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RESEARCH ARTICLE*

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Woodland ectomycorrhizal fungi benefit from large-scale reduction in nitrogen deposition in the Netherlands

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Abstract

1. Woodland ectomycorrhizal (ECM) fungal species declined considerably in the Netherlands in the late 20th century, mainly due to raised levels of atmospheric nitrogen deposition. Environmental measures have been taken to reduce this deposition, but it remains unclear whether and to what extent ECM species have benefitted from these.
2. We hypothesized that ECM species, especially those species that are known to be nitrophobic, that is, sensitive to nitrogen loading, have recovered to some extent from the reduction in nitrogen deposition after 1994. We further hypothesized that, due to legacy effects of deposition, recovery has been stronger in regions where deposition levels have previously been lower.
3. To test these hypotheses, we analysed long-term opportunistic data, that is, observations collected without standardized field method. We applied data filtering and a modified List Length method to adjust for potential biases in these data. The removal of bias left us with two periods to examine ECM species trends: before (1965–1985) and after (1994–2013) deposition reduction started [in 1994].
4. We compared trends in ECM species in 1965–1985 with those in 1994–2013. Multispecies indicators were used to summarize the findings of ECM species, and to compare these with results of litter saprotrophic species and wood saprotrophic and wood parasitic species.
5. We found that (1) most trends switched in direction from negative to positive after the reduction in nitrogen deposition began; (2) these trends were more pronounced for nitrophobic ECM species than for nitrotolerant ECM species; (3) trends for ECM species differed from those of the other functional groups; and (4) recovery was stronger in the region with a history of lower deposition.
6. *Policy implications*. Our results suggest that woodland ectomycorrhizal species benefit substantially from environmental measures to reduce nitrogen deposition. Our study is one of few scientific studies to date documenting evidence of success of large-scale (nation-wide) environmental measures. We have demonstrated that opportunistic citizen science data can be used for the detection of species trends, but it is essential to examine and control for potential bias in the data.

KEYWORDS
citizen science, ectomycorrhiza, environmental measures, legacy, mycoflora, nitrogen deposition, saprotrophs, wood parasites

1 | INTRODUCTION

In the last quarter of the 20th century, researchers reported considerable declines of woodland ectomycorrhizal (ECM) fungal species in the Netherlands and elsewhere in Western Europe (Arnolds, 1988, 1991; Nauta & Vellinga, 1995). The declines were attributed to atmospheric deposition of SO_2, NO_x and NH_3 which caused acidification and eutrophication of the soil (Arnolds, 1991; Cox, Barsoum, Lilleskov, & Bidartondo, 2010; Jansen & Van Dobben, 1987; Lilleskov, Fahey, & Lovett, 2001). The main sources of these depositions were agriculture, industry and motorized traffic (CBS, PBL & Wageningen UR, 2016a,b). Experimental research showed that ECM species were more sensitive to eutrophication of the soil than to acidification (Termorshuizen & Schaffers, 1991; Wallenda & Kottke, 1998). Eutrophication is caused by airborne deposition of nitrogen (both ammonium and nitrate), which partly accumulates in the soil and leads to the thickening of the litter layer (Knorr, Frey, & Curtis, 2005; Morrison et al., 2016). Conditions for ECM species deteriorate with increasing thickness of litter and humus layers and with high nitrogen availability in these organic layers due to several processes. High mineral nitrogen availability reduces the growth of the ECM mycelium, reduces plant investment in roots and ectomycorrhizas, shifts the competitive balance between saprotrophic and ECM species, and in combination with partly degraded organic materials generates toxic compounds (Kuyper, 2013; Lucas & Casper, 2008; Morrison et al., 2016). In the mid-1970s, environmental measures were taken by the Netherlands to reduce emissions of SO_2, NO_x and NH_3 in order to protect nature. Over the years, these measures have led to considerable reductions in the deposition of acidifying and eutrophying substances in the Netherlands (CBS, PBL & Wageningen UR, 2016a,b). The level of nitrogen deposition increased until 1975, levelled off and only lowered gradually after 1994, from 2,700 to 1,600 mol ha^{-1} $year^{-1}$ in 2015, that is from 38 to 22 kg N ha^{-1} $year^{-1}$, averaged over the Netherlands (CBS, PBL & Wageningen UR, 2016a).

