seasons, five top Swiss varieties of winter wheat were used: CH Camedo (Camedo), CH Claro (Claro), Montalbano, CH Nara (Nara), and Runal. Runal was used only in the 2021 and 2022 field seasons while all other varieties were used in every year. Previous crops were either soy (*Glycine max*), sunflower (*Helianthus annuus*), or potato (*Solanum tuberosum*), depending on location and year (Supplemental Tables A, B, and C). All varieties were sown at 350 germinable seeds m^{-2}. Row spacing was 0.19 m at Changins and Goumoëns, and 0.21 m at Reckenholz. Planting was dependent on weather and occurred in October or November and harvested in mid-July (Supplemental Table D).

A total of six fertilization regimes were tested (Table 1). An unfertilized treatment with no added N was used as a baseline in all years (0 kg N ha^{-1}; treatment $N0_{0\text{-}0\text{-}0}$). Nitrogen was applied in either two or three applications. Individual N applications are denoted with N_{app} followed by 1, 2, or 3 to denote the first, second, or third application rate (e.g., N_{app} 1 is the rate of the first N application; Table 1). $N_{app}1$ was made at the beginning of tillering (BBCH 21), the $N_{app}2$ was made at first

Table 1
Nitrogen (N) treatments by year. Within each year, all N treatments were used at all three research sites. N_{app} = N application (first (1), second (2), or third (3)).

Treatment	N_{app} 1	N_{app} 2	N_{app} 3	Total
	kg N ha^{-1}	*kg N ha^{-1}*	*kg N ha^{-1}*	*kg N ha^{-1}*
2019 & 2020				
$N0_{0\text{-}0\text{-}0}$	0	0	0	0
$N1_{40\text{-}120\text{-}0}$	40	120	0	160
$N2_{40\text{-}80\text{-}40}$	40	80	40	160
$N3_{40\text{-}40\text{-}80}$	40	40	80	160
2021 & 2022				
$N0_{0\text{-}0\text{-}0}$	0	0	0	0
$N1_{40\text{-}120\text{-}0}$	40	120	0	160
$N2_{40\text{-}80\text{-}40}$	40	80	40	160
$N4_{20\text{-}40\text{-}20}$	20	40	20	80
$N5_{20\text{-}60\text{-}0}$	20	60	0	80

detectable node (BBCH 31), and (N_{app}3 was made at flag leaf appearance (BBCH 39). Overall N regimes (i.e., the cumulative effect of multiple N_{app}) is denoted as N0 through N5 (Table 1). High N_{app} rates at the second application (N_{app} 2) were meant to stimulate yield production (treatments $N1_{40\text{-}120\text{-}0}$, $N2_{40\text{-}80\text{-}40}$, and $N5_{20\text{-}60\text{-}0}$) while the presence of a third N application (N_{app} 3) was meant to favor increased protein content (treatments $N2_{40\text{-}80\text{-}40}$, $N3_{40\text{-}40\text{-}80}$ and $N4_{20\text{-}40\text{-}20}$). Total N application rates were 0, 80, or 160 kg ha^{-1}. 160 kg ha^{-1} was chosen as a treatment to ensure no N limitation. The reduced rate of 80 total kg N ha^{-1} was added in 2021–2022 to assess the impact of lower overall N rates on yield and protein. Not all regimes were tested in each year due to space limitation; fertilization regimes differed between 2019–2020 and 2021–2022 (Table 1). N was applied as ammonium nitrate (27% N).

2.2. Measurements

Ground measurements were taken throughout the growing season to track the growth and development of the crop, primarily prior to anthesis, during anthesis, and after anthesis.

2.3. Biomass, harvest index, and grain yield

Each plot was separated into two parts; one for hand-harvested samples and one for combine harvester samples. Combine harvester width in all years was 1.5 m. In 2019 & 2020, four harvests of 7 m were made per plot; in 2021 & 2022, one harvest of 4 m was made. Total aboveground biomass (kg ha^{-1}) was measured during flowering and just before harvest. Two lengths of 0.3 m (2019–2020) and 0.6 m (2021–2022) samples were cut 2 cm from the soil surface. Samples were weighed fresh, dried at 50 °C until the mass was stable (approximately 48 h), and weighed dry. For the samples taken just before harvest, the grain was separated from the straw after drying and processed separately. Harvest index was calculated as *grain mass / total aboveground biomass*.

To determine yield, grain was harvested with a combine harvester (Zürn 150, Schöntal-Westernhausen, Switzerland). Grain was separated from chaff and other debris. Material was weighed fresh, dried at 50 °C until the mass was stable, and weighed dry. All yields are reported at 0% moisture.

2.4. Soil nitrogen

Total endogenous soil N ($NO_3^- + NH_4^+$) content was measured at Changins and Reckenholz just prior to the first fertilization (N_{endog}; approximately February at the end of winter; Supplemental Table E) and just after harvest (N_{resid}; approximately mid-July). For N_{endog}, 10 soil samples were taken across each field from 0–30 and 30–60 cm depth. For each depth, samples were combined to broadly characterize the initial N state of the soil. N_{resid} samples were taken 10 per plot from 0–30 and 30–60 cm depths and processed separately for each plot and depth. Soil samples were sent to external labs (Sol-Conseil, Gland, Switzerland in 2019 and 2020 & SADEF, Aspach-le-Bas, France in 2021 and 2022) and analyzed for NO_3^- and NH_4^+ content.

2.5. Nitrogen variables

Dried grain and straw samples were ground to 1.0 mm (grain) and 0.75 mm (straw) and N content was measured by near infrared spectroscapy (NIRS) using a ProxiMate (Buchi, Flawil, Switzerland). Protein content of the grain was calculated from the N content using a conversion factor of 6.25 (based on the wet lab chemist and calibration of our NIRS machine). Nitrogen indices were calculated as follows:

Nitrogen harvest index (NHI) $= N_{grain} / (N_{grain} + N_{straw})$ (1)

Nitrogen use efficiency (NUE) $=$ *Grain yield* $/ (N_{soil} + N_{applied})$ (Moll et al., 1982) (2)

Nitrogen uptake efficiency (NUpE) $= (N_{grain} + N_{straw}) / (N_{soil} + N_{applied})$ (Moll et al., 1982; Hawkesford, 2017) (3)

Nitrogen utilization efficiency (NUtE) $=$ *Grain yield* $/ (N_{grain} + N_{straw})$ (Gaju et al., 2011) (4)

Where N_{grain} is the N content in the grain, N_{straw} is the N content in the straw, N_{soil} is the N endogenous to the soil according to the N_{endog} samples, and $N_{applied}$ is the total amount of N applied through the season.

2.6. Climatic limiting factors

Using local daily weather data from MeteoSwiss, climatic indices were calculated for four different phenological phases of winter wheat to account for the fact that the influences of climate drivers differ depending on development stage. The four phases were estimated based on growing degree days (GDDs; McMaster, 1997, 2005) assuming a base temperature of 0 °C, and starting from planting dates as listed in Supplemental Table D. These dimensionless climatic limiting factors characterize the suitability of the crop to the environment were calculated using factor limitations suitability functions as in Holzkämper et al. (2013) and Herrera et al. (2018). Five different factors were calculated at four different physiological stages of crop growth (Table 2). Negative numbers indicate below optimal environmental conditions while positive numbers indicate above optimal environmental conditions. Zero indicates a non-limiting effect of the factor. This method has been successfully used to calculate the limiting environmental factors for winter wheat in Switzerland (Herrera et al., 2018), as well as maize

Table 2
Climatic limiting factors and phenological phases used to calculate the suitability of the winter wheat crop to each environment as in Table 2 of Holzkämper et al. (2015) using weather data from MeteoSwiss. Each factor (5 factors) is calculated for each growth stage (4 growth stages), resulting in a total of 20 limiting factors. GDD = growing degree days.

Factor	Indication
Water availability	Water stress (either drought or excess); calculated as precipitation - reference evapotranspiration.
Minimum temperature	Frost stress; average daily minimum temperature less than 0 °C.
Average temperature	Optimum temperature determining growth of the plant; average daily mean °C.
Maximum temperature	Heat stress; average daily maximum temperature above 25 °C.
Average photothermal quotient	Radiation limitation; average solar daily radiation ÷ average daily mean temperature.
Growth stage	**Time**
3-leaf	166 GDD
Double ridge	555 GDD
Anthesis	1248 GDD
Physiological maturity	1440 GDD

(Holzkämper et al., 2013, 2015).

