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Cover Crops Increase N and P Cycling and Rice Productivity in Temperate Cropping Systems

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Abstract: Cover crops can determine positive benefits on soil fertility and rice productivity, although scant attention has been devoted to evaluating the effects of hairy vetch (*Vicia villosa Roth*) and the incorporation of rice straw with different N fertilization levels on soil N and P availability and crop yields in temperate cropping systems characterized by poorly developed soils. In this study, the effects of cover crops grown before rice in a temperate mono-cropping system (NW Italy) on: (i) crop yields and yield components; (ii) apparent N fertilizer recovery and optimal level of N fertilization with hairy vetch; and (iii) temporal variation of soil available N and P forms during the hairy vetch growth and rice cropping season, have been investigated. The cultivation and incorporation of hairy vetch in the rice cropping system increased grain productivity by 12%, while reducing N mineral fertilization requirements by 33%. Combined with the incorporation of crop residues, hairy vetch provided a N and P input of 178 and 18 kg ha^{-1}, respectively, representing a readily available source for plant uptake over the whole rice cropping season, particularly under anaerobic conditions. This results in a better temporal synchronization of soil N and P availability with crop nutrient demand, leading to a better rice grain productivity and quality performance.

Keywords: cover crops; rice; nitrogen; phosphorus; soil fertility

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1. Introduction

Cereals represent the most diffuse staple food and, among them, rice feeds over 50% of the global population with a consumption of about 500 Mt [1]. With around 159 Mha under cultivation globally, rice (*Oryza sativa* L.) is typically grown in flooded paddies involving the use of large amounts of fertilizers with a low use efficiency [2,3]. The pressing challenge of providing sufficient food for a rapidly growing world population while enhancing environmental sustainability is leading to an increase in the adoption of alternative agronomic techniques [4,5] that can restore the balance in soil carbon (C) input/output and nutritional status [6].

In this regard, cover crops have been shown to have a substantial potential to improve yields and quality of the following cash crops, such as corn (*Zea mays* L.) and soybean (*Glycine max* [L.] Merr.) [7]. Cover crops can be used as green manure to add nutrients to a cropping system. Leguminous cover crops can additionally increase soil fertility through biological nitrogen fixation, i.e., the conversion of atmospheric dinitrogen gas (N_2) into plant available ammonium (NH_4^+) inside legume root nodules occupied by symbiotic rhizobia N-fixing bacteria [8].

Hairy vetch (*Vicia villosa Roth*) is a broadly winter adapted annual legume that can provide up to 100–130 kg N ha^{-1} yr^{-1} to farming systems [9]. This is especially important in rice paddies where N is one of the most yield-limiting nutrients, due to its low recovery efficiency, which is less than 40% globally [10] and around 50% in temperate rice systems [11].

Apart from limiting nitrate losses during winter compared with fallow soils [12], the incorporation of hairy vetch biomass may further influence the processes that control N availability in soil. Indeed, this biomass with a relatively low C/N ratio represents a direct source of N due to a fast N mineralization rate [13]. In addition, vetch is expected to act as primer in accelerating the decomposition of the less degradable rice straw residues with strong short-term changes in their turnover [14]. This may further promote the release of the N pool immobilized by the soil microbial biomass under anoxic conditions [15–17], although it is not clearly understood how these processes affect the temporal variations in N forms during the cropping season and their contribution to available N for plant uptake. Optimizing the synchronization between N release and the rice crop N demand would improve the potential benefits of cover crops while reducing the excessive application of N fertilizers.

Cover crops can also act as a "catch crop" to recover sparingly available nutrient forms in the soil, even in deeper horizons, thereby increasing the fertility in the surrounding rhizosphere. In particular, phosphorus (P) can be taken up by cover crops and released into more superficial soil horizons following the decomposition of plant residues and the mineralization of the microbially immobilized pool [18–21]. In rice paddies, although P availability is rarely considered limiting under continuous flooding, the balance between P mobilization during the reductive dissolution of iron (Fe) (hydr)oxides and P retention during the formation of P-Fe coprecipitates on the rice roots (due to the radial O_2 loss), can actually limit P availability more than was supposedly known until now [22]. In this context, cover crops can further increase P availability: the enhanced microbial activity following the incorporation of the plant biomass and N may boost the release of P from minerals due to the production of protons and organic acids that can dissolve or compete with phosphate for the same sorption sites of Fe and Al (hydr)oxides [23,24]. However, these effects have been poorly investigated under field conditions while relating the interplaying P and N processes as affected by the presence of legume cover crops. Moreover, the few available studies focusing on the effects of cover crops on the availability of P for paddy rice were carried out in tropical or sub-tropical regions [25,26], while the impact on temperate rice producing areas characterized by less developed soils is still scarcely studied.

Although cover crops generally show positive benefits, few studies have assessed the combined effects of hairy vetch and straw incorporation with different N fertilization levels on N and P availability and crop yields in temperate field trials characterized by poorly developed soils. Hence, this study aims to investigate the effects of cover crops grown before rice in a temperate mono-cropping system (NW Italy) on: (i) crop yields and yield components, (ii) apparent N fertilizer recovery and optimal level of N fertilization with hairy vetch; and (iii) temporal variation in soil available N and P forms during the hairy vetch growth and rice cropping season. The following hypotheses were made based on the previous considerations (i) incorporation of hairy vetch will ameliorate the availability of N and P during the whole rice cropping season, (ii) mineral N fertilizer may be reduced without compromising crop productivity, and (iii) the combination of cover crops and the incorporation of straw may increase crop yields and yield components.

2. Materials and Methods

The experimental setting and results of this research are reported from Lizcano Toledo's PhD thesis [27].

2.1. Study Site

The experimental platform was set up in 2016 and located in Nicorvo (PV) (45°17′11.5″ N 8°41′03.1″ E), close to the Rice Research Centre of Ente Nazionale Risi (Castello d'Agogna, PV, NW Italy). The data was collected over the 2019 cropping season (October 2018–October 2019). The site is situated in the low section of the river Po plain, which includes the distal part of the glacial alluvial Würmian flat and is characterized by the presence of bumps of Holocene fluvial dynamics, modified by anthropic soil levelling due to the more recent agricultural processes. The climate is temperate, characterized by hot summers and two main rainy periods in spring and autumn. The mean annual precipitation was 1070 mm (Figure 1), while the mean value for the last 20 years was 704 mm. The mean annual temperature was 17.7 °C, in line with the 20-year mean [11].

