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Large-Scale Evaluation of Official Containment Measures Against *Meloidogyne graminicola* Golden & Birchfield in European Rice Fields

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Abstract

The root-knot nematode *Meloidogyne graminicola* is a major rice pest recently introduced into Europe. In Lombardy (northern Italy), an official containment programme has been implemented to limit its spread while allowing rice cultivation to continue in infested areas. This study provides a large-scale evaluation of the field performance of the phytosanitary measures implemented under commercial farming conditions between 2022 and 2025. Field performance differed among management measures and according to the phytosanitary status of fields at the beginning of each annual observation. Trap-cropping showed high operational performance while allowing continued rice production, and crop rotation was also associated with favourable outcomes. In contrast, prolonged post-harvest flooding showed more variable performance under local environmental and operational conditions. Analysis of three-year management programmes further indicated substantial differences among the management sequences implemented in the monitored fields. Programmes combining crop rotation and trap-cropping were among those with the highest observed proportions of hectares meeting the official eradication criterion, although management sequences were not experimentally assigned. Overall, the results show that official containment measures can support the progressive recovery of infested fields under commercial farming conditions. The Lombardy experience provides large-scale operational evidence to inform the development of containment-based strategies for *M. graminicola* in European rice-growing areas, while highlighting the need to adapt management programmes to local agronomic and environmental conditions.

Keywords: *Meloidogyne graminicola*; pest control; pest management; rice; root-knot nematode; measures

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1. Introduction

Rice (*Oryza sativa* L.) is a staple food for a large share of the global population and sustains the livelihoods of many rural communities. Despite its adaptability to diverse environments, its productivity remains sensitive to water availability and to the emergence of pests whose impact is increasingly influenced by environmental change. Among these, the root-knot nematode *Meloidogyne graminicola* Golden & Birchfield has become one of the most important constraints to rice production worldwide [1,2].

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M. graminicola induces galls and multinucleated giant cells that impair root function. While rice is its major host, the nematode can infest more than 150 plant species across several botanical families, including common rice-field weeds that act as reservoirs [3]. Weed species such as *Alisma plantago-aquatica* L., *Cyperus difformis* L., *Echinochloa crus-galli* P. Beauv. and *Heteranthera reniformis* Ruiz & Pav. can maintain nematode populations between rice crops, making weed management an essential component of integrated control strategies [4].

The nematode survives in the soil mainly as eggs, which contain the first juvenile stage. Under favourable environmental conditions, second-stage juveniles invade rice roots and induce the characteristic hook-shaped terminal galls. A distinctive feature of *M. graminicola* is its ability to survive flooding by persisting within the aerenchyma of graminaceous hosts, allowing the nematode to remain active even under prolonged submergence [5]. Infested plants typically show reduced vigour, stunting, chlorosis, early flowering and poor grain filling, with severe early infestations potentially causing crop failure [6–8]. Because morphological traits overlap among *Meloidogyne* species, molecular diagnosis is required for reliable identification [9].

Yield losses caused by *M. graminicola* may range from 28% to 87%, depending on cultivar, environmental conditions and infestation severity [7,10]. Climate projections further suggest that increasing temperatures and changes in rainfall patterns may favour the establishment and spread of this species in temperate rice-growing regions, including Europe.

M. graminicola is not seed-borne and spreads naturally only over short distances. Nevertheless, long-distance dispersal may occur through the movement of infested soil attached to agricultural machinery, footwear, livestock or migratory birds [3].

Until 2016, *M. graminicola* had been reported only from Asia, parts of the Americas, Madagascar and South Africa. Its first detection in the EPPO (European and Mediterranean Plant Protection Organization) region occurred in northern Italy (Piedmont) in 2016 [11], followed by the first outbreaks in Lombardy in 2018 [12,13]. Italy remains the only EPPO country where the pest is currently established [14]. Following the first detections in Lombardy, severe symptoms associated with *M. graminicola* infestation and the phytosanitary implications of its establishment prompted the implementation of official eradication measures in infested fields [13,15]. These restrictions highlighted the need to develop management strategies capable of controlling the pest while maintaining rice production.

Management options include chemical nematicides, cultural practices and biological control agents. Synthetic nematicides commonly used in Asia are not authorised in the European Union, while biological control agents have shown inconsistent efficacy [5,7]. Although prolonged flooding may reduce nematode populations, it rarely provides complete control. In response to the emergence of *M. graminicola* in northern Italy, the Lombardy Regional Plant Protection Service, in collaboration with CREA-DC, developed an innovative trap-crop strategy based on the use of rice as a temporary host. Preliminary field experiments demonstrated that this approach could substantially reduce nematode populations while allowing rice cultivation to be maintained, providing the first practical management strategy specifically developed for European rice systems [15].

Following the introduction of *M. graminicola* into the European Union, specific phytosanitary measures were established under Commission Implementing Regulation (EU) 2022/1372 [16]. The trap-crop strategy described by Sacchi et al. [15] subsequently became one of the official containment measures adopted by the Lombardy Regional Plant Protection Service, together with crop rotation, prolonged flooding and complementary phytosanitary actions. While the previous study demonstrated the biological effectiveness of trap-cropping under experimental conditions, evidence of its long-term performance after implementation within an official regional containment programme has remained unavailable. More gener-

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ally, little information exists on the effectiveness of phytosanitary measures when applied at regional scale under routine commercial farming conditions, where management decisions are driven by operational needs rather than experimental design.