2.7. Weather data

Daily weather data was obtained from the Swiss Federal Office of Meteorology and Climatology (MeteoSwiss, Zurich, Switzerland). The weather station was “CGI Changins/Nyon” for Changins (46.40°N, 6.23°E; 458 m a.s.l.), “PUY Pully” for Goumoëns (46.52°N, 6.67°E; 455 m a.s.l.), and “REH Zürich/Affoltern” for Reckenholz (47.43°N, 8.52°E; 433 m a.s.l.). Reference evapotranspiration (ET_o) was calculated on a daily time-step using the *Evapotranspiration* package in R (Guo et al., 2016, 2022) and the radiation daily mean ($W\ m^{-2}$), air temperature daily maximum (°C), air temperature daily minimum (°C), precipitation daily total (mm), relative air humidity daily maximum (%); relative air humidity daily minimum (%), and wind speed daily mean ($m\ s^{-1}$). Average yearly precipitation and temperature for each site are as follows: Changins 1050 mm and 11.5 °C; Goumoëns 1200 mm and 11.0 °C; Reckenholz 1180 mm and 10.0 °C.

2.8. Economic components

Partial profit was calculated as revenue minus fertilizer cost where revenue was calculated as the yield per hectare times price received, plus a protein payment based on the protein content of the grain (swiss granum, 2020). To simplify the economic analysis, all costs other than N fertilizer were assumed constant between treatments. When calculating protein payment, we use protein as measured on the whole grain to reflect practice at grain elevators (Supplemental Table F). Since winter wheat is a crop that spans two years (planted in the fall and harvested the following summer), fertilizer prices were taken as the yearly average fertilizer price in the year prior to harvest; e.g., wheat that was harvested in 2019 used the average yearly fertilizer price for 2018. Average yearly fertilizer prices were 1.37, 1.50, 1.35, and 1.64 Swiss Franc (CHF; 1.45, 1.59, 1.43, and 1.73 €) per kilogram for harvest years 2019, 2020, 2021, and 2022, respectively (Union suisse des paysans, 2022).

2.9. Data analysis

We used PROC MIXED to fit a mixed model in SAS (SAS Institute, Cary, NC) for yield, protein, harvest index (HI), N harvest index (NHI), N left in the soil post-harvest (N_{resid}), partial profit, N use efficiency (NUE), N utilization efficiency (NUtE), and N uptake efficiency (NUpE). Each site (Changins, Goumoëns, and Reckenholz) was analyzed separately due to variation in environmental conditions and experimental design. The structure of the data did not allow for the separation of each individual N application, so each N_{treat} was taken as a package of three N applications (treatments N0 through N5; Table 1). Variety (fixed), N_{treat} (fixed), and repetition nested within year (random) were treated as covariables. Interactions between variety, N_{treat}, and year were also tested. Because Reckenholz used a split-plot design with N_{treat} as the main effect, we nested N_{treat} within block at this site. Because an incorrect block randomization was applied in 2021, the data of Reckenholz 2021 was not suitable to be included in the mixed model. However, this does not preclude its use in the random forest. Least squares (LS) means were calculated for each observation based on year, location, N_{treat}, and variety; Tukey groupings were added as a post-hoc test.

Additionally, a variance components analysis was performed using PROC VARCOMP in SAS using the same model as in PROC MIXED. Restricted maximum likelihood was used as the estimation method in both PROC VARCOMP and PROC MIXED. The percent variation for each estimate and its contribution to the variance was computed.

Further, we used random forest to tease apart the effects of a myriad of environmental and management variables (Table 3). Random forest is a non-parametric ensemble supervised machine learning technique developed by Breiman (2001). Because random forest utilizes a process called bagging (bootstrap + aggregating), it is an excellent option for analyzing data that is unbalanced, does not assume a normal distribution, or has an unknown distribution. Random forest is an excellent complement to a traditional mixed model analysis because of its ability to tease apart important factors in determining the response variable. Here, random forest can provide additional clarity on the impact of environmental factors. Partial dependence plots allow the user to view the response to a single variable, holding all other variables constant. Compared to a traditional linear regression or mixed model analysis, random forest can provide additional insight and further resolution of the data, which may not be possible with the aforementioned methods.

Table 3
Variables used in the random forest. Both forests (for yield and for protein) contained 33 variables each. Since the relationship between grain yield and grain N content is well characterized, grain yield was included as an independent variable in the grain N content forest and vice versa.

Factor	Units	Meaning
Grain yield	kg ha^{-1}	Yield of grain
Grain N content	%	Nitrogen content of the grain at maturity.
Site & environmental characteristics		
Altitude	m	Altitude of the study location
Latitude	deg.	Latitude of the study location
Longitude	deg.	Longitude of the study location
Slope	%	Slope of the study site
Percent sand	%	Percent sand in the top 20 cm of soil at study site
Percent silt	%	Percent silt in the top 20 cm of soil at study site
Percent clay	%	Percent clay in the top 20 cm of soil at study site
Climatic limiting factors	unitless	20 factors as listed inTable 2
Management & nitrogen		
Variety	unitless	Variety of winter wheat planted
N application 1	kg ha^{-1}	Rate of the first nitrogen application
N application 2	kg ha^{-1}	Rate of the second nitrogen application
N application 3	kg ha^{-1}	Rate of the third nitrogen application
Endogenous soil N	kg ha^{-1}	Total soil N content in February before N applications

Data from all years and sites were used for the random forest. Nitrogen treatment could be separated into individual applications (N_{app} 1, N_{app} 2, and N_{app} 3), thus revealing the effects of each individual treatment. Two random forests were built from the same dataset: one for yield (RF_{yield}) and one for N_{grain} (RF_{Ngrain}). Random forest analysis was carried out using R (RStudio, Boston, MA) and the *randomForest* package (Liaw and Wiener, 2002). The entire dataset included 642 observations across three sites and four years. We removed observations with missing data, resulting in 607 observations for RF_{yield} and RF_{Ngrain} (95% inclusion rate).

One thousand trees were grown with 6 variables tried at each split. The number of variables tried at each split was calculated as the $\sqrt{n}$ where n is the number of independent variables in the model, rounded up to the nearest whole number. Partial dependence plots (pdp) were created using the R package *pdp* (Greenwell, 2017). A description of variables included in the random forest model is found in Table 3. The *boruta* package in R was used to compare to the random forest results (Kursa and Rudnicki, 2010). *Boruta* is a wrapper for the *randomForest* package that can help identify important features.

3. Results

3.1. Environmental conditions

In general, winter wheat was well suited to the environments tested. Across all sites, 2021 had greater precipitation than any other year while 2022 had particularly dry conditions (Fig. 1). Crop evapotranspiration (ET) vs. precipitation (PP) revealed that Changins was more likely to be water-limited during key developmental phases (March – July; Supplemental Table G). However, the limiting factors revealed adequate to

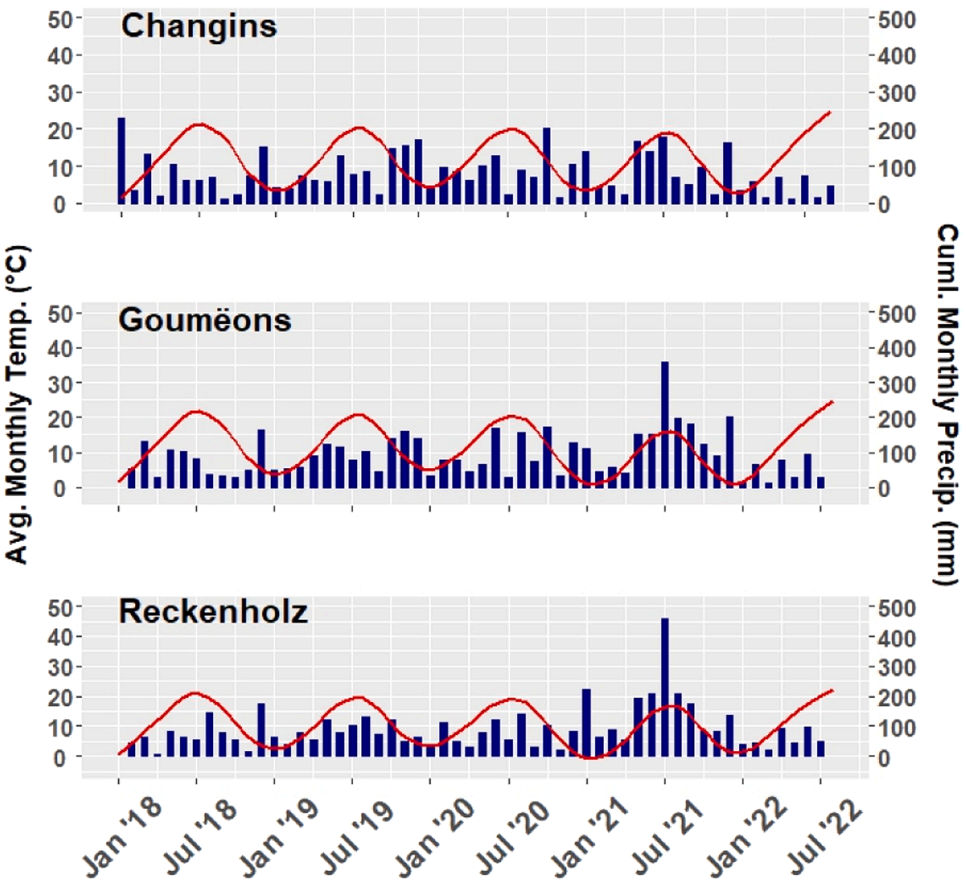

Fig. 1. Weather data for Changins, Goumoëns, and Reckenholz from 2018 to 2022. Red line represents average monthly temperatures (°C; left axis). Blue bars represent cumulative monthly precipitation (mm; right axis). Data from MeteoSwiss, the Swiss Federal Office for Meteorology and Climatology.

excessive water availability across all sites and years at all stages except for physiological maturity (dry down), which showed some deficit in 2022. Additionally, at all sites and years, radiation was in deficit.