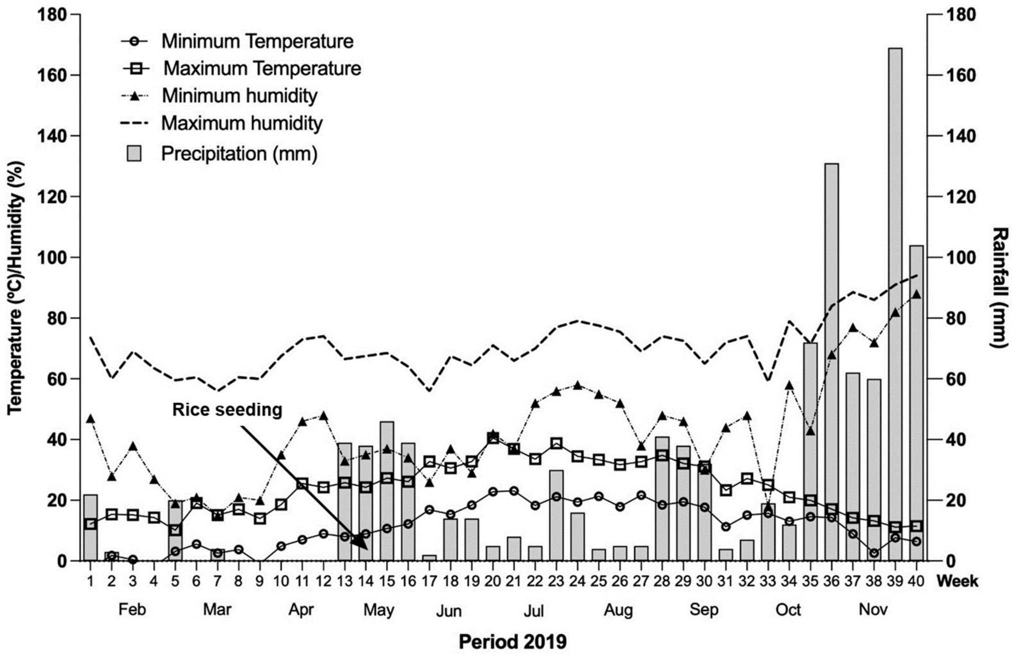

Figure 1. Average weekly air temperature with the maximum, medium and minimum values (°C), and precipitation with maximum, median and minimum humidity (%) over the 2019–2020 experimental period.

The topsoil (0–30 cm) was characterized by a sandy loam texture, with a pH in H_2O (1:2.5 w/v) of 5.0. The average organic C and total N contents were 12.0 and 1.0 g kg^{-1}, respectively, while the cation exchange capacity (CEC) was 12.7 cmol$_{(+)}$ kg^{-1} with 1.5% K saturation.

2.2. Experimental Design

The experimental design was a randomized complete block design, in split-plot arrangement with four replicates. Each replicate included two main factors: (i) with and without hairy vetch in the main plots and (ii) four levels of N fertilization in sub-plots 5 × 6 m each, which were randomly assigned.

Each October, the species *Vicia villosa* var. *Villano* was sown broadcasting seeds directly on the rice straw residues at a density of 50 kg ha^{-1} and was green mulched at the beginning of May. Immediately after cover crop termination, SOLE CL rice variety was dry seeded at a density of 150 kg ha^{-1} in rows spaced 12.5 cm. The field water management involved the maintenance of dry conditions after dry seeding for approximately one month until the tillering stage, towards the second half of June. The plots were subsequently flooded except for a short period in the second half of June and July for the application of herbicide and fertilizer, respectively. Drainage was allowed at the ripening stage (grain moisture between 26–28%), around 20 days before harvest that was carried out in October.

For each treatment, four levels of N fertilization (as urea, 46% N, Yara, Italy) were tested (0, 80, 120 e 160 kg N ha^{-1}), and split in two times during the cropping season (70% at tillering and 30% at panicle differentiation). Phosphorus and potassium were applied at tillering at a dose of 18 kg P ha^{-1} and 75 kg K ha^{-1}.

2.3. Crop Yields, Yield Components and Nutrient Contents

Crop yields, yield components and the N, P and K contents in grain and straw at harvest were determined. The grain yields and straw biomass were determined by harvesting a 0.25 m^2 area in each plot with a combined harvester plot machine, and expressing the results on the basis of a 14% moisture content. The yield components, i.e., the number of panicles m^{-2}, spikelet $panicle^{-1}$, sterility and 10^3 grain weight were determined. The panicle density and the harvest index (i.e., the ratio of the dry grain yield to the dry aboveground plant biomass at harvest), were calculated on three replicated 0.25 m^2 sampling areas within each plot. The tillering rate was calculated as the ratio between the panicle density and the number of plants. The number of spikelets per panicle and the percentage sterility were determined on a sample of 20 panicles for each plot, while the weight of 10^3 grains was determined on two replicates per plot.

The head milled rice and the milled rice yield were obtained using a G390/R dehuller (Colombini & Co. Srl, Abbiategrasso, Milano, Italy) and a TM-05 grain testing mill (Satake Engineering Co., Tokyo, Japan), respectively, through a milling degree of 11.25–12.75%. The broken kernels were separated by a rice length grader (TRG, Satake Engineering Co., Tokyo, Japan). The total N content in the grain and straw was determined by an elemental analysis (NA 2500, Carlo Erba Instruments, Milano, Italy) and expressed on a dry weight basis. The apparent N recovery was calculated as the difference between the aboveground plant N uptake in the fertilized and control plots, normalized to the amount of applied fertilizer N. The total P and K were also determined in the grain and straw after acid digestion and determination by the UV-vis colorimetric method [28] and atomic absorption spectrometry, respectively. A chlorophyll meter (SPAD-502, Minolta Camera Co., Osaka, Japan) was used to obtain SPAD values on the Y leaf. The SPAD readings were acquired every two weeks during rice growth until the maturation stage.

2.4. Soil and Vetch Sampling and Analyses

The soil samples were collected from those plots, with and without vetch, that were fertilized with 120 kg N ha^{-1}. Three soil samples from each replicate were randomly collected and pooled into a composite sample on a monthly basis, from February to September 2019, with a hand corer at 0–20 cm. The composite samples were then immediately sieved at 2 mm and partly used as such for N analyses while an aliquot was air dried for P analyses.