The present study represents the natural continuation of the work published by Sacchi et al. [15]. Building on the experimental evidence previously obtained, we analyse four consecutive years (2022–2025) of official phytosanitary surveillance data collected during the regional containment programme implemented in Lombardy. Rather than assessing the biological efficacy of individual measures under controlled experimental conditions, this study evaluates their operational performance under real farming conditions. Specifically, we assess the annual field performance of the officially adopted containment measures in maintaining or restoring nematode-negative status and evaluate alternative three-year management programmes designed to achieve the criteria for official eradication under the regional phytosanitary protocol. The overall objective is to provide evidence-based guidance for the implementation of sustainable containment strategies against *M. graminicola* in European rice-growing systems.

2. Materials and Methods

2.1. Study Area

The study was conducted in Lombardy (northern Italy), one of the major rice-growing regions in Europe. Approximately 100,300 ha are cultivated with rice, accounting for more than 40% of the total Italian rice-growing area [17]. Rice production is concentrated mainly in the province of Pavia, where *M. graminicola* has been officially present since 2018 (Figure 1).

Figure 1. Geographical location of the Province of Pavia in Italy, representing the major rice-producing area of Lombardy and indicating sites where *Meloidogyne graminicola* is present.

Rice cultivation plays an important role not only in the regional economy but also in the environmental context of the area. The extensive network of paddy fields and irrigation canals contributes to groundwater recharge and provides a mosaic of aquatic and terrestrial habitats that can support biodiversity [18–20].

M. graminicola was first detected in the province of Pavia in 2018. Following its introduction, the officially infested area increased rapidly, reaching 705 ha in 2023. This progressive expansion raised major concerns because of the high specialisation of the region in rice production and the potential economic, environmental, and phytosanitary consequences of further spread. Consequently, the Lombardy Regional Plant Protection Service implemented an official containment programme based on the phytosanitary measures described in Section 2.2.

The present study analyses the outcomes of this official containment programme. Following the implementation of the management measures, the officially infested area showed a marked decline, with a 36.9% reduction in 2024 and a 60.1% reduction in 2025 compared with 2023 (Figure 2) [21].

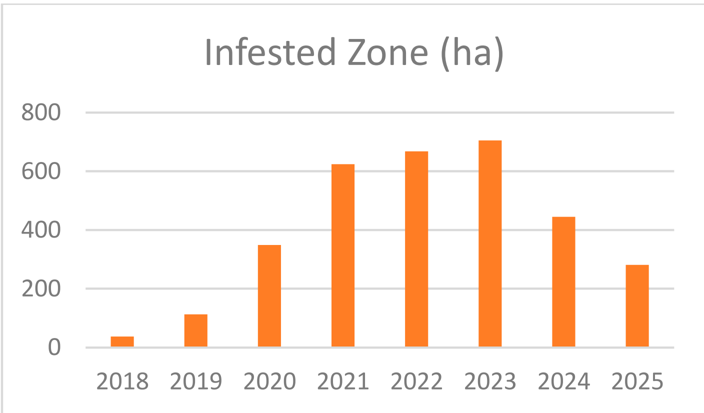

Figure 2. Temporal dynamics of area infested by *Meloidogyne graminicola* in Lombardy (2018–2025).

2.2. Pest Control Measures Implemented in Lombardy

Following the official detection of *M. graminicola* in Lombardy, phytosanitary management was implemented in accordance with Commission Implementing Regulation (EU) 2022/1372, which requires eradication where feasible or, alternatively, the implementation of official containment measures. Given the extent of the infestation and the strategic importance of rice production in the affected area, the Lombardy Regional Plant Protection Service adopted a containment programme.

Because chemical nematicides are not authorised for use against *M. graminicola* in the European Union and currently available biological control agents provide inconsistent results, the containment programme relies primarily on cultural practices. Since 2022, rice cultivation in infested areas of the Province of Pavia has been permitted only when at least one of the following officially approved management measures was implemented:

- Continuous flooding, consisting of maintaining flooded conditions throughout the entire field for at least six months after rice harvest. Flooding typically began immediately after harvest, in September–October, and continued until the following spring, before field preparation for the next crop in March–April.
- Trap-crop, applied at the whole-field scale. Rice was sown over the entire field to stimulate egg hatching and juvenile penetration into the roots and was subsequently destroyed with herbicide within five weeks of sowing, before completion of the nematode life cycle, following the protocol described by Sacchi et al. [13].

- Crop rotation, in which rice was replaced by a non-host or selected poor-host crop, including *Brassica* spp., *Allium cepa* L., *Glycine max* (L.) Merr., *Hordeum vulgare* L., *Panicum miliaceum* L., *Sorghum bicolor* (L.) Moench, *Triticum aestivum* L., and *Zea mays* L.

The choice among these management measures was not predetermined by the study but resulted from decisions taken by farmers in agreement with the Lombardy Regional Plant Protection Service, according to local agronomic, operational, and phytosanitary conditions.

These main management measures were complemented by mandatory phytosanitary practices applied to all monitored fields, irrespective of the main management measure adopted. These included systematic removal of host weeds and thorough cleaning of agricultural machinery, tools, vehicles, and footwear before leaving infested fields to reduce the risk of pest spread through contaminated soil. Because the implementation of these complementary practices did not vary among monitored fields, they could not be included as explanatory variables in the statistical analyses.

Under Regulation (EU) 2022/1372, official phytosanitary restrictions may be lifted only after three consecutive years without detection of *M. graminicola* during official inspections. Consequently, the regional containment programme was designed not only to suppress nematode populations but also to support the progressive recovery of infested fields towards the conditions required for the removal of official restrictions.

2.3. Detection and Severity Assessment

The data analysed in this study were collected as part of the official phytosanitary surveillance programme implemented by the Lombardy Regional Plant Protection Service between 2022 and 2025. Annual inspections were conducted in early July, when rice plants were at the tillering stage and symptoms of *M. graminicola* are most readily detectable. Each monitored field was visually inspected over its entire area by trained phytosanitary inspectors. Inspections were based on visual assessment of both aboveground and belowground symptoms. Aboveground observations included reduced plant vigour, stunting, chlorosis and wilting, whereas belowground assessments focused on the presence of the characteristic hook-shaped galls and root swellings associated with *M. graminicola* infestation.