3.2. Yield, HI, grain N content, and partial returns

Across all sites, N_{treat} had a significant and positive effect on yield. As total N applied increased from 0 to 80 and to 160 kg N ha^{-1}, average yields across all years for each site also increased from 3100 to 5000 and 6400 kg dry grain ha^{-1} at Changins, 4500, 6300, and 6600 kg ha^{-1} at Goumoëns, and 6100, 8000, and 7600 kg ha^{-1} at Reckenholz. As expected, the unfertilized treatment produced the lowest yields, while $N3_{40\text{-}40\text{-}80}$ produced the greatest (4400 vs. 7000 kg ha^{-1} across all years and sites; Supplemental Table H). Additionally, when N treatments were separated by total N applied (80 or 160 kg ha^{-1}) by site and the ANOVA run again, N_{treat} was only significant for yield at Goumoëns at the 160 kg ha^{-1} level (Supplemental Tables I and J). Variety was a significant factor in determining yield only at Reckenholz ($P < 0.05$; Table 4). Here, on average and across all years, Claro, Montalbano, and Nara resulted in the greatest yield (7400 kg ha^{-1}) while Camedo resulted in the lowest (7100 kg ha^{-1} Supplemental Table H). The interaction of N_{treat} and variety was not significant for any site with respect to yield. In general, the lowest grain yield and endogenous soil N was found at Changins, while the greatest was found at Reckenholz (Supplemental Tables H and E).

Variety strongly influenced HI at all sites ($P < 0.05$), but N_{treat} was only significant at Reckenholz where N_{treat} 3 had a significantly lower HI compared to all other treatments (0.37 v. 0.42 kg kg^{-2}; Table 4 and

Table 4
Analysis of variance for grain yield, N content in the grain, harvest index, and partial profit at Changins (CH), Goumoëns (GO), and Reckenholz (RE) from 2019 to 2022. Repetition (rep) was analyzed as nested within year. * = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.0001$. df = degrees of freedom. Least squares means by year, site, variety (var), and N treatment (N_{treat}) with post-hoc Tukey test significant differences shown in Supplemental Table H.

ANOVA	df	CH	GO	RE	CH	GO	RE	CH	GO	RE	CH	GO	RE
		Grain yield			*N content*			*HI*			*Partial Profit*		
Var	4	0.5266	0.0727	***	***	***	***	**	***	***	0.2505	0.4068	***
N_{treat}	5	***	***	***	***	***	0.1880	0.4898	0.2743	*	***	***	***
Year	3	*	0.0867	**	***	***	0.6104	***	**	0.1574	0.0565	0.0872	**
Year × N_{treat}	9	*	***	0.1004	**	0.0616	***	0.4844	0.7042	0.1546	0.0574	***	0.2258
Year × Var	10	0.1532	0.1035	***	**	0.2617	***	0.1814	0.9430	**	0.1667	0.1158	**
N_{treat} × Var	19	0.8504	0.1783	0.6377	0.0933	0.7428	**	0.5394	0.4575	**	0.9119	0.2242	0.5992
Year × N_{treat} × Var	31	0.4911	0.0037	0.7259	0.5974	0.9977	0.8441	0.7472	0.4030	0.0561	0.5040	**	0.7879
Error terms													
Year(rep)	6	0.14	0.27	0.05	0.18	0.10	0.68	0.05	0.02	0.03	803	1651	312
Residual	126	0.07	0.05	0.03	0.10	1.14	0.08	0.05	0.02	0.02	427	330	225

Supplemental Table H). Like grain yield, the year had a strong effect on HI ($P < 0.05$), except at Reckenholz. Across all years, Nara had the highest HI at all sites (0.46, 0.47, and 0.44 kg kg^{-1} at Changins, Goumoëns, and Reckenholz) and Runal had the lowest (0.43, 0.41, and 0.39 at Changins, Goumoëns, and Reckenholz, respectively). The N_{treat} × variety interaction was only significant at Reckenholz ($P < 0.05$).

Nitrogen content in the grain was strongly influenced by variety, N_{treat}, and year at Changins and Goumoëns and by variety at Reckenholz ($P < 0.05$; Table 4 and Supplemental Table H). Across all years, Runal had the greatest average N_{grain} content at all sites (CH 2.11%; GO 2.43%; RE 2.64%). Camedo had the lowest average N_{grain} content at Changins and Goumoëns (1.89% and 2.12%), while Claro had the lowest at Reckenholz (2.40%). As expected, $N0_{0\text{-}0\text{-}0}$ resulted in the lowest N content (1.78%, 1.92%, and 2.09% at Changins, Goumoëns, and Reckenholz), while $N3_{40\text{-}40\text{-}80}$ resulted in the greatest N_{grain} at Changins and Reckenholz (2.13% and 2.65%), and $N2_{40\text{-}80\text{-}40}$ at Goumoëns (2.44%; Supplemental Table H). The interaction between year and N_{treat} was only significant at Changins and Reckenholz (Table 4 and Supplemental Table H). In general, N_{grain} was lowest at Changins (1.98%) and greatest at Reckenholz (2.43%), with Goumoëns intermediate (2.21%). When the data was split by site and total N applied, N_{treat} was significant for N_{grain} at Changins, but only at the 160 kg ha^{-1} N level (Supplemental Tables I and J). A visual analysis of the influence of N_{treat} and variety on N_{grain} versus grain yield by site and year is shown in Fig. 2. ANOVA results for N_{yield} (calculated as N_{grain} × yield) closely resembled that of yield, with the notable exception of variety at Reckenholz becoming insignificant (Supplemental Table K).

The variance components analysis revealed that N_{treat} contributed the most to the variance of yield and N_{grain}, with the exception of Reckenholz where year contributed more variation to yield (Fig. 3). Similar to the mixed model, variety contributes more to N_{grain} than to yield at all sites (Table 4 and Fig. 3). For Reckenholz, the mixed model indicates that among the varieties tested here, variety is significant in determining yield, but the variance components analysis indicates that the percent variance contributed by variety is very small (less than 1%). Variance unexplained by the model also play a large role, with approximately 20% on average across all models.

Partial returns shared a pattern similar to that of yield with the lowest partial returns at Changins (2968 CHF; 3138 €) and the greatest at Reckenholz (3978 CHF; 4206 €; Table 4 and Supplemental Table H). Similar to yield, N_{treat} but not variety had a strong effect on partial profits, with the exception of Reckenholz where variety had a significant effect. Here, Claro had the greatest average returns across all years (4092 CHF; 4327 €) and Camedo had the lowest (3904 CHF; 4128 €; Supplemental Table H). Across all sites, the significance of N_{treat} was largely due to the total N applied (0, 80, or 160 kg N ha^{-1}) with greater average partial profits with 160 kg N ha^{-1} and the least with 0 kg N ha^{-1}. The exception was at Reckenholz where 80 kg N ha^{-1} resulted in slightly greater partial returns. However, the difference in average partial profits between the 80 and 160 kg N ha^{-1} was only 127 and 241 CHF (134 and 255 €) at Goumoëns and Reckenholz, respectively, and 698 CHF (738 €) at Changins.

3.3. NHI, NUE, NUtE, NUpE, N_{resid}

Variety and N_{treat} were not significant for NHI at Changins ($P > 0.05$; Supplemental Table K). However, the N_{treat} × variety interaction was significant. At Changins, NHI was greatest in 2020 and least in 2019 (0.85 and 0.69 kg kg^{-1}). Interestingly, the two stressed years, 2021 (excessively wet) and 2022 (excessively dry), had similar average NHIs (≈ 0.79 kg kg^{-1}; Supplemental Table K). Goumoëns and Reckenholz revealed a different pattern, where variety, N_{treat}, and year were all significant for NHI at Goumoëns, and only variety at Reckenholz. In general, higher NHIs were found with treatments with less than 160 kg N ha^{-1} (0.80 v. 76 kg kg^{-1}; Supplemental Table K). Montalbano had the lowest NHI compared to all other varieties (0.75 v. 0.79 kg kg^{-1}),

In general, NUE was greater for treatments with 80 kg ha^{-1} of total N compared to 160 kg ha^{-1} total N (Table 5 and Supplemental Table L). N_{treat} had the strongest effect on NUE, and variety was only significant at Reckenholz while year was only significant at Changins and Reckenholz. At Reckenholz across all years, Camedo had the lowest (39 kg kg^{-1}) and Runal the greatest (44 kg kg^{-1}) NUE. Changins had the most stable NUE across N treatments (≈ 40 kg kg^{-1}) while Goumoëns had the greatest (≈ 49 kg kg^{-1}, 2021).