The nitrate and ammonium contents were determined on fresh and sieved soil samples collected during the cropping season. Nitrate and ammonium were extracted from the soils with 1 M KCl for 1 h and determined spectrophotometrically as described by Cucu et al. [16]. Bicarbonate extractable P (P_{Olsen}), assumed to represent the readily bioavailable phosphate, was determined on air-dried soils according to Olsen et al. [29]. An extraction with 0.1 M NaOH + 1.0 M NaCl (1:1) (P_{NaOH}) was also performed to determine the labile P adsorbed on mineral surfaces, precipitated as Fe and Al phosphates [30], and P associated with humic substances. The phosphate content was determined in both extracts by molybdate colorimetry [31].

The vetch biomass and rice residues were also collected from three replicated 0.25 m^2 sampling areas only once, just before soil incorporation. The cover crop roots were carefully separated from the soil, washed with deionized water until soil particle free and then the roots and shoots were separated and air dried. Similarly, the rice residues were washed and dried. The fresh and air-dried biomass was determined as well as the C, N and P contents as described above.

The changes in net N mineralization as well as the total potential mineralizable nitrogen (PMN), as a function of the cover crop and/or the rice straw incorporation, were also evaluated by means of a laboratory soil microcosm experiment in order to elucidate the potential contribution of the incorporated residues on available N for the next rice crop. The soil samples were collected from the 0–15 cm layer of the plots both with and without vetch in April 2019 (prior to tillage). The respective soils received (i) hairy vetch residues and rice straw, or (ii) only rice straw, by applying the biomass in proportions comparable to those estimated in the field. The soils were then incubated under both oxic (maintained at 50% water-filled pore space) and anaerobic (water saturated) conditions for 100 days at a temperature of 25 °C (modified by Sahrawat and Ponnamperuma [32] and Waring and Brenmer [33]). The kinetics of organic N net mineralization were assessed by the determination of available inorganic N at different times, and the data fit into an exponential increase to a maximum (i.e., PMN) model.

2.5. Statical Analysis

The applied statistical model was a two-way ANOVA accounting for the presence of vetch and the N fertilization levels, and their two-way interactions. When the F test was significant ($p < 0.05$), the means were compared using the Bonferroni test. The statistical analysis was performed using SPSS version 26.

3. Results

3.1. Rice Yields and the Yield Components

The introduction of vetch strongly affected the grain yield ($p < 0.05$), with more enhanced effects at low N fertilization levels (Table 1) ($p < 0.01$). The total and straw biomass increased in the presence of vetch as well ($p < 0.01$ and $p < 0.05$, respectively), following the fertilization with significant differences at all levels ($p < 0.05$ and $p < 0.01$ for total and straw biomass, respectively). The harvest index was hence lower in the presence of vetch at the highest N levels, with a significant interaction cover crop × fertilization ($p < 0.05$).

Table 1. Performance of the cover crop management (+Vetch or −Vetch) alone or in interaction with the four levels of N fertilization in terms of grain yield, total and straw biomass, harvest index and the significance of the different analyzed effects.

N Fertilization Level (kg N ha^{-1})	Cover Crop Management	Grain Yield (t ha^{-1})	Total Biomass (t ha^{-1})	Straw Biomass (t ha^{-1})	Harvest Index (%)
Average	+Vetch	8.5 ^{a}	17.2 ^{a}	8.7 ^{a}	50 ^{a}
	−Vetch	7.6 ^{b}	15.2 ^{b}	7.6 ^{b}	50 ^{a}
0		6.5 ^{c}	12.8 ^{c}	6.3 ^{c}	51^{a}
80		8.3 ^{b}	16.4 ^{b}	8.2 ^{b}	50 ^{ab}
120		8.8 ^{a}	17.7 ^{a}	9.0 ^{a}	49 ^{ab}
160		8.7 ^{a}	17.8 ^{a}	9.1 ^{a}	49 ^{b}
0	+Vetch	7.6 ^{b}	14.5 ^{c}	6.9 ^{e}	52 ^{a}
	−Vetch	5.5^{c}	11.0 ^{d}	5.5 ^{f}	50 ^{ab}
80	+Vetch	8.8 ^{a}	17.4 ^{ab}	8.7 ^{bc}	50 ^{ab}
	−Vetch	7.8 ^{b}	15.5 ^{c}	7.7 ^{de}	50 ^{ab}
120	+Vetch	8.9 ^{a}	18.4 ^{a}	9.5 ^{ab}	48 ^{bc}
	−Vetch	8.6 ^{a}	17.1 ^{b}	8.5 ^{cd}	50 ^{ab}
160	+Vetch	8.9 ^{a}	18.6 ^{a}	9.7 ^{a}	48 ^{c}
	−Vetch	8.5 ^{a}	17.0 ^{b}	8.5 ^{cd}	50 ^{abc}
	Sources		***P*(F) values**		
	Cover crop management	0.050	0.010	0.050	ns
	Fertilization	0.010	0.050	0.010	ns
	Cover crop management × fertilization	0.010	ns	ns	0.050

Within a column for each cover crop management or for each fertilization level, the means followed by different letters are significantly different according to Bonferroni's post hoc test. ns: not significant.

The presence of vetch also influenced the yield components (Table 2). The different grain yields were related to the number of spikelets on average, which showed higher values in the presence of vetch ($p < 0.05$), with no significant differences due to the fertilization levels. An increase in the 10^3 grain weight was also observed with vetch ($p < 0.05$), whereas a progressive decrease was caused by the increasing N levels ($p < 0.01$). Conversely, the panicle density did not show any effect determined by vetch, but the increasing values from 426 to 562 panicles m^{-2} were due to the different levels of N fertilization ($p < 0.01$). This was reflected on the tillering rate with no differences with or without vetch, but the increasing values with N fertilization levels ($p < 0.01$). On the other hand, the increasing values of the applied N caused a higher sterility reaching 19.5% in the highest fertilized plot ($p < 0.01$).

Table 2. Performance of the cover crop management (+Vetch or −Vetch) alone or in interaction with the four levels of N fertilization in terms of yield components (spikelets, 10^3 seeds weight, panicle density, sterility), tillering rate, milled rice yield, damaged kernels and chalkiness and the significance of the different analyzed effects.