To support visual assessment during field inspections, inspectors used a photographic reference based on the 1–10 root-galling index proposed by Bridge et al. [8] (Figure 3). For the purposes of the official surveillance procedure, a score of 4 represented the operational threshold for diagnostic investigation. Plants showing symptoms corresponding to scores ≥ 4 were collected and submitted to the laboratory, where the presence of *M. graminicola* was assessed using morphological and molecular diagnostic procedures according to the official protocol of the Italian National Plant Protection Organization (NPPO) [9]. The visual scale was therefore used to support field screening and sample selection rather than to quantify infestation severity. Individual galling index scores were not used as response variables, and the analyses presented in this study were based exclusively on the official detection status (presence/absence) recorded for each field.

2.4. Field Performance of the Officially Implemented Management Measures

The analysis covered the period 2022–2025 and included all fields inspected annually within the official phytosanitary surveillance programme. At the beginning of the study (2022), the dataset comprised all fields classified as infested at the end of the 2021 growing season. In subsequent years, newly detected infested fields were incorporated into the monitoring programme, ensuring that the dataset reflected the temporal and spatial evolution of the infestation. Overall, the annual field-performance assessment covered a cumulative monitored area of 4593 ha over the 2022–2025 period. Because individual fields could contribute data in multiple years, this value represents the cumulative area monitored across years rather than 4593 ha of unique fields.

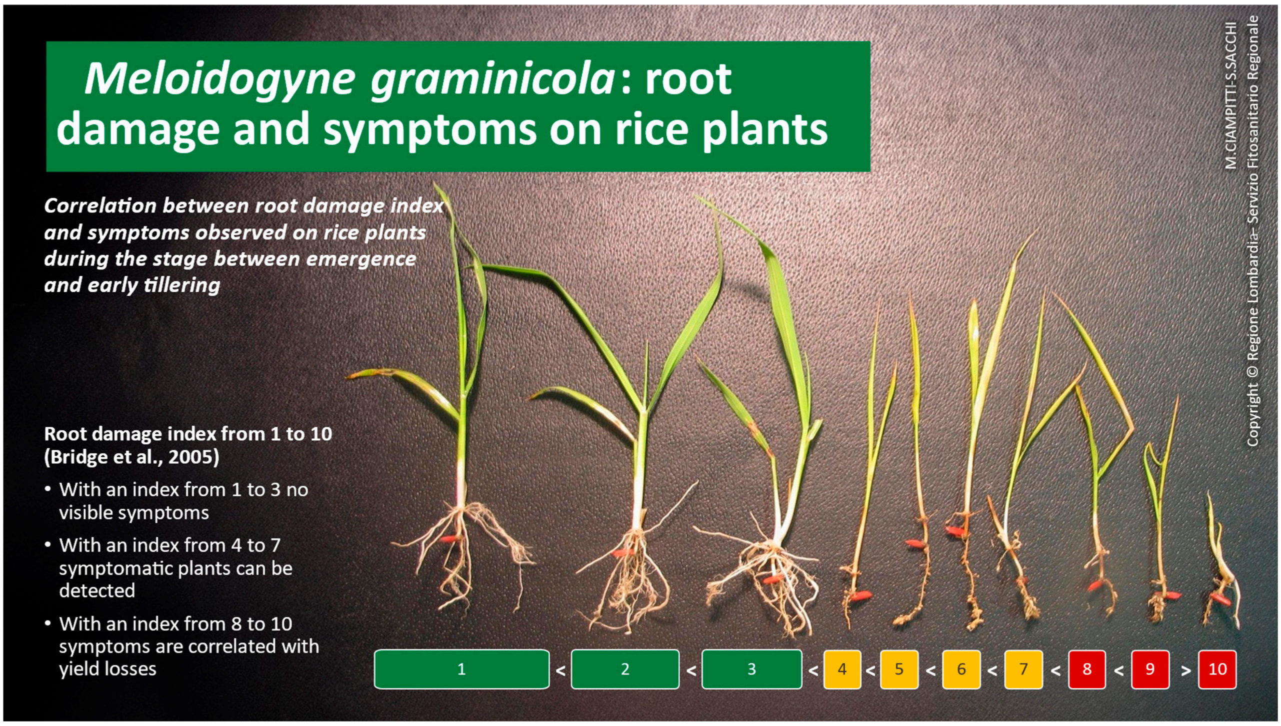

Figure 3. Photographic reference used during official field inspections, based on the 1–10 root-galling index proposed by Bridge et al. [8]. Scores 1–3 indicate no visible aboveground symptoms, scores 4–7 indicate visually detectable symptoms, and scores 8–10 indicate severe symptoms associated with yield losses. A score $\geq$ 4 was used as the operational threshold for diagnostic sampling.

For each inspected field, the management measure applied, the cultivated area (ha), and the official phytosanitary status were recorded. Although both the number of fields and the cultivated area were available, all analyses were performed using hectare-based data because the objective was to evaluate field performance in terms of managed agricultural area rather than the number of cadastral units.

At the beginning of each growing season, each field was assigned an initial phytosanitary status according to the results of the official inspection conducted in the previous year. Fields where *M. graminicola* had been detected were classified as Red, whereas previously infested fields in which the nematode was no longer detected during official inspections were classified as Green.