At Changins, year was significant for both N utilization and uptake efficiency ($P < 0.05$; Table 5 and Supplemental Table L). NUtE was similar in years with moderate precipitation like 2019 and 2020 (approximately 37.7 kg kg^{-1}), but lower in the wet year (2021; 36.0 kg kg^{-1}) and greater in the dry year (2022; 42.6 kg kg^{-1}). For NUpE at Changins, 2020 revealed a NUpE of 1.01 kg kg^{-1} while 2021, the comparatively wet year, was greater at 1.08 kg kg^{-1}. In 2022, the comparatively dry year, NUpE was less at 0.93 kg kg^{-1}. N_{treat} was significant for both NUtE and NUpE at Changins and Goumoëns, and for NUpE at Reckenholz. For NUtE, treatments with less overall N had a greater utilization efficiency (42.3 v. 37.1 kg kg^{-1} for 80 and 160 kg N ha^{-1}, respectively). For NUpE, lower amounts of total N resulted in greater NUpE (1.12 v. 0.94 kg kg^{-1} for 80 and 160 total kg N ha^{-1}, respectively). Similar results were found at Goumoëns with respect to significant factors. However, at Reckenholz, variety was an important factor in NUE and NUpE, which was not the case at Changins and Goumoëns.

For the amount of N left in the soil post-harvest (N_{resid}), the year was important at Changins, but not Reckenholz ($P < 0.05$, Supplemental Table K). Variety did not impact N_{resid} at either site, but the year × variety interaction was significant at Changins. On average and across all years, N_{resid} for $N0_{0\text{-}0\text{-}0}$ was 37 kg N ha^{-1} (Changins) and 62 kg N ha^{-1} (Reckenholz). Interestingly, $N3_{40\text{-}40\text{-}80}$ left close to the same amount as $N0_{0\text{-}0\text{-}0}$ at Reckenholz (62 kg N ha^{-1}) and less than $N0_{0\text{-}0\text{-}0}$ at Changins (21 kg N ha^{-1}). All other N treatments left ≈ 45 kg N ha^{-1} at Changins and ≈ 72 kg ha^{-1} at Reckenholz. Overall, N_{resid} was roughly 50% greater at Reckenholz compared to Changins.

3.4. Random forest

For yield, the random forest revealed that the model explained 78% of the variation in yield and that the most important variables in determining yield were the rate of the first and second N applications (1st & 2nd most important), the N content in the grain (3rd), and temperature during anthesis, which was generally below the optimal temperature for development (4th & 5th; Table 6). The rate of the third N application was of moderate importance (ranked 7th) and the variety was of lowest importance (Supplemental Fig. 1). Additionally, partial dependence plot for yield v. N_{grain} revealed an increase in both yield and N_{grain} until approximately 8000 kg ha^{-1} yield and 2.24% N_{grain} (Fig. 4). Yield v. N_{app} 1 revealed a continual increase up to 40 kg N ha^{-1}, beyond which point a higher application rate was not tested. N_{app} 2 revealed a leveling off around 30 kg ha^{-1}, while N_{app} 3 showed a continual increase in yield with additional applied N up to 80 kg ha^{-1}, though the increase was very small (approximately 0.02 kg ha^{-1} additional yield; Fig. 5, panels A-C).

For N_{grain}, the model explained 76% of the variation in N_{grain} and the most important variables for determining N_{grain} were N applications 1 & 2 (1st and 2nd most important), grain yield (3rd), variety (4th), and the rate of the 3rd N application (5th; Table 6). The endogenous soil N was ranked 6th out of a total 33 variables in both the yield and the N_{grain} models, indicating that the effect of endogenous soil N in our experiments was considerable (Supplemental Table E). Partial dependence plots showed that N_{grain} increased sharply up to 40 kg N ha^{-1} (larger quantities for N_{app} 1 were not tested) and a more gradual increase up to 120 kg N ha^{-1} for N_{app} 2 (Fig. 5, panels D & E). N_{app} 3 revealed a very modest increase in N_{grain} with increasing rates of applied N, and a

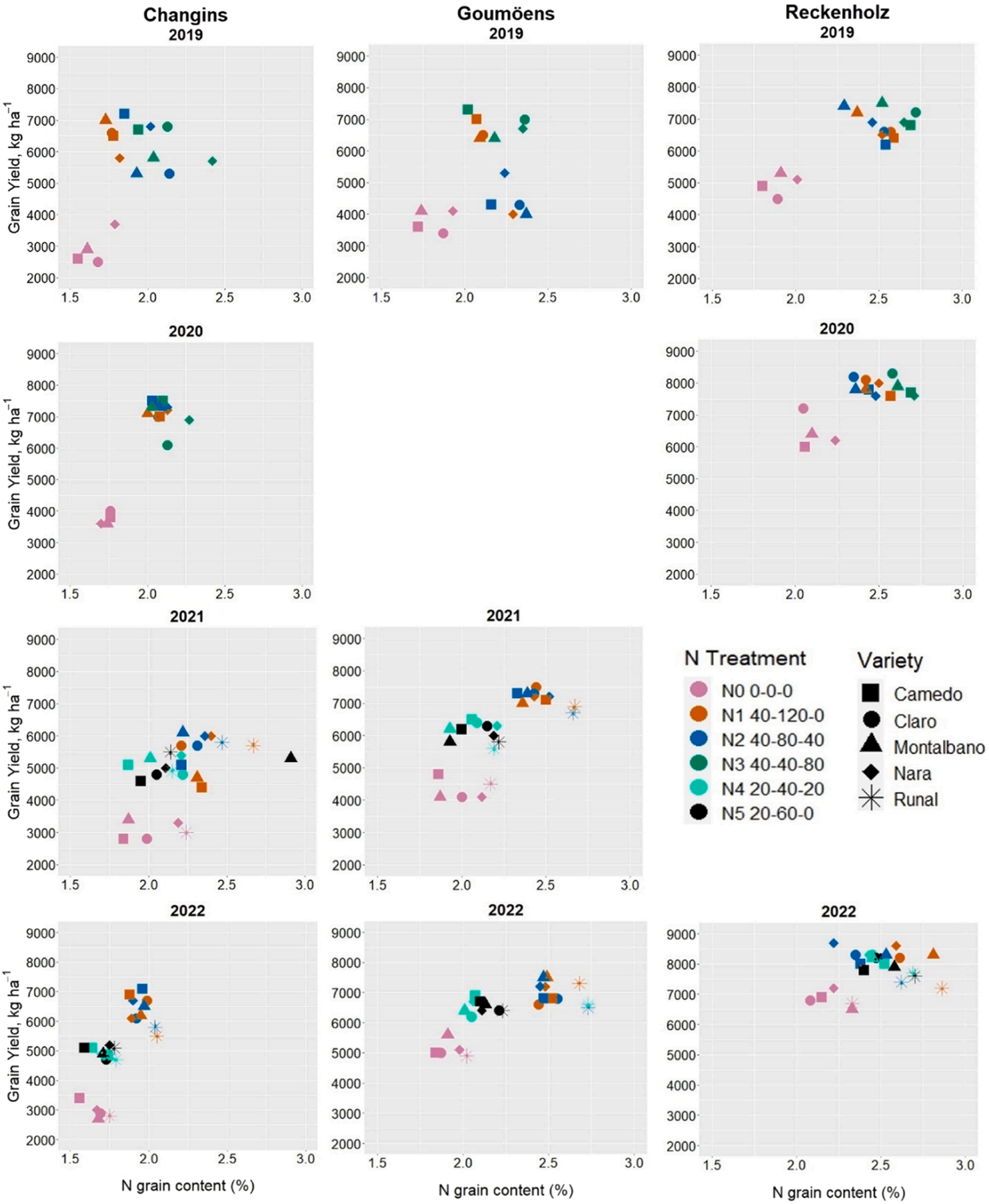

Fig. 2. LS means for N grain content (%) versus grain yield (kg ha^{-1}) for each site and year given N treatment and variety. For grain yield, N_{treat} had a significant and positive effect on yield while variety was only significant at Reckenholz. Year was significant at Changins and Reckenholz. For N_{grain}, N_{treat}, variety, and year were significant factors at Changins and Goumoëns, but only variety was significant at Reckenholz.