N Fertilization (kg N ha^{-1})	Cover Crop Management	Spikelets (panicle^{-1})	10^3 Seeds Weight (g)	Panicle Density (m^{-2})	Sterility (%)	Tillering Rate	Milled Rice Yield (%)	Damaged Kernels (%)	Chalkiness (%)
Average	+Vetch	142 ^{a}	25.1 ^{a}	509 ^{a}	16.0 ^{a}	2.3 ^{a}	71.4 ^{a}	0.48 ^{a}	1.3 ^{a}
	−Vetch	129 ^{b}	24.3 ^{b}	490 ^{a}	14.2 ^{a}	1.9 ^{a}	71.5 ^{a}	0.54 ^{a}	1.0 ^{a}
0		134 ^{a}	25.5 ^{a}	426 ^{c}	10.1 ^{c}	1.7 ^{c}	71.6 ^{a}	0.61 ^{ab}	0.9 ^{b}
80		132 ^{a}	24.8 ^{b}	483 ^{b}	14.2 ^{b}	2.0 ^{bc}	71.5 ^{ab}	0.63 ^{a}	1.2 ^{ab}
120		133 ^{a}	24.4 ^{c}	528 ^{ab}	16.7 ^{b}	2.3 ^{ab}	71.3 ^{b}	0.42 ^{bc}	1.2 ^{ab}
160		145 ^{a}	24.1 ^{d}	562 ^{a}	19.5 ^{a}	2.4 ^{a}	71.3 ^{b}	0.39 ^{c}	1.3 ^{a}
0	+Vetch	143 ^{ab}	25.0 ^{bc}	432 ^{de}	11.0 ^{d}	1.8 ^{bc}	71.6 ^{a}	0.69 ^{ab}	1.1 ^{ab}
	−Vetch	124 ^{b}	26.0 ^{a}	420 ^{e}	9.1 ^{d}	1.5 ^{c}	71.6 ^{a}	0.53 ^{abc}	0.7 ^{b}
80	+Vetch	133 ^{ab}	24.6 ^{cd}	497 ^{bcd}	15.7 ^{bc}	2.1 ^{ab}	71.5 ^{a}	0.52 ^{abc}	1.2 ^{ab}
	−Vetch	131 ^{b}	25.0 ^{b}	469 ^{cde}	12.7 ^{cd}	1.9 ^{bc}	71.5 ^{a}	0.74 ^{a}	1.2 ^{ab}
120	+Vetch	141 ^{ab}	24.0 ^{e}	530 ^{abc}	18.6 ^{ab}	2.4 ^{a}	71.1 ^{b}	0.40 ^{bc}	1.3 ^{a}
	−Vetch	124 ^{b}	24.9 ^{bc}	527 ^{abc}	14.7 ^{c}	2.2 ^{ab}	71.5 ^{a}	0.41 ^{bc}	1.1 ^{ab}
160	+Vetch	151 ^{a}	23.8 ^{e}	579 ^{a}	19.0 ^{ab}	2.6 ^{a}	71.3 ^{ab}	0.31 ^{c}	1.5 ^{a}
	−Vetch	138 ^{ab}	24.4 ^{d}	545 ^{ab}	20.0 ^{a}	2.2 ^{ab}	71.3 ^{ab}	0.47 ^{abc}	1.1 ^{ab}
	Sources				***P*(F) values**				
	Cover crop management	0.050	0.050	ns	ns	ns	ns	ns	ns
	Fertilization	ns	0.010	0.010	0.010	0.010	0.050	0.050	ns
	Cover crop management × fertilization	ns	ns	ns	ns	ns	ns	ns	ns

Within a column for each cover crop management or for each fertilization, the means followed by the different letters are significantly different according to Bonferroni's post hoc test. ns: not significant.

Conversely to the grain yield, the derived milled rice yield was not affected by vetch and N fertilization level, except for the control plots (0 kg N ha^{-1}, $p < 0.05$) (Table 2). Vetch did not affect either the quality of the rice in terms of damaged kernels and chalkiness. Notwithstanding the low observed variability, the highest percentage of damaged kernels was observed at the lowest N fertilization levels ($p < 0.05$), whereas the highest percentage of chalkiness was at the highest N fertilization level, although not statistically significant.

3.2. Rice Nutrient Uptake and N Apparent Recovery

The grain N content was significantly affected by the presence of vetch ($p < 0.01$) as well as by the level of fertilization ($p < 0.01$) with increasing values up to 120 kg N ha^{-1} (Table 3). However, significant differences between the plots with and without vetch were observed even at the highest N fertilization level. In general, the straw N content followed the same trend with significant differences due to the cover crop management ($p < 0.05$) and N fertilization levels ($p < 0.01$), although smaller differences were evidenced at 0 or 80 kg N ha^{-1}. In terms of the total N uptake, higher values were always obtained with vetch, being on average 45% more than the plots without vetch ($p < 0.01$) at all N fertilization levels ($p < 0.01$). Nevertheless, the apparent fertilizer N recovery was not affected by the treatments and, although the values were in general higher with vetch than without, the differences were not statistically different.

Table 3. Performance of the cover crop management (+Vetch or −Vetch) alone or in interaction with the four levels of N fertilization in terms of the grain and straw N contents, the total N uptake and the apparent N recovery, the grain and straw P and K contents and the total P and K uptake, and the significance of the different analyzed effects.