Five annual management categories were distinguished for the analysis: Trap-crop, Flooding, Other crop, Rice after other crop, and No main measure. Trap-crop and Flooding were implemented at the whole-field scale as described in Section 2.2. "Other crop" identified years in which rice was replaced by a non-host or selected poor-host crop as part of a crop-rotation strategy. "Rice after other crop" did not represent a separate control measure but identified the first year in which rice cultivation was reintroduced after one or more years of rotation. This category was considered separately because cultivation of rice, the most susceptible host, allows the phytosanitary status of the field to be verified under conditions favourable for detection. "No main measure" identified years in which none of the principal field-level management categories was implemented; mandatory complementary phytosanitary practices were nevertheless applied, as described in Section 2.2.

Field performance was evaluated separately according to the phytosanitary status at the beginning of each annual observation. For initially Red fields, conversion performance was defined as the proportion of hectares changing from Red to Green status between two consecutive annual inspections. For initially Green fields, maintenance performance was defined as the proportion of hectares remaining Green between two consecutive annual inspections.

Among the evaluated categories, Trap-crop was considered of particular practical relevance because it enables farmers to continue rice cultivation while simultaneously implementing an official phytosanitary measure. Its field performance was therefore used as the reference for comparison with the other management categories under commercial rice-growing conditions.

Differences in field performance among management categories were assessed separately for conversion and maintenance using chi-squared tests for equality of proportions (prop.test in R), pooling the annual observations over the 2022–2025 study period. The 95% confidence intervals for the hectare-based success proportions were calculated using the same function, with the area achieving Green status as the number of successes and the total managed area as the number of trials. Thus, the comparisons assessed differences among management categories rather than differences among years. Following the overall comparison, pairwise tests were performed using Trap-crop as the reference category. All statistical analyses were performed using R version 4.5.0 [22].

2.5. Field Evaluation of Three-Year Management Programmes

The long-term field performance of the implemented management programmes was evaluated using fields that were classified as infested at the end of the 2021 growing season and subsequently monitored throughout 2022–2024. Under the regional phytosanitary protocol, a field met the criteria for official eradication when it remained Green for three consecutive years, corresponding to the absence of *M. graminicola* during annual official inspections. Official eradication could only be confirmed when the third consecutive Green year occurred under rice cultivation, allowing verification of pest absence in the presence of the most susceptible host crop. A total of 302 fields, corresponding to 625 ha, were included in this analysis.

Each three-year management programme was reconstructed from the chronological sequence of measures actually implemented in the same field over three consecutive years; these sequences were not assigned in advance as experimental treatments. The abbreviations used for the annual measures are reported in Table 1. Accordingly, each three-letter code describes the sequence applied from the first to the third year; for example, AAT indicates Other crop in the first two years followed by Trap-crop in the third year. Because official eradication could only be confirmed when the field was cultivated with rice, programmes ending with Other crop (A) or Flooding (S) in the third year were excluded from the analysis. Furthermore, only programmes including at least one year of Trap-crop (T) or Rice after other crop (R) were retained, as these programmes allowed the phytosanitary status to be verified under rice cultivation.

Mandatory complementary phytosanitary practices, including host-weed control and cleaning of agricultural machinery, were implemented in all fields irrespective of the annual management measure and are therefore not represented in the programme codes.

As in the annual analyses, field performance was evaluated using cultivated area (ha) as the unit of analysis. For each management programme, the proportion of hectares achieving three consecutive Green years and the corresponding 95% binomial confidence intervals were calculated. Differences among programmes were assessed using chi-squared tests for equality of proportions (prop.test in R). Because some programmes were implemented over relatively small areas and showed extreme success rates, their estimated performance should be interpreted with caution.

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Table 1. Codes used to describe the annual measures.

Code	Management Measure	Description
A	Other crop	Cultivation of a non-host or selected poor-host crop instead of rice
T	Trap-crop	Rice trap-crop followed by crop destruction before completion of the nematode life cycle
R	Rice after other crop	Rice cultivation following a year with a non-host or selected poor-host crop
S	Flooding	Continuous flooding for at least six months after the previous rice harvest
N	No main measure	None of the four main field-level management measures evaluated in the study was applied during that year; mandatory complementary phytosanitary measures were nevertheless implemented

To facilitate comparison among programmes with similar management approaches, individual programmes were grouped into three categories: Trap-crop programmes, including programmes containing Trap-crop (T) but no Rice after other crop (R); Rice-after-rotation programmes, including programmes containing Rice after other crop (R) but no Trap-crop (T); Combined programmes, including at least one year of both Trap-crop (T) and Rice after other crop (R). For each category, the total managed area, the area achieving official eradication, and the overall success proportion were calculated. Differences among categories were evaluated using chi-squared tests on the aggregated hectare data.

Finally, a binomial generalised linear model (GLM) with a logit link was fitted to examine the association between the composition of the three-year management programmes and the probability of meeting the official eradication criterion. The response variable consisted of the number of hectares that met and the number of hectares that did not meet the official eradication criterion, while the explanatory variables were the number of years (0–3) within each programme in which Trap-crop (T), Rice after other crop (R), and No main measure (N) were implemented. Model coefficients were expressed as odds ratios (ORs) with corresponding 95% confidence intervals (CIs). Because management sequences were not experimentally assigned, the estimated associations were interpreted within the operational context of the regional containment programme rather than as causal effects of individual management measures.

3. Results

During the period 2022–2025, a total of 1084 field inspections were conducted across 517–625 ha of rice-growing land each year. Table 2 summarises the annual observations for each management measure, reporting the number of fields and monitored area (ha) overall and according to their phytosanitary status at the beginning of each annual observation. These values represent the starting conditions for the subsequent conversion and maintenance analyses.

Monitoring covered a large area throughout the study period, with only limited interannual variation in the number of inspected fields, providing a consistent basis for comparing the field performance of the different management measures.