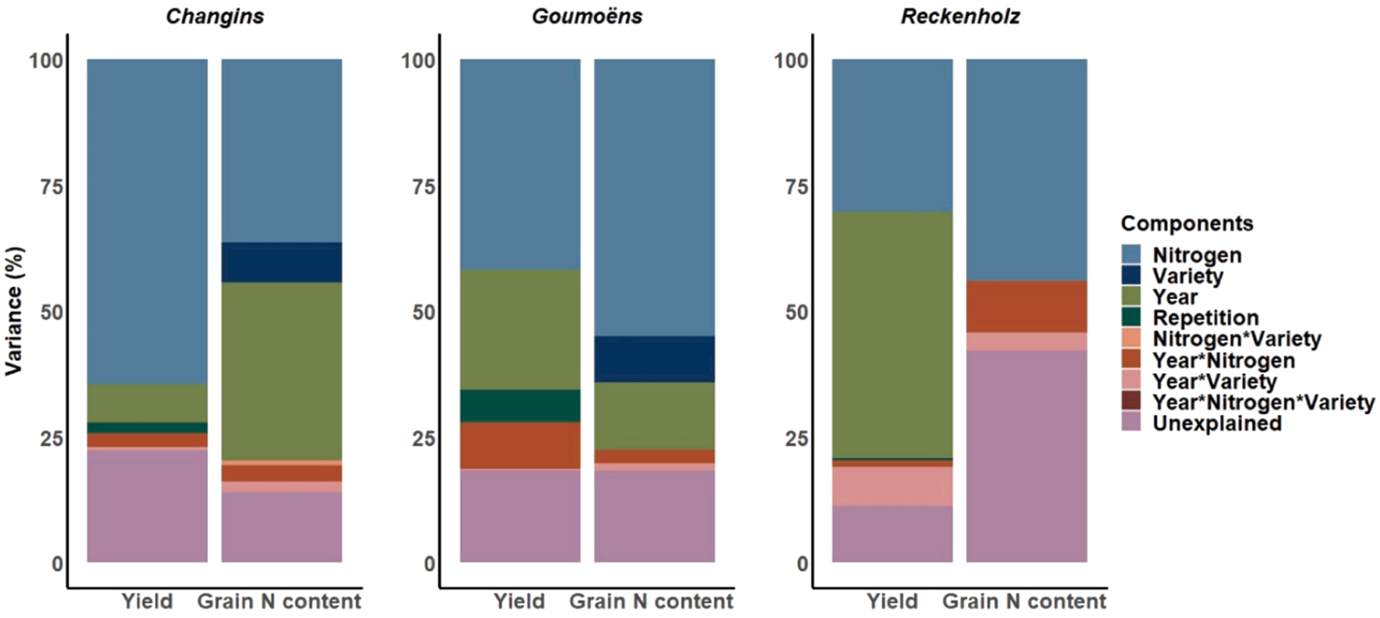

Fig. 3. Variance components analysis for yield and protein models at Changins, Goumoëns, and Reckenholz. The percent of each factors' contribution to variance is shown on the Y axis.

Table 5
Analysis of variance for Nitrogen Use Efficiency (NUE), Nitrogen Utilization Efficiency (NUtE), and Nitrogen Uptake Efficiency (NUpE) at Changins (CH), Goumoëns (GO), and Reckenholz (RE) from 2019 to 2021. * = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.0001$. df = degrees of freedom. Least squares means by year, site, variety (var), and N treatment (N_{treat}) with post-hoc Tukey test significant differences shown in Supplemental Table L.

ANOVA	df	NUE			NUtE			NUpE		
		CH	*GO*	*RE*	*CH*	*GO*	*RE*	*CH*	*GO*	*RE*
Var	4	0.5117	0.0839	***	*	***	***	0.4105	0.1159	***
N_{treat}	4	***	***	***	***	***	0.6133	***	***	*
Year	2	*	0.3022	**	**	0.7980	0.4061	**	0.3583	0.1381
Year × N_{treat}	4	**	0.6393	0.4592	0.1398	0.2642	0.2981	*	0.2373	0.5523
Year × Var	7	0.1734	*	**	0.2289	*	***	0.1403	**	*
N_{treat} × Var	15	0.7186	0.6001	0.9728	0.2907	0.5278	*	0.9797	0.8837	0.5331
Year × N_{treat} × Var	15	0.9414	0.8650	0.8592	0.5665	0.5789	0.1589	0.9640	0.8643	0.3458
Error terms										
Year(rep)	5	7.4	11.1	3.6	6.6	2.7	9.7	0.20	0.34	0.40
Residual	85	4.8	3.5	1.6	3.2	2.8	1.5	0.15	0.16	0.09

Table 6
Results from the random forest for both yield and grain N content (N_{grain}) models. Top 5 factors for each model are shown. Both models used 607 out of 642 possible observations (95% inclusion rate). Observations were deleted when not all factors in the random forest were included in the observation. Percent increase in the mean squared error (% Inc. MSE) is the change in the MSE when a variable is randomly shuffled within a column. Greater % Inc. MSE indicates greater importance in the model.

Yield		N_{grain} content	
Variable	*% Inc. MSE*	*Variable*	*% Inc. MSE*
N_{app} 1	35	N_{app} 1	41
N_{app} 2	35	N_{app} 2	36
N_{grain} content	31	Yield	35
Avg. temp anthesis	24	Variety	29
Min. Temp anthesis	23	N_{app} 3	20
Mean squared residual	0.005	Mean squared residual	0.025
Variation explained	78%	Variation explained	76%

leveling off around 50 kg N ha^{-1} and 2.22% N in the grain.

The boruta showed similar results to the random forest. For yield, the boruta algorithm confirmed the importance of the first and second N applications, as well as the N content of the grain and temperature

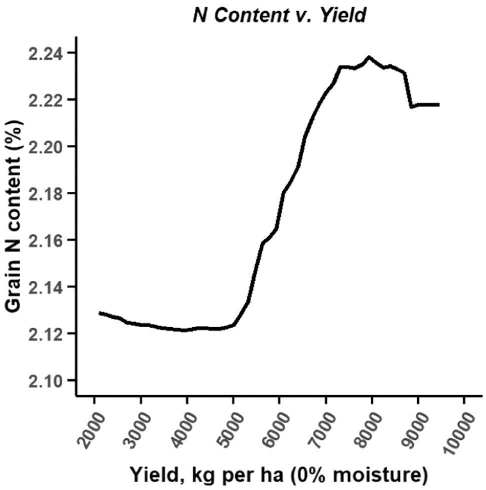

Fig. 4. Grain N concentration v. yield, derived from the N_{grain} random forest. A peak with maximum yield and N_{grain} is observed around 8000 kg ha^{-1} and 2.24% N in the grain.