N Fertilization (kg N ha^{-1})	Cover Crop Management	Grain N (%)	Straw N (%)	Total N Uptake (kg ha^{-1})	Apparent N Recovery (%)	Grain P (%)	Straw P (%)	Total P Uptake (kg ha^{-1})	Grain K (%)	Straw K (%)	Total K Uptake (kg ha^{-1})
Average	+Vetch	1.40 ^{a}	0.84 ^{a}	195 ^{a}	67.3 ^{a}	0.26 ^{a}	0.16 ^{a}	38.5 ^{a}	0.28 ^{a}	2.21 ^{a}	218 ^{a}
	−Vetch	1.21 ^{b}	0.74 ^{b}	149 ^{b}	62.0 ^{a}	0.28 ^{a}	0.16 ^{a}	32.0 ^{b}	0.27 ^{a}	1.69 ^{b}	150 ^{b}
0		1.11 ^{c}	0.65 ^{c}	114 ^{c}	-	0.24 ^{c}	0.14 ^{b}	24.4 ^{b}	0.23 ^{c}	1.77 ^{d}	127 ^{b}
80		1.26 ^{b}	0.75 ^{bc}	166 ^{b}	63.3 ^{a}	0.28 ^{b}	0.15 ^{b}	34.7 ^{a}	0.29 ^{b}	1.87 ^{c}	179 ^{ab}
120		1.40 ^{a}	0.85 ^{ab}	199 ^{a}	71.2 ^{a}	0.29 ^{a}	0.17 ^{a}	41.3 ^{a}	0.30 ^{a}	2.04 ^{b}	211 ^{a}
160		1.45 ^{a}	0.92 ^{a}	209 ^{a}	59.5 ^{a}	0.28 ^{b}	0.18 ^{a}	40.6 ^{a}	0.28 ^{b}	2.11 ^{a}	218 ^{a}
0	+Vetch	1.17 ^{cd}	0.66 ^{c}	134 ^{c}	-	0.27 ^{ab}	0.13 ^{bc}	29.7 ^{bc}	0.28 ^{ab}	1.97 ^{b}	158 ^{bc}
	−Vetch	1.05 ^{d}	0.65 ^{c}	93.9 ^{d}	-	0.21 ^{b}	0.14 ^{bc}	19.1 ^{c}	0.18 ^{b}	1.58 ^{c}	97.3 ^{c}
80	+Vetch	1.38 ^{b}	0.80 ^{abc}	190 ^{b}	65.7 ^{a}	0.28 ^{a}	0.15 ^{b}	37.4 ^{ab}	0.28 ^{ab}	2.12 ^{b}	208 ^{ab}
	−Vetch	1.13 ^{d}	0.70 ^{c}	143 ^{c}	60.9 ^{a}	0.28 ^{a}	0.14 ^{bc}	32.1 ^{b}	0.30 ^{a}	1.63 ^{c}	150 ^{bc}
120	+Vetch	1.52 ^{a}	0.96 ^{a}	227 ^{a}	77.1 ^{a}	0.29 ^{a}	0.19 ^{a}	43.5 ^{a}	0.30 ^{a}	2.33 ^{a}	247 ^{a}
	−Vetch	1.27 ^{bc}	0.74 ^{bc}	172 ^{b}	65.3 ^{a}	0.30 ^{a}	0.16 ^{b}	39.0 ^{ab}	0.31 ^{a}	1.76 ^{b}	175 ^{abc}
160	+Vetch	1.53 ^{a}	0.96 ^{a}	229 ^{a}	59.1 ^{a}	0.29 ^{a}	0.18 ^{ab}	43.2 ^{a}	0.28 ^{ab}	2.42 ^{a}	259 ^{a}
	−Vetch	1.36 ^{b}	0.88 ^{ab}	189 ^{b}	59.9 ^{a}	0.26 ^{bc}	0.18 ^{ab}	38.0 ^{ab}	0.27 ^{ab}	1.81 ^{b}	178 ^{abc}
	Sources					***P*(F) values**					
	Cover crop management	0.010	0.050	0.010	ns	ns	ns	0.050	ns	0.010	0.001
	Fertilization	0.010	0.010	0.010	ns	0.050	0.050	0.001	0.050	0.050	0.010
	Cover crop management × fertilization	ns	ns	ns	ns	ns	ns	0.001	ns	ns	0.001

Within a column for each cover crop management or for each fertilization, the means followed by different letters are significantly different according to Bonferroni's post hoc test. ns: not significant.

The different N uptake was confirmed by the SPAD measurements in the leaves during the growing season (Table 4), which values were increasingly higher with vetch than without, starting from tillering to harvesting with a progressively more enhanced effect of the fertilization level. The interaction cover crop management × N fertilization was also significant at DAS 42 ($p < 0.05$). The greatest differences were recorded in the second phase of the crop cycle.

Table 4. Chlorophyll content (SPAD) in the rice leaves at different times (day after seeding, DAS) during the cropping season as affected by the cover crop management (+Vetch or −Vetch) alone or in interaction with the four levels of N fertilization, and the significance of the different analyzed effects.

N Fertilization (kg N ha^{-1})	Cover Crop Management	Days after Seeding						
		31	42	55	67	81	94	109
Average	+Vetch	38.6 ^{a}	42.9 ^{a}	37.1 ^{a}	34.5 ^{a}	38.0 ^{a}	40.2 ^{a}	39.2 ^{a}
	−Vetch	35.0 ^{b}	41.9 ^{a}	36.0 ^{a}	31.8 ^{b}	35.3 ^{b}	37.5 ^{b}	36.1 ^{b}
0		36.4 ^{a}	36.0 ^{c}	32.0 ^{d}	30.9 ^{c}	32.1 ^{d}	34.7 ^{c}	33.3 ^{d}
80		37.1 ^{a}	43.6 ^{b}	35.7 ^{c}	32.8 ^{b}	35.7 ^{c}	38.4 ^{b}	36.6 ^{c}
120		36.9 ^{a}	45.0 ^{a}	38.4 ^{b}	33.9 ^{b}	38.1 ^{b}	40.6 ^{a}	39.2 ^{b}
160		36.9 ^{a}	45.3 ^{a}	40.1 ^{a}	35.1 ^{a}	40.6 ^{a}	41.6 ^{a}	41.5 ^{a}
0	+Vetch	38.2 ^{a}	37.4 ^{a}	32.9 ^{a}	32.2 ^{a}	33.7 ^{a}	36.1 ^{a}	34.3 ^{a}
	−Vetch	34.7 ^{b}	34.6 ^{b}	31.2 ^{a}	29.6 ^{b}	30.6 ^{b}	33.3 ^{b}	32.2 ^{b}
80	+Vetch	39.1 ^{a}	44.4 ^{a}	36.5 ^{a}	34.6 ^{a}	36.8 ^{a}	39.7 ^{a}	38.1 ^{a}
	−Vetch	35.1 ^{b}	42.8 ^{b}	34.9 ^{b}	31.0 ^{b}	34.7 ^{b}	37.1 ^{b}	35.1 ^{b}
120	+Vetch	38.7 ^{a}	45.0 ^{a}	38.7 ^{a}	35.4 ^{a}	39.5 ^{a}	42.1 ^{a}	40.9 ^{a}
	−Vetch	35.2 ^{b}	45.0 ^{a}	38.1 ^{a}	32.5 ^{b}	36.7 ^{b}	39.1 ^{b}	37.5 ^{b}
160	+Vetch	38.6 ^{a}	45.1 ^{a}	40.4 ^{a}	36.0 ^{a}	42.1 ^{a}	42.8 ^{a}	43.5 ^{a}
	−Vetch	35.2 ^{b}	45.4 ^{a}	39.9 ^{a}	34.3 ^{b}	39.2 ^{b}	40.4 ^{b}	39.5 ^{b}
	Sources	***P*(F) values**						
	Cover crop management	0.050	ns	ns	0.010	0.010	0.010	0.010
	Fertilization	ns	0.010	0.010	0.010	0.010	0.010	0.010
	Cover crop management × fertilization	ns	0.050	ns	ns	ns	ns	ns

Within a column for each cover crop management or for each fertilization, the means followed by different letters are significantly different according to Bonferroni's post hoc test. ns: not significant.