Currently, there is only limited scientific evidence that ECM species have benefitted from the nation-wide reduction in nitrogen deposition. Some authors have reported positive trends of a number of ECM species and attributed this positive trend to lower nitrogen deposition (Arnolds, 2010; Arnolds & Veerkamp, 2008). Experimental data on recovery rates of ECM fungi after cessation of nitrogen inputs are equally rare (Hasselquist & Högberg, 2014; Strengbom, Nordin, Näsholm, & Ericsson, 2001). The mentioned reports describing recovery of ECM species in the Netherlands are based on analysis of a large database maintained by the Dutch Mycological Society ("Nederlandse Mycologische Vereniging"; NMV), consisting of opportunistic data, that is, observations collected without standardized field protocol. These type of data need to be treated with caution to prevent biased trend estimates (Isaac, Van Strien, August, De Zeeuw, & Roy, 2014). We detected several sources of bias in the opportunistic data of mushrooms that were to date insufficiently identified and remedied (see Methods).

Our aim is to evaluate whether ECM species in the Netherlands have benefitted from the nation-wide reduction in nitrogen deposition. Given that general reduction in nitrogen deposition started after 1994, we hypothesize a different trend in ECM species before and after 1994. We also hypothesize that, if nitrogen deposition is the driver of changes, nitrophobic species, that is, species that prefer nutrient-poor conditions, will be most affected. Thus, we expect trends to be more pronounced in this group than in the group of nitrotolerant species. We also predict that recovery is more pronounced in ECM species, given their higher sensitivity to N deposition, than in litter saprotrophic species and wood saprotrophic and parasitic species. Finally, we predict a stronger positive trend in regions on sandy soils that have undergone a lower degree of nitrogen accumulation, because both the general reduction in deposition will more likely be closer to the critical loads (Bobbink & Hettelingh, 2011), and because with a lower legacy of nitrogen deposition the rates of recovery increase.

The analysis was done in two steps. In the first step, we assessed the trends of individual species, using the same opportunistic data as in earlier analyses (Arnolds, 2010; Arnolds & Veerkamp, 2008), complemented with data of recent years, but treating all sources of bias we could find. In a second step, we summarized the trends of species and created multispecies indicators using the Living Planet Index method (Loh et al., 2005; Van Strien et al., 2016).

2 | MATERIALS AND METHODS

2.1 | Study species

From all species that could be considered woodland species (app. 1,600 in total), we selected 130 study species that can be relatively easily recognized in the field by most (amateur) mycologists and for which we have sufficient data over a long period. Species were classified into functional groups based on www.verspreidingsatlas.nl (see Table S1). Most of them were ECM species (n = 75), others were parasites and saprotrophs on wood (n = 30), and terrestrial saprotrophs (n = 25). The classification of ECM species as nitrophobic (n = 45) was also taken from www.verspreidingsatlas.nl. This listing was based on expert judgement, but is supported by experimental studies on forest fertilization and short-distance nitrogen deposition gradients, stable-isotope signatures of fruit bodies, the ability to access nitrogen from organic sources, and the exploration type of the mycorrhizal mycelium (Hobbie & Agerer, 2010; Lilleskov, Hobbie, & Horton, 2011; Lilleskov et al., 2001; Taylor et al., 1997). The classification of ECM species (n = 9) as nitrotolerant was based on work by Kuyper (2013), and Suz et al. (2014).

2.2 | Data

Similar to most other studies of fungi, changes in the occurrence of fruit bodies were used as proxy for changes in the occurrence of species. The Netherlands is probably the most data-rich country regarding mushrooms, through the collection of many records by amateur and professional mycologists over many years. We used the NMV-database containing more than 2 million records from 1965 to 2013. We quantized observations at a 1 km × 1 km resolution and compiled "year lists" from all observations. We define year lists as all species encountered within a calendar year by a single observer. We henceforth refer to the 1 km × 1 km geo-referenced sample areas as "quadrats". On average about 1,300 1 km × 1 km quadrats were visited annually since 1965 and the number of quadrats has increased over time (Table 1). From some quadrats only a single species record was obtained in a year, while in other quadrats >200 species were recorded in a year.