Figure 4 provides a graphical comparison of the relative use of the different management measures according to the phytosanitary status at the beginning of each annual observation. By expressing the monitored area as percentages, the figure facilitates comparison of the management measures applied to initially Green and initially Red fields.

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Table 2. Number of fields and monitored area (ha) associated with each management measure, overall and according to phytosanitary status (Green or Red) at the beginning of each annual observation.

Applied Measure	All		Starting Status: Green		Starting Status: Red	
	N° Fields	ha	N° Fields	ha	N° Fields	ha
Trap-crop	58.88 ± 62.83 [0–181]	131.12 ± 128.18 [0–375]	63 ± 44.86 [0–106]	139.76 ± 96.11 [0–207.17]	54.75 ± 84.58 [4–181]	122.48 ± 170 [11.6–375]
Flooding	5 ± 2.56 [0–8]	14.69 ± 8.59 [0–26]	5 ± 3.56 [0–8]	12.47 ± 10.42 [0–22.56]	5 ± 1.63 [3–7]	16.9 ± 7.1 [8.69–26]
Rice after other crop	8.63 ± 14.69 [0–36]	21.71 ± 38.29 [0–100.3]	17.25 ± 17.46 [0–36]	43.42 ± 46.52 [0–100.3]	0	0
Other crop	58.13 ± 57.06 [0–128]	103.12 ± 100.07 [0–223.03]	84.5 ± 57.46 [0–128]	146.76 ± 103.27 [0–223.03]	31.75 ± 49.41 [2–105]	59.48 ± 87.3 [1.99–187]
No main measure	4.88 ± 4.26 [0–12]	16.42 ± 16.62 [0–38.19]	5.5 ± 5 [0–12]	13.11 ± 17.1 [0–38.19]	4.25 ± 4.03 [0–9]	19.72 ± 17.98 [0–37]

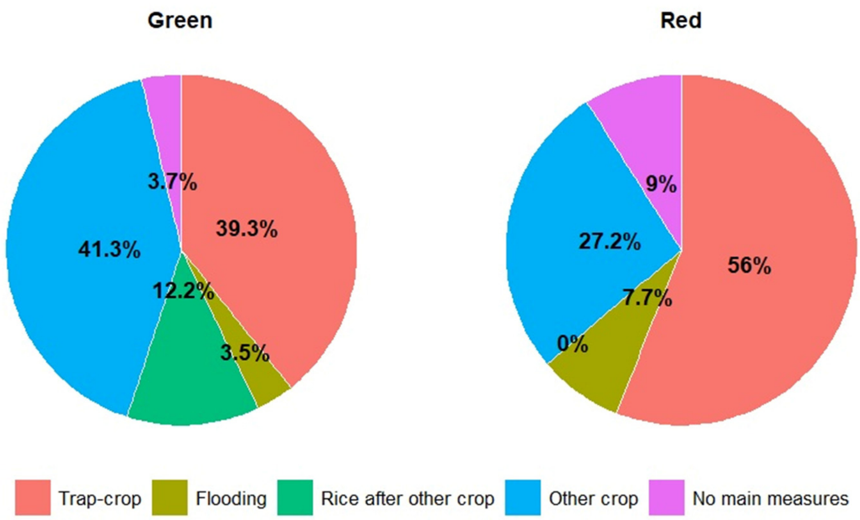

Figure 4. Relative use of the different management measures according to phytosanitary status at the beginning of each annual observation. Pie charts show the percentage of the monitored area associated with each management measure in initially Green and initially Red fields over the 2022–2025 study period.

Trap-crop was the predominant measure among initially Red fields, whereas Other crop and Trap-crop accounted for most of the monitored area among initially Green fields. Rice after other crop was applied exclusively to initially Green fields, while Flooding and No main measure represented relatively small proportions under both initial phytosanitary conditions.

3.1. Field Performance of Individual Management Measures

The field performance of the management measures differed according to the initial phytosanitary status of the fields (Table 3).

For fields with Green starting status, field performance represented maintenance of Green status at the subsequent annual inspection. Maintenance was generally high across management measures, although lower performance was observed for Flooding. For fields with Red starting status, field performance represented conversion to Green status at the subsequent annual inspection. Conversion varied more markedly among management measures, with the highest performance observed for Other crop and the lowest for No main measure. Rice after other crop was not evaluated because this measure was not applied to fields with Red starting status (Table 3; Figure 5).

Table 3. Field performance of the five management measures according to the phytosanitary status at the beginning of each annual observation. For fields with Green starting status, performance represents maintenance of Green status, whereas for fields with Red starting status, it represents conversion to Green status. Values are reported as treated area (ha), final Green area (ha), and percentage of the treated area achieving Green status, with 95% confidence intervals (CI).

Measure	Starting Status: Green			Starting Status: Red		
	Treated Area (ha)	Final Green Area (ha)	Success (%) [CI95]	Treated Area (ha)	Final Green Area (ha)	Success (%) [CI95]
Trap-crop	811	769	95 [93–96]	709	513	72 [67–76]
Flooding	69.9	53.8	77 [65–86]	87.6	48.8	56 [45–66]
Rice after other crop	243	224	92 [88–95]	0		0[-]
Other crop	925	925	100 [99–100]	365	365	100 [98.7–100]
No main measure	74.4	67.2	90 [81–96]	95.9	37.2	39 [29–49]