The grain and straw were also analyzed for their P and K content (Table 3). The presence of vetch did not affect the content of the two macronutrients in both compartments, except the K content in the straw, which was higher with vetch at all N levels. Independently of the presence of vetch, P and K contents in both the grain and straw generally increased with N fertilization levels. Notwithstanding the similar content, the total P and K plant uptake resulted to be significantly higher with vetch (Table 3).

3.3. Vetch Biomass and Nutrient Uptake

As expected, the vetch total biomass increased from 0.6 to 1.5 g DW plant^{-1} before the paddy ploughing, corresponding to a final value of 4.5 t DW ha^{-1} (Figure 2). This increase was mostly related to the shoot development although the shoot/root ratio remained around 3 for the whole period. Both N and P concentrations remained almost constant in the shoots and roots with growth. Nitrogen reached 35 and 42 mg g^{-1} in the shoot and root, respectively, corresponding to 165 kg N ha^{-1}. Phosphorus reached 2.4 and 3.3 mg g^{-1} in shoot and root, respectively, corresponding to 11.2 kg P ha^{-1} while C was 427 and 387 mg g^{-1}, corresponding to 1.87 t C ha^{-1}.

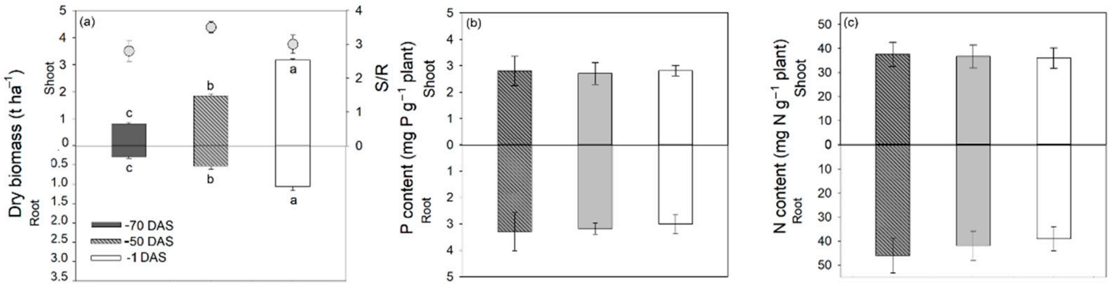

Figure 2. Vetch shoot and root biomass (bars) and shoot/root ratio (circles) (**a**), Total P (**b**) and N content (**c**) during the winter growth (February–May 2019). Error bars represent ± standard deviation. Bars with different letters differ for $p < 0.05$.

3.4. Soil Nitrogen and Phosphorus Forms and Temporal Dynamics

Prior to crop residue incorporation, the vegetal biomass present on the plots in which the vetch was cultivated was composed of 3.2 t ha^{-1} of aboveground cover crop biomass (C/N ≈ 12.0) and 6.5 t ha^{-1} of rice crop residues (C/N ≈ 30.4) equivalent to a total N input of around 178 kg N ha^{-1} (C/N of the mixture = 24.4) and corresponding to 82 mg N kg^{-1} considering a bulk density of 1.44 g m^{-2} and a soil depth of 15 cm. The plots that were kept fallow during the winter period only had a similar amount of rice crop residues equivalent to 61 kg N ha^{-1} (28 mg N kg^{-1}) and some weeds that however did not contribute substantially to the total N inputs and were therefore excluded.

The changes in net N mineralization as well as the total potential mineralizable nitrogen (PMN) as a function of the cover crop and/or rice straw incorporation were evaluated by means of a laboratory soil microcosm under anaerobic and aerobic conditions. Under anaerobic conditions, a faster and more intense net N mineralization was observed in the presence of vetch compared with the control (Figure 3a), with greater values of potentially mineralizable N even in the last stages of the growing season, reaching 108 ± 10 mg N kg^{-1} with respect to 78 ± 10 mg N kg^{-1} without vetch. This was related to the prompt net release of ammonium accompanied by a rapid disappearance of nitrate forms under these conditions. Moreover, the release of the mineral N (mainly in the form of nitrate) was slower under aerobic conditions and resulted in a generally lower net N mineralization (Figure 3b). Nitrification during the first weeks after residue incorporation seemed to be limited in the presence of vetch with respect to the control (Figure 3b inlay). Overall, aerobic conditions resulted in lower values of potential mineralizable N with respect to anaerobic conditions, however the values were nonetheless higher in the presence of vetch (71 ± 5 mg N kg^{-1}) with respect to the control (37 ± 3 mg N kg^{-1}).

Considering the soil nutrient availability in the field during both the hairy vetch and rice cropping seasons (Figure 4), it was observed that extractable nitrate-N concentrations were relatively low with values <1 mg kg^{-1} soil until vetch incorporation (Figure 4b). Then, a peak to 13 and 7 mg N kg^{-1} was recorded in the soil with and without vetch, respectively, before the first fertilization, and then the values decreased, to around 1–2 mg N kg^{-1}. Ammonium showed slightly, but the significantly higher values during the whole crop season with a peak two weeks later than that registered for nitrate (at DAS 48), corresponded to the mineral N fertilization at tillering stage.

With regards to phosphorus, prior to crop residue incorporation, the hairy vetch and crop residue biomass present on the plots in which vetch was cultivated showed P contents of 2.4 and 1.6 mg g^{-1}, respectively, contributing to a total P input of approximately 18 kg ha^{-1}. The plots that were kept fallow during the winter period only had the contribution of rice crop residues equivalent to 10 kg P ha^{-1}.

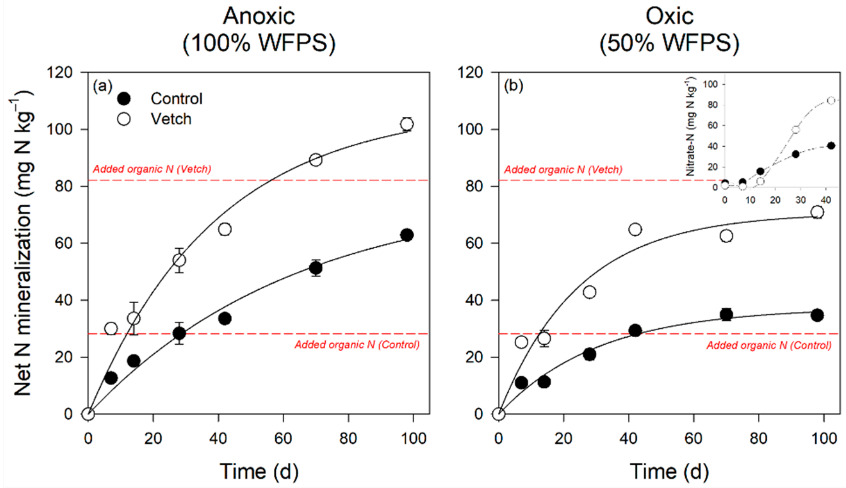

Figure 3. Potentially mineralizable nitrogen kinetics in the microcosm soils with the incorporation of hairy vetch + crop residues (black circles) and only crop residues (gray circles) under anaerobic (**a**) and aerobic conditions (**b**). Dashed lines indicated the input of N with the straw residues and hairy vetch + straw residues. In the inlay (**b**) nitrate-N concentrations in the soil subjected to incubation under aerobic conditions.