While exploring these data, we assessed whether sampling methods have changed systematically over time. We detected three changes in searching behaviour (Table 1). First, the frequency of year lists without any woodland species record has gradually increased (Table 1; Pearson's $r = .84$; $p < .01$), indicating that observers have gradually shifted their attention from exclusively woodland localities to now also include non-woodland localities (grasslands, bogs, dunes). Second, the number of species on year lists in woodland localities has gradually increased (Table 1; Pearson's $r = .94$; $p < .01$). This increase suggests that sampling effort and/or observer expertise may have increased over time. Third, in mid 1980s a conspicuous increase happened in the frequency of year lists from roadside verges and forest lanes with planted trees. At that time, observers became attracted to these localities because conditions for ECM species were better in roadsides than in the forest. The higher species richness (particularly of ECM species) is caused by the fact that a large part of litter in exposed roadsides is removed by wind, leading to less litter accumulation (Keizer & Arnolds, 1994). The shift to non-woodland localities may easily produce false estimates of declines of woodland, and especially ECM species, while the shift to roadsides may lead to false increases of ECM species. Longer lists due to searching behaviour may also lead to false increases, but would not be biased in favour of ECM species. Therefore, it is compulsory to take these systematic changes into account.

The changing preference for non-woodland localities was corrected for by data filtering. Field work in other habitats does not affect the probability of finding a woodland species, so we filtered out all non-woodland species records. The variation in number of species on year lists was addressed by incorporating list length (LL) as a covariate in our statistical model (see below). Information of habitat was lacking for many lists, so we could not use this as another covariate in the model. Instead, we only used data before 1985 and after 1994, because we assessed no systematic change in frequency of lists from roadsides within each of these periods. As a consequence, the annual estimates of occurrence can no longer be compared between these two periods, because after 1994 more observations came from roadsides than before 1985. But trends in occurrence may still be compared between the two periods.

An additional analysis was performed with non-roadside species to circumvent any effects of the changing preference of observers for roadsides and yet to cover the entire period 1965–2013. We used the data for all years and assessed trends in ECM species associated with conifers or birch. These trees are seldom planted in roadside verges or forest lanes, so trends in their associated fungi will not be confounded by the shift of observers to roadsides in the 1980s.

2.3 | Study regions

Woodland in the Netherlands mainly occurs in regions with Pleistocene sandy soils (Figure 1), which were originally poor in nitrogen. Nitrogen deposition in woodland mainly came in the form of NH_3 emitted by intensive animal husbandry nearby. In order to provide a geographically differentiated analysis we divided the area in three regions (Figure 1). In the northern region, little intensive animal husbandry occurs and current nitrogen deposition is relatively low at approximately 1,500 mol ha^{-1} $year^{-1}$ (CBS, PBL & Wageningen UR, 2016a). The central and southern sandy soil regions have more intensive animal husbandry, which emits considerable amounts of NH_3. Current levels of nitrogen deposition are approximately 2,200 mol ha^{-1} $year^{-1}$ for the central region and approximately 2,500 mol ha^{-1} $year^{-1}$ for the southern region (CBS, PBL & Wageningen UR, 2016a), with values that may exceed 5,000 mol ha^{-1} $year^{-1}$ in particular areas. Overall, eutrophying deposition has decreased in all regions since 1994. For a proper comparison of trends between the three regions, we only included species that occur in all three regions.

TABLE 1 Some characteristics of the macrofungi dataset in three periods of equal length (mean ± SE). A list comprises all species observed in a year in a 1 km × 1 km quadrat

	1965–1980	1981–1996	1997–2013
No. of annual year lists (in all data)	585 ± 40	1702 ± 130	2050 ± 121
% year lists without woodland species (in all data)	41.5 ± 0.6	46.2 ± 0.3	48.3 ± 0.3
Mean no. woodland species per year list (in woodland species data)	10.9 ± 0.4	17.2 ± 0.6	24.2 ± 0.7
% year lists from roadsides (in woodland species data with habitat information)	11.0 ± 1.0	22.4 ± 2.5	29.2 ± 1.5

FIGURE 1 Location of the northern, central and southern sandy soil regions in the Netherlands