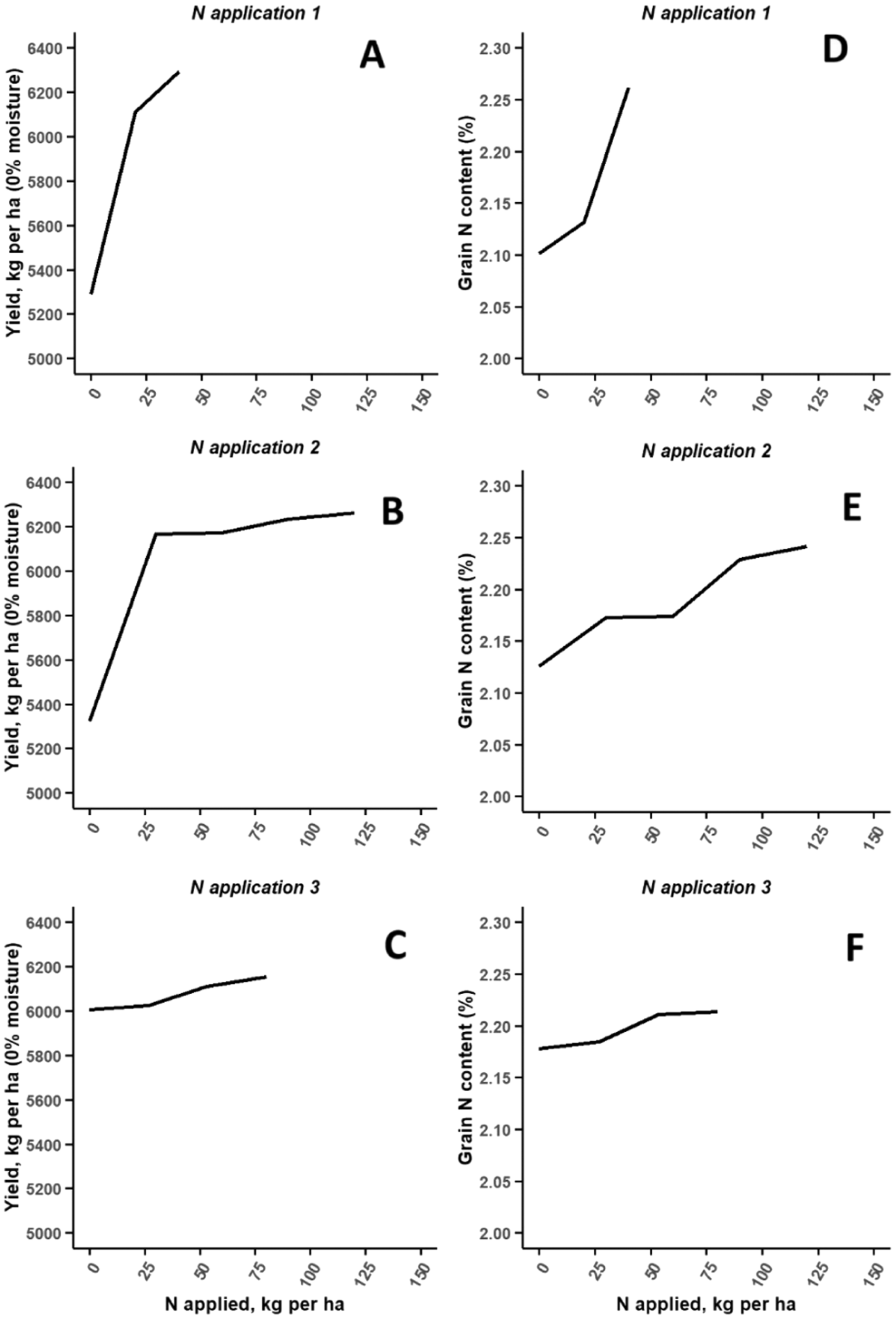

Fig. 5. Yield (panels A-C) and grain N content (panels D-F) response to N application rate for applications 1, 2, and 3 from the random forest analysis. The N applied in kg ha^{-1} is given on the x axis and yield (kg ha^{-1} at 0% moisture) or grain N content (%) is given on the y axis.

during anthesis (Supplemental Fig. 2). A similar result was found for N_{grain}, with variety as a highlighted factor (Supplemental Fig. 4). In both models, climatic limiting factors at the double ridge stage and physiological maturity were deemed unimportant.

4. Discussion

4.1. Yield, Quality & Partial Profit

Our aim was to determine if different varieties or locations needed different N application regimes to produce a high yielding and high-quality wheat crop. Results show that, given the yields observed across all years and sites in this study, variety may only be significant for yield under conditions that are more optimal for winter wheat growth. Variety was only significant at Reckenholz (Table 4 and Supplemental Table H), which consistently had the greatest yields across all years (7300, 5300, and 6100 kg ha^{-1} at Reckenholz, Changins, and Goumoëns, respectively), hinting at the potential of site-specific variety recommendations under certain growing conditions. Here, averaged across all sites and years, Runal yielded approximately 6300 kg ha^{-1}, with all other varieties with an approximate yield of 6200 kg ha^{-1}. Interestingly, Runal is one of the oldest registered varieties used here that is reputed for its high quality (high N_{grain}) grain, but not necessarily high yield. The interaction between variety and N_{treat} was not significant at any site, indicating that fertilization regimes based on variety within the top class may be unnecessary at these sites or under the conditions experienced in this study. A similar result was found by Levy et al. (2007) where N fertilization and variety across several winter wheat classes did not affect protein content.

Importantly, we found that when analyzed by total N applied, N_{treat} (and thus the N split application) was only significant for yield at Goumoëns at the 160 kg N ha^{-1} level, with $N2_{40\text{-}80\text{-}40}$ producing a lower yield (approximately 6300 v. 6800 kg ha^{-1} for $N1_{40\text{-}120\text{-}0}$ and $N3_{40\text{-}40\text{-}80}$). This may indicate that though fertilization strategies based on variety that include N splits may not be advantageous, strategies based on location may provide some benefit. Previous studies found that yields may be unaffected when a single dose of N is applied around tillering compared to multiple doses of N around tillering, shooting, heading, and just post flowering (Woolfolk et al., 2002; Makary et al., 2020). However, other studies show a significant difference in yield between single applications made at tillering and split applications made at booting or just prior to flowering (Gravelle et al., 1988). Our study reveals that yield may be unaffected by late season N applications, as long as adequate N is applied earlier in the spring (first and second N applications around tillering and first node growth stages). However, it is important to note that late season N applications may be risky due to potential limited precipitation in the late spring and summer, preventing N uptake by the plant (Beniston, 2012; Vittoz et al., 2013; Brabant and Levy Häner, 2016).

We found that biomass partitioning to the grain, as calculated by harvest index (HI), was largely affected by variety, but not necessarily by N_{treat}, indicating strong genetic control similar to other studies (Zhang et al., 2012; Dai et al., 2016). However, at Reckenholz the N_{treat} × variety interaction was significant, indicating that the interaction may be more important in a well-suited growing region. This is supported by the variance components analysis (Fig. 3), which revealed that at Reckenholz, the year (a proxy for environmental conditions) had a greater influence on yield than N_{treat}, thus highlighting the importance and effects of suitable growing conditions. Environmental conditions like precipitation during the growing season can play a significant role in determining optimal yield and protein levels and the level of N applied to achieve them (Rasmussen and Rohde, 1991). Our study confirmed similar findings with respect to growing season conditions where temperature during anthesis was important in determining yield (Table 6). However, the greater variance in year and the lower variance in N_{treat} at Reckenholz compared to other sites may be due in part to the greater levels of endogenous N compared to Changins or Goumoëns (Fig. 3 and Supplemental Table E).

Contrary to yield, we found that variety had a strong influence on the N content of the grain (N_{grain}), and thus quality, at all sites (Table 4 and Supplemental Table H). The random forest also supports this result, where variety was the fourth most important variable in determining N_{grain}, indicating that Swiss top winter wheat varieties may have, intentionally or not, been bred to have more similar yields than N_{grain} contents (Table 6). The impact of genetics on N_{grain} (or protein) is apparent, though may play a lesser role than the environmental factors (Kramer, 1979; Peterson et al., 1992). Even under identical fertilization regimes, varieties may produce disparate protein levels in the grain (Johnson et al., 1973). In Switzerland, producers choose to grow top varieties because of their proven high protein content. However, our research shows that with respect to N_{grain}, selecting the most suitable variety is still important, even within the top variety category. Historical data from variety trials in Germany show that yields have increased significantly over the past several decades, but there has also been a reduction in protein content in the grain (Laidig et al., 2017), perhaps an indication of priorities of breeding efforts. The pattern of high yielding cultivars correlating with lower protein is not new (Kramer, 1979; Guthrie et al., 1984), and it appears that breeding efforts for new winter wheat cultivars in Switzerland may want to focus on closing the protein gap among high-yielding varieties.

N_{treat} also impacted N_{grain} at Changins and Goumoëns but not at Reckenholz, which may be a result of the greater amount of N_{endog} at that site (Table 4 and Supplemental Table H). However, when the total amount of N applied was taken into account, N_{treat} only had a significant effect on N_{grain} at greater levels of N fertilization at Changins at 160 kg N ha^{-1} total N (Supplemental Tables I and J). Goos et al. (1982) showed a transition zone representing N deficiency and N sufficiency with respect to grain protein production between 11.1 to 12.0% protein content (or approximately 1.84% to 1.92% N_{grain}, assuming a Jones factor of 6.25; Goos et al., 1982). Across all years, all of our N_{treat} resulted in an N_{grain} above the transition zone, with the exception of $N0_{0\text{-}0\text{-}0}$ at Changins and Goumoëns (1.78% and 1.92% respectively, averaged across all years) and Changins $N4_{20\text{-}40\text{-}20}$ (1.91% N_{grain}; Supplemental Table H). This indicates that our crop may be receiving adequate supply to avoid N deficiency, even under no fertilization at Reckenholz and low fertilization at Changins and Goumoëns. This is likely due to large amounts of endogenous soil N as measured early in the spring each year (Supplemental Table E).