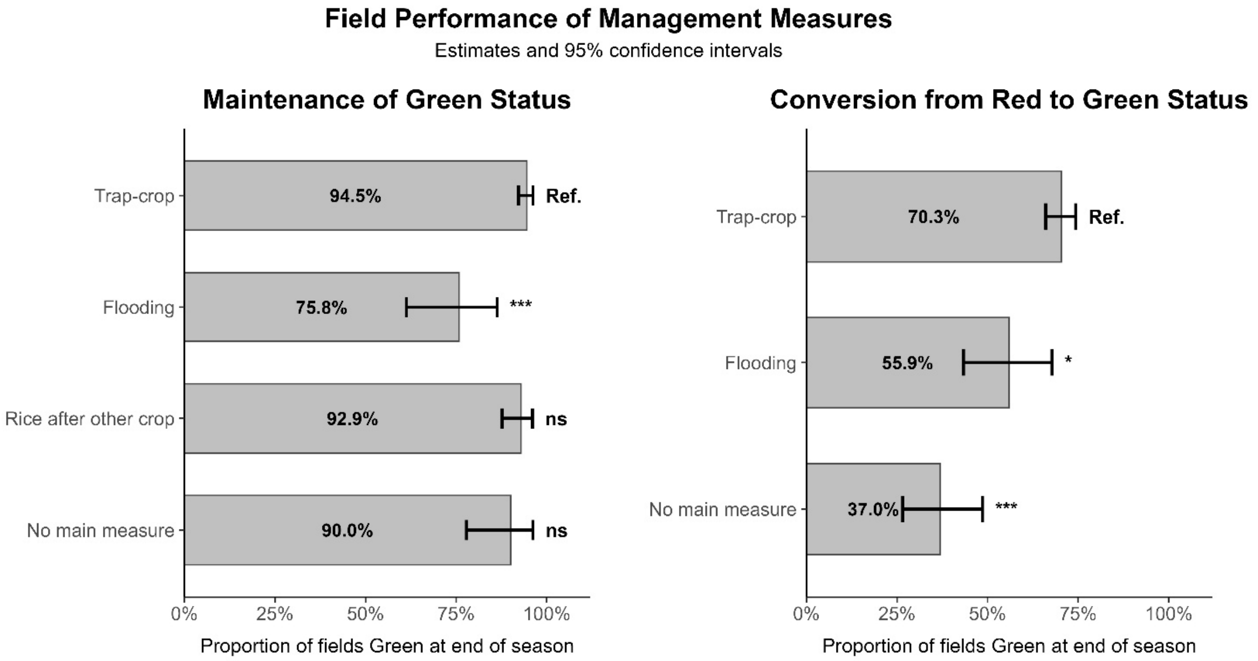

Figure 5. Field performance of the evaluated management measures according to yearly initial phytosanitary status. Bars represent the proportion of fields maintaining Green status (initially Green fields; **left** panel) or converting from Red to Green status (initially Red fields; **right** panel). Error bars indicate 95% confidence intervals. Statistical annotations refer to pairwise comparisons with Trap-crop as the reference measure (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$; ns, not significant).

Overall differences among management measures were significant for both maintenance of Green status ($\chi^2 = 84.91$, $p < 0.001$) and conversion from Red to Green status ($\chi^2 = 155.85$, $p < 0.001$). Pairwise comparisons using Trap-crop as the reference measure are reported in Table 4 and Figure 5.

Table 4. Pairwise comparisons between Trap-crop and alternative management measures. Estimated success proportions, differences relative to Trap-crop (Δ), and *p*-values from chi-squared tests are reported separately for maintenance of Green status and conversion of Red fields to Green status (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$; ns, not significant).

	Initial Status: Green				**Initial Status: Red**			
	Estimated Proportion	**Δ Trap Proportion**	***p*-Value**		**Estimated Proportion**	**Δ Trap Proportion**	***p*-Value**	
Trap-crop (reference)	0.945				0.703			
Flooding	0.758	−0.187	<0.001	***	0.559	−0.144	0.023	*
Rice after other crop	0.929	−0.016	0.534	ns		n.a.		
Other crop	1.000	0.055	<0.001	***	1.000	0.297	<0.001	***
No main measure	0.900	−0.045	0.311	ns	0.370	−0.333	<0.001	***

For fields with Red starting status, conversion was significantly higher with Other crop than with Trap-crop, whereas Flooding and No main measure showed significantly lower conversion. For fields with Green starting status, maintenance did not differ significantly between Trap-crop and either Rice after other crop or No main measure, whereas it was significantly lower with Flooding and higher with Other crop.

3.2. Field Performance of Three-Year Management Programmes

After applying the selection criteria described in Section 2.5, eight three-year management programmes containing at least one Trap-crop year (T) or one Rice after other crop year (R) were retained for analysis: AAT, AAR, ART, TTT, TTN, TAT, ATT, and TAR.

The field performance of the management programmes varied considerably (Figure 6 left panel). Programmes AAT and ART achieved complete attainment of the official eradication criterion, with all managed hectares remaining Green for three consecutive years. AAR also showed a high proportion of hectares meeting the eradication criterion (94.6%). In contrast, TTT, TAT, and ATT showed intermediate performance, with 60–65% of the managed area achieving three consecutive Green years, whereas TTN and TAR showed substantially lower proportions (35.7% and 44.0%, respectively). Overall differences among programmes were significant ($\chi^2 = 23.7$, df = 7, $p = 0.0013$).

To facilitate comparison among programmes with similar management approaches, the individual programmes were grouped into three categories (Figure 6 right panel). Rice-after-rotation programmes (AAR) showed the highest proportion of hectares meeting the official eradication criterion, with 10.4 of 11.0 ha (94.6%) remaining Green for three consecutive years. Trap-crop programmes (AAT, TTT, TTN, TAT, and ATT) covered approximately 170 ha, of which 111 ha (65.2%) met the eradication criterion. Combined programmes (ART and TAR) covered 48.7 ha, with 24.3 ha (49.9%) achieving three consecutive Green years. Differences among the three programme categories were significant ($\chi^2 = 8.68$, df = 2, $p = 0.013$), although the difference between Rice-after-rotation and Trap-crop programmes was not statistically significant ($p = 0.09$).