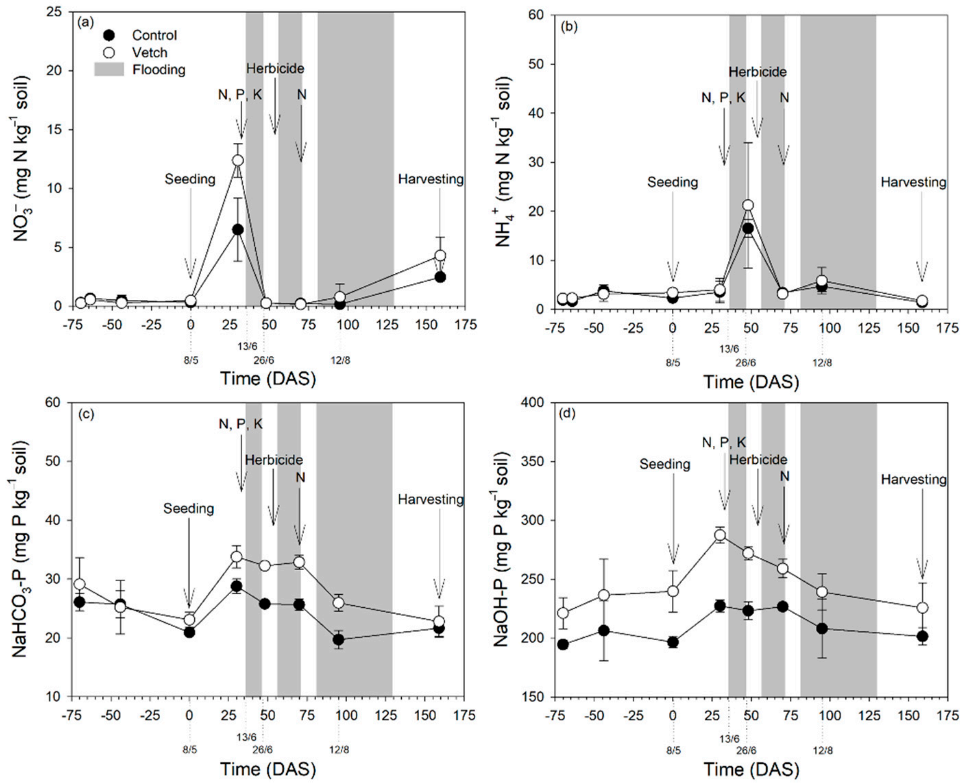

Figure 4. Extractable nitrate-N (**a**), ammonium-N (**b**), $NaHCO_3$-P (**c**) and NaOH-P (**d**) concentrations in the soil during the hairy vetch and rice growing season.

Compared with inorganic N, soil P was more affected by the cover crop management. Both $NaHCO_3$-P, which represents the readily available P, and NaOH-P, i.e., the moderately available P forms, were higher with vetch than without, with a strong increase corresponding to the tillering stage. The values thereafter progressively decreased maintaining, however, strong differences between the two treatments.

4. Discussion

4.1. Hairy Vetch Increases the Rice Yield Performance as a Function of N Fertilizer Levels

The leguminous cover crop green mulching strongly affected the rice productivity. These results were in agreement with a number of works [34,35], that found that cover crops significantly stimulate rice growth and plant yield. Interestingly, the benefit of the cover crop was more evident at low N fertilization levels with a cover crop × fertilizer interaction. With 80 kg N ha^{-1}, the rice crop associated with hairy vetch reached indeed a great performance in grain productivity, being 12% higher than in the plot without vetch and similar to that obtained with 120 and 160 kg N ha^{-1}, that would imply saving up to 33% of mineral N addition. Similarly, Yang et al. [14] found that the incorporation of green manure improved early and late rice yields in Northern China allowing a reduction of 20% of the recommended amount of chemical fertilizer.

Apart from a higher grain productivity, the plots with vetch also showed a higher straw biomass, producing on average 1.1 t ha^{-1} more than without vetch, with significant differences at all N levels. The highest straw biomass was reached when vetch was combined with a fertilization of 120 kg N ha^{-1}, probably due to a greater allocation of N in the grain, requiring higher N input for maintaining an adequate availability to build the straw N budget [36,37].

Within the components of the rice yield, the only factors affected by vetch were the number of spikelets per panicle and, to a lower extent, the weight of 10^3 seeds as found by Kaewpradit et al. [38], Yang et al. [14] and Nie et al. [39] following the different combinations of legume residues and grasses. This implies that the increase in rice grain yield determined by the vetch was a result of a more vigorous growth of rice plants mainly at or before the tillering stage [40], although no cover crop management effect was highlighted on the panicle density. The cover crop, rather than the N fertilization levels, indeed seemed to favor rice growth during the first stages of the crop development, as confirmed by the higher chlorophyll content observed at 31 DAS. However, at the mid-tillering stage, the positive effect of vetch on the SPAD values was negligible, and at panicle initiation, when the rice growth rate increased, the effect was reversed with greater differences due to the N fertilization levels. Thereafter, vetch incorporation combined with the N levels again affected chlorophyll content, probably suggesting the positive effect of the legume residues on maintaining and supporting an appropriate soil N availability even at the late growth stages. The lower SPAD values observed at the reproductive stage (67 DAS) in both treatments, with and without vetch, could be attributed to a higher extraction of N from the leaves for filling the grain [41].

In general, the higher SPAD values were mirrored by a greater total N uptake with vetch at all N fertilizer levels, indicating that under integrative techniques, the increased yield sink capacity and the N use efficiency were closely related to the improvements in the physiological characteristics of rice, such as the increases in the percentage of productive tillers and the leaf photosynthetic rate [42].