Many studies with the aim of increasing N_{grain} focus on N applications made later in the season, like after anthesis or with use of foliar N (Woolfolk et al., 2002; Wyatt et al., 2018). In Germany, producers may delay the last N application until the start of flowering (BBCH 60) in an effort to increase N_{grain} (Makary et al., 2020), which is later than the last application made here (made at the appearance of the flag leaf). Additionally, a single application around BBCH 27 resulted in lower N_{grain} compared to treatments which received three N applications (applied around tillering (BBCH 27), shooting (BBCH 31), and heading (BBCH 51) (Makary et al., 2020). Though we did not have a treatment with a single application, we found that when considering all treatments tested here, a split application with 40 kg N ha^{-1} placed at tillering and 120 kg N ha^{-1} placed at first node, and no third N application resulted in lower overall N_{grain} at Changins (Supplemental Table H). This may have been due in part to high levels of endogenous N in the soil at Reckenholz compared to the other sites (approximately 45 v. 25 kg N ha^{-1}; Supplemental Table E), or the effect of year where Goumoëns and Reckenholz were missing observations in 2020 and 2021, respectively. Further, Brown and Petrie (2006) show that protein increases associated with late season N application was highly dependent on previous N applications and soil available N. Conversely, Levy et al. (2016) showed that the N_{grain} potential is largely driven by the total N available to the plant, rather than the split of the N applications. Our results show that there may be lower N_{grain} with earlier N applications under some conditions, but future work may want to explore varying N application levels with a later third application than used here.

With respect to partial profits, we found a similar pattern as grain yield (Table 4 and Supplemental Table H). Like yield, variety was only significant in determining partial profits at Reckenholz. This indicates that overall yield, rather than protein content of the grain, is a more important factor for profit, despite a monetary incentive to produce high-protein wheat. Winter wheat with less than 13.9% protein (approximately 2.22% N_{grain}) will not receive a supplemental payment at the elevator (swiss granum, 2020). Additionally, grains with less than 12.8% protein receive a deduction in payment, but this did not lead to a large impact on partial profits (−51 CHF (−54 €) ha^{-1} on average and a range of −188 to +204 CHF (199 to 216 €) ha^{-1}). N_{treat} was significant in determining partial profits, but this was largely due to total N applied and not individual N regimes (Supplemental Table H and M). However, at Reckenholz, $N4_{20\text{-}40\text{-}20}$ resulted in approximately 3% more partial profits compared to $N5_{20\text{-}60\text{-}0}$. It is possible that the N splits have an effect on yield, N content of the grain, and profits, but to have a clearer response, more disparate N application rates and timings need to be considered.

Our first goal aimed to assess if producers should select varieties based on location to maximize yield and grain N content, and if there is evidence for a genotype-specific N fertilization regime. In general, our results do not support variety specific N fertilization regimes, but site-specific fertilization provides promise at sites and conditions used in this trial. We also found that top Swiss winter wheat varieties are more similar in yield production than quality (N_{grain}).

4.2. Random forest identifying maximum yield and N_{grain}

Our second goal was to identify both a point where yield and quality are maximized, and a N application regime to achieve this. We used random forest to tease apart various environmental, genetic, and management variables, and identify the most important factors determining yield and N_{grain}. Random forest has been used extensively in agriculture to classify crop cover (Ok et al., 2012; Tatsumi et al., 2015), predict changes in environment (Kreakie et al., 2021), and analyze and represent crop yields in response to climate change (Hoffman et al., 2020) as well as identify key variables in determining yield (Burton and Kemanian, 2022). We identified environmental variables at anthesis, particularly water deficit and temperature, as major drivers of yield (Supplemental Fig. 1). Day and Intalap (1970) found that water deficit at flowering resulted in lower yields, mostly due to lower grain weights. Though drought can have a significant effect on all stages of crop growth including early in the season like jointing (Day and Intalap, 1970; Singh and Malik, 1983; Farooq et al., 2014), it is especially critical around and after flowering (Farooq et al., 2014), when drought stress can accelerate leaf senescence and shorten the grain filling period (Blum, 1996). Additionally, we found that water stress around anthesis ranked in the top third of variables for yield and N_{grain} in terms of importance (9th in both models; Supplemental Fig. 1). Temperature during flowering was also of high importance in both random forests. Wheat crop growth models show that under climate change, wheat must exhibit tolerance to heat stress, particularly in central and southern Europe, likely by extending their grain filling period and adapting time of flowering (Kamran et al., 2014; Stratonovitch and Semenov, 2015; Rogger et al., 2021). Losses from heat stress mainly occur due to accelerated senescence and reduced grain number (Talukder et al., 2014). In sum, random forest revealed N applications 1 and 2, as well as environmental variables during flowering as key variables in determining yield and N_{grain}.

Using the partial dependence plot for each N application in our random forest and working within the maximum allowable N amount for Swiss farmers under which an entitlement to direct payments from the state still applies (not to exceeded 90% of the crop requirement or ≈ 160 kg N ha^{-1}; (Fedlex, 2013), we found that an optimal N fertilization regime for our locations was approximately 40-40-40 kg N ha^{-1} to attain high yield, and 40-90-30 kg N ha^{-1} to attain high N_{grain} (Fig. 5). However, our fields had high levels of endogenous N, especially at Reckenholz, that was likely available at the beginning of the season, potentially explaining the low suggested 1st and 2nd N applications for yield. Additionally, in the dataset the first N application was never more than 40 kg ha^{-1}, which is why the first N application rate suggestion for both yield and N_{grain} is 40 kg ha^{-1}. Future tests may include first N applications greater than 40 kg ha^{-1} since the random forests indicates an upward trend for both yield and N_{grain} (Fig. 5). These N regime results may be useful for testing in virtual environments based on our sites using crop modeling (e.g., digital twins), which allows for the testing of multiple years and locations to assess the utility of a fertilization regime. The random forest also reveals that as yield increased beyond 8000 kg ha^{-1}, N_{grain} diminished precipitously, with both parameters reaching a maximum around 8000 kg ha^{-1} for yield and ≈ 2.24% N_{grain} (Fig. 4), pointing toward a yield and N_{grain} goal for producers under the conditions tested here. Current average production since 2000 is 6000 kg ha^{-1} for yield (Herrera et al., 2020; FAO, 2022) while average protein content of top Swiss varieties used here range from approximately 12% to 13.5% (1.92% to 1.16% N_{grain}; swiss granum, 2022b). A reduction in grain protein concentration with increasing yields across multiple varieties has been identified by others (Fowler, 2003; de Oliveira Silva et al., 2020), indicating that setting targets that balance yield and N_{grain} may be necessary for a successful crop.

4.3. Efficient use of nitrogen

Beyond producing a suitable yield and quality, we aimed to assess the N efficiency of each N application regime. We found that, in agreement with current literature, parameters related to the efficient use of N followed general trends with lower levels of applied N leading to greater efficiency of the system, and thus less N prone to loss.

One of the goals of this research was to identify a N application regime that would help improve the efficient use of N resources. Some modern cultivars of wheat have become more efficient under constrained N conditions compared to older cultivars (Guarda et al., 2004; Hategekimana et al., 2012). But overall NUE for winter wheat, and cereals in general, remains low and is estimated to be around 35% globally, but precision crop management and genetics may push NUE as high as 41% (Omara et al., 2019). This indicates that over 60% of N that is applied to a crop is not utilized by the plant and thus prone to environmental loss or used elsewhere (growth of weeds, microbes, etc.). In this study, the average NUE of fertilized plots across all years and sites was 54 and 36 kg kg^{-1} for plots totaling 80 and 160 kg N ha^{-1}, respectively. When fertilizer recovery is high, it is largely associated with greater biomass production (Noulas et al., 2004). With respect to NUE, the N uptake efficiency (NUpE) and the N utilization efficiency (NUtE) are critical processes that determine the way N flows through the soil-plant system (Hawkesford, 2017), where NUpE is the ratio of total N in the crop biomass over the total available N in the soil and applied (Eq. 3), NUtE is the mass of grain produced given the mass of N taken up by the plant (Eq. 4) (Hawkesford and Riche, 2020). Many studies point towards genetic manipulation focused on increasing the N use efficiency (Eq. 2) of crops as a method to increase production efficiency (Good et al., 2004; McAllister et al., 2012; Hawkesford and Riche, 2020). However, we aimed to assess whether N use parameters may be manipulated based on using different N split applications.

Our results show that N_{treat} was a significant driver of NUE, NUpE, and NUtE (Table 5 and Supplemental Table L). However, that was largely due to the total amount of N applied, rather than the rate of the individual splits since N_{treat} was largely not significant between treatments with the same total N applied (Supplemental Tables N and O). The one exception was NUtE at Changins, where $N3_{40\text{-}40\text{-}80}$ resulted in a greater NUtE compared to other 160 kg N ha^{-1} treatments (39 v. 35 kg kg^{-1}). This may be a hint that under some conditions, placing a larger amount of N closer to the period of greatest uptake (near grain filling) may be beneficial in increasing N utilization by the plant. The NUE of cereals can change based on N application splits, for example a single application versus multiple (Sylvester-Bradley and Kindred, 2009). However, we did not detect a difference between two and three nitrogen applications with this parameter. Overall, variety was a significant driver for the utilization of N (as measured by NUtE), similar to previously reported results (Bingham et al., 2012). However, variety was only significant for NUE and NUpE at Reckenholz, indicating a strong dependency on environment × genotype interactions for these variables. Unlike other research (Le Gouis et al., 2000; Cormier et al., 2013), the variety × N_{treat} interaction was not significant for any N use parameter, except for NUtE at Reckenholz, indicating a strong similarity between varieties at similar N levels, potentially due to the background genetics (breeding) of each variety (Table 5 and Supplemental Tables L, N, and O).