The generalised linear model identified significant associations between programme composition and the probability of meeting the official eradication criterion. The number of years including Trap-crop, Rice after other crop, and No main measure was significantly associated with the odds of achieving three consecutive Green years. The estimated odds ratios were 0.30 (95% CI: 0.10–0.67) for Trap-crop years, 0.06 (95% CI: 0.01–0.34) for Rice after other crop years, and 0.09 (95% CI: 0.02–0.42) for years with no main measure. Because management measures were not randomly assigned and their selection reflected field-specific agronomic, operational, and phytosanitary conditions, these odds ratios should be interpreted as associations within the observed management programmes rather than as causal effects of individual measures.

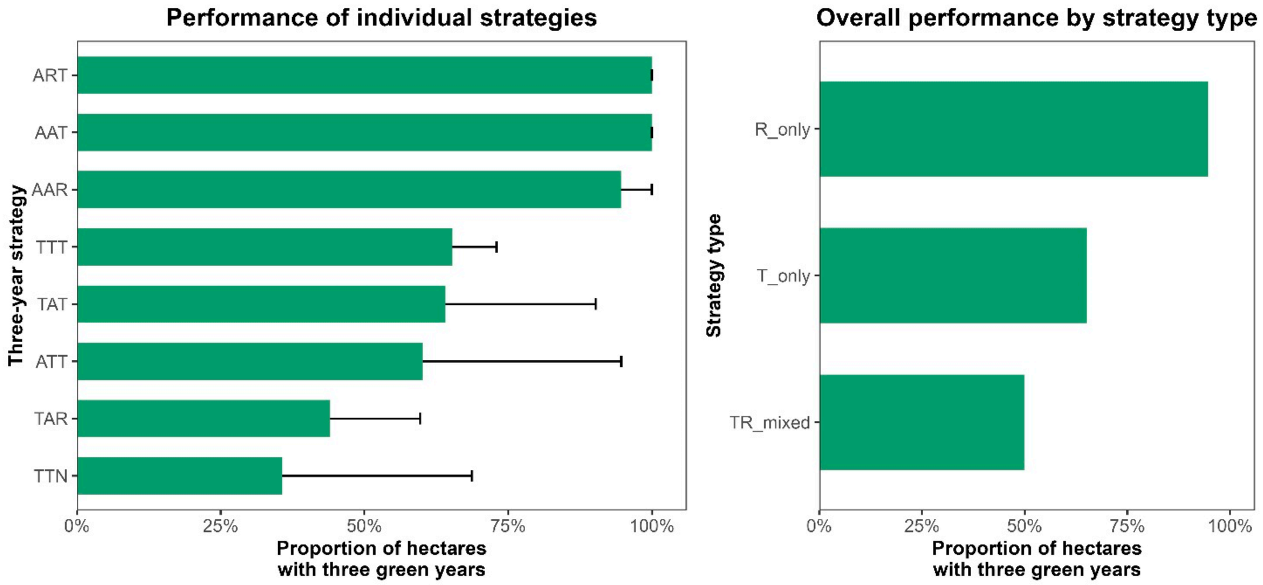

Figure 6. Left panel: Proportion of hectares achieving three consecutive Green years for each three-year management programme containing at least one Trap-crop (T) or Rice-after-other-crop (R) year, with 95% binomial confidence intervals. **Right** panel: Overall performance of the three programme groups (Trap-crop programmes, Rice-after-rotation programmes, and Combined programmes), expressed as the proportion of hectares achieving three consecutive Green years.

4. Discussion

This study provides one of the first evaluations of the field performance of an official regional containment programme for *M. graminicola* under commercial rice-growing conditions. Unlike previous experimental studies assessing the biological efficacy of individual management measures, the present work analysed four years of routine phytosanitary surveillance, allowing the operational performance of different management approaches to be evaluated under real farming conditions.

The relative use of management measures indicates that Trap-crop and crop rotation formed the core of the regional containment programme, accounting for most of the managed area throughout the study period. Their widespread adoption reflects not only their compatibility with local rice production systems but also the need to balance phytosanitary objectives with the economic sustainability of farms.

Management measures were not used uniformly among fields. Trap-crop and, to a lesser extent, Flooding were implemented predominantly in fields where *M. graminicola* had already been detected (Red fields), whereas crop-rotation measures were more frequently adopted in fields that had already achieved Green status. This pattern reflects the operational management of the regional containment programme, in which phytosanitary measures were selected according to the infestation status and local agronomic conditions rather than being assigned within an experimental design.

Despite this non-random allocation, clear differences in field performance emerged among the management measures. In fields already classified as Green, Trap-crop and Other crop were associated with high maintenance of Green status. Flooding showed greater variability, suggesting that its field performance under commercial farming conditions may depend on local soil and hydrological characteristics.

Among initially infested (Red) fields, Trap-crop showed one of the highest conversion rates from Red to Green status, second only to Other crop. This result extends the findings of Sacchi et al. [15], who demonstrated under experimental conditions that repeated Trap-crop cycles substantially reduced nematode populations. The present study shows that the same approach also performs well after implementation within an official phytosanitary programme, where management decisions are constrained by operational, agronomic and economic considerations. Unlike other available measures, Trap-crop allows farmers to continue rice cultivation while simultaneously reducing nematode pressure, which probably explains its extensive adoption throughout the monitored area.

Continuous Flooding was used only on a limited proportion of the monitored area. This was largely due to local agronomic constraints, as many soils in the study area have limited water-retention capacity and irrigation schedules are regulated by irrigation consortia. Although Flooding showed intermediate field performance in the present study, previous experimental evidence indicates that prolonged flooding can substantially suppress *M. graminicola* populations [5,23,24]. Its potential value under European rice-growing conditions therefore deserves further investigation where suitable hydrological conditions allow its practical implementation.