The vetch did not significantly affect grain sterility, which was instead strongly influenced by the N fertilization levels [43], increasing consistently from 10.1 up to 19.5%. This suggests that vetch could indirectly prevent the disease, by reducing the amount of chemical fertilizer required for obtaining optimal productivity performances. Combining vetch with 80 kg N ha^{-1}, the grain sterility was indeed reduced by 15 and 27%, with respect to 120 and 160 kg N ha^{-1}, respectively. This, together with a reduced percentage of chalkiness, may guarantee a higher grain quality while maintaining a sustainable productivity.

4.2. Hairy Vetch Increases Soil N and P Availability

The positive aforementioned results were related to the vetch biomass input. In fact, hairy vetch showed a great capacity to accumulate aerial biomass and N in temperate climate conditions [44], corresponding to 4.5 t ha^{-1} of dry biomass and 165 kg N ha^{-1}. Apart from increasing the total N input to the soil, the vetch biomass appeared to rapidly contribute to the mineral soil N pools promptly increasing the available N during the first vegetative phases of rice growth. Indeed, net N mineralization, as a result of the balance between gross mineralization and immobilization (and other losses) processes, in the oxic microcosms with vetch biomass incorporation, reached values in excess of 60 mg N kg^{-1} after 40 days (equivalent to around 140 kg N ha^{-1}), while the potential mineralizable N reached a maximum estimated value of 70 mg N kg^{-1} (about 150 kg N ha^{-1}) comparable to the N input derived from the highest mineral fertilization level (160 kg N ha^{-1}). This was not only related to the intrinsic greater decomposability of vetch characterized by a relatively low C/N ratio [45], but due to the synergic effect on the co-decomposition and/or positive priming of the rice straw residues [41,46]. In fact, the incorporation of rice straw alone having a relatively high C/N ratio resulted in a lower estimated potential mineralizable N of 37 mg N kg^{-1} due to both the lower total N input and the higher N immobilization [3,16,17]. The net N mineralization under anaerobic conditions was generally greater than under aerobic conditions with the estimated potential mineralizable N in the presence of hairy vetch reaching a value of 108 mg N kg^{-1} equivalent to 234 kg N ha^{-1}, confirming a net release of plant available N over the whole rice cropping season. This could be attributed to the low metabolic N requirements of the anaerobic microbial community (i.e., less microbial N immobilization [47]), as well as the positive feedback of the labile OM addition on the subsequent desorption and mineralization of soil-derived dissolved organic N [48].

The increasing N availability can explain the greater tillering rate observed in the field and the higher SPAD values found at the vegetative stage in the presence of vetch [49]. However, more interestingly was the fact that, under anaerobic conditions, the incorporation of vetch continuously fed the N available pool even in the 60–100 days span, favoring net N mineralization beyond the amount of organic N incorporated into the soil. This may lead to a continuous source of NH_4^+ during the periods of high N requirement by rice, corresponding to the flowering and grain filling stage [34,50]. The benefit of the incorporation of the cover crop biomass on N availability was thus evident not only during the early stages of the rice crop, with an increase in soil nitrate under oxic conditions (typical of dry seeded rice), but also during the late phenological stages. This was not mirrored by a consequent trend in the soil extractable NH_4^+ observed in the field that was constantly low throughout the whole cropping season, probably undermined by the rice N uptake. The N dynamics occurring in the controlled microcosms which excluded plant N uptake, clearly highlighted the positive effect of vetch over the long term period.

Regarding P, although no differences were found in its content in both the rice grain and straw, the total P uptake was significantly affected by the presence of vetch, as a consequence of the greater biomass. The higher P input derived by the hairy vetch was accompanied by a greater soil P availability acquired over the years of cover crop management. Significant changes in the $NaHCO_3$-P content were indeed found starting from the seeding date and reaching values 25% higher with hairy vetch than without at the tillering stage. This P fraction is considered as labile P and its changes are largely influenced by biocycling, which is favored in the presence of a highly degradable residue, such as hairy vetch [51,52], thereby enhancing the fast recycling of P immobilized in both the hairy vetch and straw biomass. In this respect, hairy vetch is also known to exude organic acids and protons that can increase the release of P from minerals feeding the available pool [45,53]. This can create the conditions for improving the ability of rice roots to acquire P from the solid phase [54].

The fraction of P extracted with NaOH was in general ten times higher than that solubilized with $NaHCO_3$. The NaOH-Pi fraction, which includes P adsorbed on the Fe and Al (hydr)oxide surfaces or precipitated as Fe and Al salts, is generally reported to be the highest in paddy environments [55]. This is due to the reducing conditions during the flooding period, which cause the reductive dissolution of Fe (hydr)oxides by Fe-reducing microorganisms, the transportation of soluble Fe^{2+}, and redistribution of Fe (hydr)oxides [56–58], facilitating the release of P associated to their surfaces. Under prolonged reducing conditions, the fraction of P released after the reduction of the Fe minerals can also be subsequently readsorbed on, or co-precipitated with, the newly formed, scarcely stable Fe phases [59,60], which are easily extractable with NaOH. The presence of hairy vetch emphasized the P release, since NaOH-P content was significantly higher even during the vetch growth. Organic acid anions exuded by vetch can contribute to the P release by competing with phosphate for the same sorption sites [24,61]. Moreover, the incorporation of fresh cover crop biomass can further enhance the activity of Fe-reducing microorganisms, which are C limited, favoring the indirect P release via Fe reduction during flooding [62]. The higher values found before vetch incorporation may highlight the legacy P effect due to repeated conditions over the years.

5. Conclusions

The cultivation and incorporation of hairy vetch in rice cropping systems showed important beneficial effects in the soil-plant system, providing a substantial source of N for the rice plant that was readily available for plant uptake over the whole rice cropping season, particularly under anaerobic conditions. The potential of vetch to provide an easily degradable biomass also enhanced rice P uptake, by increasing the available pool with an important contribution from P forms that are considered sparingly accessible in aerobic soils. This may result in a better temporal synchronization of soil N and P availability with the crop demand, leading to a better grain productivity and quality performance, while reducing the N mineral fertilization requirements from 120 to 80 kg N ha^{-1}, corresponding to 33.3% saving in the mineral N inputs.

Cultivating vetch during the cold fallow season thus represents a recommendable agronomic practice for improving soil fertility and nutrient use efficiency, especially in monocropping temperate rice systems, although further investigation is required to better understand the interacting and co-regulated processes that control the N and P availability.

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