Further, we observed a downward trend with N utilization efficiency as N uptake efficiency increased (Supplemental Table L), indicating that greater uptake does not necessarily translate into a greater grain yield. It is possible that although the additional N uptake does not contribute to yield, it may contribute to increasing the N content of the grain. We also found an inverse relationship between NUtE and N_{grain} (Supplemental Fig. 4), which was also observed by Barraclough et al. (2010). This indicates that the physiological response of winter wheat to N uptake and utilization may inhibit producer goals of attaining high quality crops while simultaneously increasing N efficiency. Overall, NUE, NUtE, and NUpE all decreased when total N applied was 160 compared to 80 kg N ha^{-1} (NUE: 36 v 54 kg kg^{-1}; NUtE: 34 v 39 kg kg^{-1}; NUpE 1.1 v 1.4 kg kg^{-1}; Supplemental Table L).

These results point towards the overall importance of total N application rates in determining the efficient use of N. Though N_{treat} did not influence N use variables between treatments of the same total N application rate, future work should assess the volatilization and leaching losses associated with each application regime since application timing is a factor for preventing N loss to the environment and maximizing plant uptake. Additionally, in regions that are more well-suited to growing winter wheat, like Reckenholz, variety choice may play an important role in increasing N use, utilization, and uptake efficiency.

4.4. Environmental impacts (N_{resid} & N_{yield})

Our study aimed to assess the impact of N fertilization regimes on the N left in the soil post-harvest (residual soil N; N_{resid}) that is then prone to loss to the environment. In Switzerland, much attention has been given to reducing the environmental impact of agriculture through emissions reduction and improving the efficient use of nutrients (Decrem et al., 2007; Herzog et al., 2008; FOEN, 2021). N_{resid} as nitrate (NO_3^-) is highly mobile in the soil due to the negative charge and prone to loss through leaching, and N_{resid} as ammonium (NH_4^+) is prone to volatilization. In Switzerland, risks of N leaching under arable land were estimated to be strongly dependent on soil conditions (Klein et al., 2014), but of course also rotational management (in particular the inclusion of cover crops) and amounts and types of fertilizers applied matter (Nemecek et al., 2008; Herzog et al., 2008; McConnell et al., 2023).

We found that N_{resid} was not strongly affected by N_{treat} at either Changins or Reckenholz ($P < 0.05$), and that year had a stronger effect (Supplemental Table K). Furthermore, across all years, average N_{resid} for plots that received a total of 80 kg N ha^{-1} was ≈ 46 and 73 kg ha^{-1} at Changins and Reckenholz respectively, while the average for $N1_{40\text{-}120\text{-}0}$ and $N2_{40\text{-}80\text{-}40}$, which received a total of 160 kg N ha^{-1} was ≈ 41 and 71 kg ha^{-1} at Changins and Reckenholz (Supplemental Table K). At both sites, $N3_{40\text{-}40\text{-}80}$ left less N in the ground (21 and 62 kg N ha^{-1}), but this is likely a year effect due to this N_{treat} was only tested in 2019 and 2020, which were relatively adequate growing years and may have encouraged more complete take up of N from the soil. Further, we found that the suggested fertilization rate from random forest for increasing yield (which accounted for endogenous soil N) resulted in 120 kg ha^{-1} of applied total N while fertilization rates for increasing N_{grain} required higher levels of applied N (Fig. 5). This may indicate that adequate yields may require less N than current standards, but that achieving a high-quality crop requires greater N applications.

Large quantities of N were left in the soil for the $N0_{0\text{-}0\text{-}0}$ treatment as well (37 and 62 kg ha^{-1} at Changins and Reckenholz). These results indicate a potential large amount of soil N mineralization during the growing season in addition to the relatively high levels of endogenous N prior to the period of significant plant growth (Supplemental Table E), or an incomplete use of available resources. At Changins, soy was frequently used as a previous crop (Supplemental Table A), which may add significant amounts of N to the soil with a range of 35–55 kg N ha^{-1} (Power, 1987; Brune et al., 2022). However, the soil at this site tended to be shallow and very stony, which may explain the lower yield and N_{grain} at this site despite residual soil N after harvest. At Reckenholz, the previous crop was potatoes in all years (Supplemental Table B), which require high levels of N fertilization and have low N recovery from the soil and reduced N uptake efficiency (Errebhi et al., 1998; Maltas et al., 2018), thus potentially explaining the relatively large quantities of residual N.

Variety was not significant for the total amount of N taken up by the grain (N_{yield}; Supplemental Table K). Previous work found that the total plant N (N per unit area) can vary considerably with variety (Austin et al., 1977). Our results show that top varieties used in our study are more closely related in terms of the genetics that help control N_{yield}, and thus do not exhibit much variation. N_{treat} was significant for N_{yield} across all sites, but this was partially dictated by the total amount of N applied. N_{treat} was only significant at Goumoëns with 160 total kg N ha^{-1} (Supplemental Tables P and Q; $P < 0.05$). Despite significance, these differences were small (typically less than 10 kg N ha^{-1}). Additionally, on average across all years for each site, the N removed from the soil (N_{yield}) was greater than or equal to the available soil N (calculated as N applied + N_{endog}) for $N0_{0\text{-}0\text{-}0}$ at all sites, $N4_{20\text{-}40\text{-}20}$ and $N5_{20\text{-}60\text{-}0}$ treatments at Goumoëns, and all N_{treats} at Reckenholz (Supplemental Table R). This indicates that, under the conditions tested here, there was likely a considerable amount of N left in the soil, mineralized, and made available to the crop during the season.

In sum, we found that residual soil N is largely a factor of total N applied to the crop rather than the rate of the N splits. However, the N_{yield} of the crop may be influenced by variety in locations and under conditions used in this trial. Additionally, there was considerable N uptake from the soil, likely due to a combination of high levels of endogenous N in the soil, a limited first N application, and a well-suited growing region, which may have contributed to greater yields and protein levels.

5. Conclusion

In this field study we identified a fertilization regime of 40-40-80 kg N ha^{-1} to be optimal with regard to both yield quantity and yield quality (in terms of grain N content), but this needs to be validated across more years, including years with challenging environmental conditions. However, our results could not prove that variety- and region-specific fertilization recommendations could provide additional benefits for increasing nutrient use efficiency. The choice of variety affected grain yield quantity only at the most productive site (Reckenholz). For yield quality, however, varietal choice played a significant role: Runal consistently had a higher grain N content at all sites. Importantly, we identified that Swiss top winter wheat varieties may have been bred to be more similar in yield than in N_{grain}, but that the overall N_{grain} can be manipulated by management practices. Further, a point of maximum yield and N_{grain} was identified at 8000 kg ha^{-1} and ≈ 2.24% N_{grain}. Future breeding efforts may wish to address this N gap between varieties.

Additionally, we aimed to meet Swiss goals of improving N efficiency in cropping systems. Our work revealed that the overall N application throughout the season has a greater bearing on the efficient use of N compared to different N splits. However, in general, we found that small differences between varieties may be more apparent at lower N fertilization rates. If producers adopt low-N input systems, further investigating the differences that emerge between varieties at low N levels is worth exploring. Additionally, environmental impacts (i.e., N losses through leaching and volatilization) should be quantified in these future studies to evaluate potential tradeoffs between production and environmental objectives.

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CRediT authorship contribution statement

Amanda Burton: Data curation, Formal analysis, Investigation, Methodology, Project administration, Writing – original draft, Writing – review & editing. **Lilia Levy Häner:** Conceptualization, Funding acquisition, Methodology, Supervision, Writing – review & editing. **Noémie Schaad:** Data curation, Investigation, Methodology, Project administration. **Silvan Strebel:** Data curation, Investigation, Methodology, Project administration, Writing – review & editing. **Nicolas Vuille-dit-Bille:** Data curation, Investigation, Methodology, Project administration, Writing – review & editing. **Paola de Figueiredo Bongiovani:** Investigation, Methodology, Writing – review & editing. **Annelie Holzkämper:** Methodology, Writing – review & editing. **Didier Pellet:** Conceptualization, Funding acquisition, Methodology, Supervision, Writing – review & editing. **Juan Manuel Herrera:** Conceptualization, Formal analysis, Funding acquisition, Methodology, Supervision, Writing – review & editing.

Declaration of Competing Interest

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Data Availability

Data will be made available on request.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.fcr.2024.109251.

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