The analysis of three-year management programmes further showed considerable variability in long-term outcomes among the management sequences implemented, with the proportion of managed area meeting the official eradication criterion ranging from complete attainment to less than 40%.

The generalised linear model also identified significant associations between programme composition and the probability of achieving three consecutive Green years. These associations should not be interpreted as estimates of the intrinsic effectiveness of the individual measures. Management decisions were made according to local agronomic, operational, and phytosanitary conditions, and repeated use of a particular measure may therefore also reflect the underlying characteristics of the fields in which it was implemented. The results consequently provide evidence on the performance of different management sequences within the operational context of the Lombardy containment programme rather than causal estimates of the contribution of individual interventions.

From an economic perspective, the distinction between eradication and containment has important practical implications. Complete eradication programmes require the prohibition of rice cultivation, with potentially important economic consequences for affected farms. Furthermore, rice cultivation is supported by public subsidies that may no longer be available when rice production is suspended. Consequently, compensation payments differ markedly between the two approaches, reaching EUR 2000 ha^{-1} under eradication measures [25,26] compared with EUR 250 ha^{-1} under the current containment programme [27]. These payments represent policy support and should not be interpreted as estimates of the actual costs or economic profitability of the respective management strategies. The surveillance dataset did not include measure-specific implementation costs, crop yields, production losses, revenues, or farm net income; consequently, the present study cannot provide a quantitative comparison of the economic performance of alternative management programmes. A dedicated farm-level economic assessment would be required to determine their relative costs, benefits, and effects on farm income.

Beyond its agronomic implications, maintaining rice cultivation may also be relevant in the broader environmental context of European rice landscapes, where flooded rice fields can provide complementary wetland habitats for several groups of organisms [28,29]. However, the present study did not include monitoring of biodiversity, habitat quality, water retention, or other ecosystem functions. Therefore, the present data do not allow the ecological benefits or impacts of the evaluated containment measures to be assessed.

Assessing such effects would require dedicated ecological monitoring under alternative management strategies.

Overall, the present study shows how a management approach initially validated under experimental conditions was subsequently implemented within an operational regional phytosanitary programme. This transition from experimental evidence to routine implementation represents one of the main contributions of the study and provides practical evidence to support the development of containment programmes adapted to commercial rice-growing conditions in Europe.

5. Limitations

The findings of this study should be interpreted in light of the characteristics of the underlying surveillance programme. First, this was an observational study based on an official phytosanitary monitoring programme rather than a controlled field experiment. Consequently, management measures were not randomly assigned but selected by farmers, in agreement with the Lombardy Regional Plant Protection Service, according to local agronomic, operational, and phytosanitary conditions. As a result, fields receiving repeated or more intensive interventions were also likely to be those facing greater infestation pressure, potentially confounding comparisons among management programmes.

Second, the primary objective of the surveillance programme was to determine the official phytosanitary status of each field (presence or absence of *M. graminicola*) rather than to quantify nematode population density. Although inspections followed a standardized protocol and diagnostic confirmation was performed according to the official NPPO procedures, quantitative estimates of initial nematode populations or galling severity were not systematically collected. Consequently, differences in programme performance may partly reflect variation in initial infestation levels that could not be explicitly accounted for in the analyses.

Mandatory complementary phytosanitary practices, including host-weed control and machinery cleaning, were applied across all monitored fields. Because these practices did not vary among fields, their specific contribution to programme outcomes could not be evaluated independently.

Despite these limitations, the study provides a unique large-scale assessment of the implementation of official containment measures under commercial farming conditions. The combination of four consecutive years of monitoring, more than 1000 field inspections, and over 600 ha included in the surveillance programme provides substantial empirical evidence on the field performance of management programmes implemented under routine phytosanitary practice.

6. Conclusions

This study provides the first large-scale evaluation of the field performance of an official *M. graminicola* containment programme under European rice-growing conditions. The results show that nematode-negative status can be achieved and maintained under commercial farming conditions while allowing rice cultivation to continue, providing a practical alternative to strict eradication measures that require suspension of rice cultivation.

Among the available management options, Trap-crop showed high field performance while allowing commercial rice production to continue. Crop rotation also showed high field performance, whereas the performance of prolonged flooding was more variable under local environmental and operational conditions. The suitability of these measures in other European rice-growing regions should therefore be assessed according to local conditions. Prolonged flooding may be appropriate where water availability, soil characteristics, and field infrastructure allow flooded conditions to be maintained for the required period, whereas crop rotation may be particularly suitable where agronomically and economically viable non-host or poor-host crops can be integrated into local cropping systems. Trap-crop may represent a particularly relevant option where continued rice production is a priority, although its performance should also be validated under local environmental and agronomic conditions.

The analysis of three-year management programmes further showed that long-term outcomes were associated with the sequence of measures implemented over time. Among the programmes represented in the dataset, Other crop–Other crop–Trap-crop and Other crop–Rice after other crop–Trap-crop achieved the highest observed field performance, with all monitored hectares meeting the official eradication criterion, while Other crop–Other crop–Rice after other crop also showed high performance (94.6%). These results provide practical examples of multi-year sequences associated with favourable outcomes but should not be interpreted as evidence of an optimal or universally applicable programme, given the observational nature of the study and the unequal representation of management sequences.

The Lombardy experience provides a practical framework for the sustainable management of *M. graminicola* and offers evidence to support the development of containment-based strategies in other European rice-growing regions facing future introductions of this quarantine pest. However, the selection and combination of management measures should be adapted to regional water availability, soil and field characteristics, cropping systems, and agronomic constraints.

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