2.4 | Statistical analysis

We first deduced non-detection records for each of our 130 study species by assuming all cases in which the species was not on a year list for a certain 1 × 1 km quadrat as non-detected in that particular quadrat and year. The obtained detection/non-detection data require a method to account for the unstandardized observer effort and the variation in quadrats surveyed between years. Occupancy modelling is currently the best statistical correction method available for such data (Isaac et al., 2014; MacKenzie et al., 2006; Van Strien, Van Swaay, & Termaat, 2013). This method separates occupancy (the presence of a species in a quadrat) from detection (the observation of the species in that quadrat) when analysing field survey data. Occupancy models properly address the aforementioned changes in searching behaviour; these changes would be reflected in changes in detection rather than changes in occurrence. Occupancy models require separate visits to the same quadrats within the same season to estimate detection probability; unfortunately such replicated visits are scarce in the mushroom data, ruling out the use of occupancy modelling. An alternative is the LL method that uses the number of species recorded per visit to correct for variation in observer effort (Szabo, Vesk, Baxter, & Possingham, 2010). This method performs reasonably well in studies with simulated data (Isaac et al., 2014), although it cannot cope with all inherent properties of opportunistic data, so it is necessary to further examine potential bias thoroughly. The LL method developed by Szabo et al. (2010) takes into account the number of species in a logistic regression model:

$$\text{logit}(P_{it}) = \alpha + \text{year}_t + \log(\text{no. species}_{it})$$

where P_{it} is the probability of a species observed in quadrat i in year t, α is the intercept, year_t is estimated as fixed year effect and no. species_{it} is the number of species observed in a quadrat in a year. The use of the log expresses that P increases with the number of species observed but with a reduced magnitude. However, it is plausible that this relation is asymptotic rather than logarithmic, that is, after a particular LL is obtained, a further increase in the number of species on the list will not any longer affect P. Therefore, we modified this part of the LL method to a form that is similar to the Michaelis–Menten equation (e.g. Raaijmakers, 1987). We also added quadrat_i as random quadrat effect in the model, to account for spatial differences in surveys over time. This leads to a modified LL model:

$$\text{logit}(P_{it}) = \text{year}_t + (b_1 \times \text{no. species}_{it})/(b_2 + \text{no. species}_{it}) + \text{quadrat}_i$$

where b_1 and b_2 are the two parameters describing the Michaelis–Menten equation. Mean number of woodland species per year list is about 20 (Table 1) and maximum species number is about 400. Parameters b_1 and b_2 are meant to describe the observational process and have no particular biological meaning; they will therefore not be reported in this paper. As expected, a longer LL increased the probability of a species to be observed. Generally, this probability hardly increased if year lists consist of more than 200 species.

For each species, only quadrats were included in the analysis where the species had been recorded at least once in 1900–2013. For species for which we had sufficient data (i.e. occurred in >50 quadrats per region), we ran a separate model for each of three regions in the Netherlands with sandy soils (north, central, south).

We used R 3.2.3 (R Development Core Team 2015) and fitted models in a Bayesian mode of inference using JAGS with vague priors for all parameters (Plummer, 2009). We standardized the number of species in the model to enhance model convergence (Kéry, 2010). Posterior means are reported as point estimators of occurrence probability and associated Bayesian standard deviations as standard errors (SE) and 1.96 × SE as 95% confidence intervals (CI). Our interest is in estimating the year effects and trends therein. Occurrence probability was converted into annual indices with first year (1965, 1994) = 100. We assessed the trend per species as a derived parameter in the model by applying a linear trend regression to the year effects, thereby ensuring error propagation in trend estimation. If CI of trends do not overlap zero, trends are reported as significant. For each analysis, we ran three Markov chains with 6,000 iterations each and discarded the first half as burn-in. These specifications were sufficient to achieve convergence based on the Gelman–Rubin Rhat statistic (Rhat < 1.1).

2.5 | Multispecies indicators

We summarized changes in trends of groups of species by calculating the geometric mean per year of the annual species indices. This procedure is widely adopted to create indicators for biodiversity

change (e.g. global LPI, Loh et al., 2005; Van Strien et al., 2016). The geometric mean is stable when positive and negative trends, as well as their magnitude, are in balance. This applies, for example, in a situation where the distribution/population of one species in the indicator increases by 200% over a certain time period, the distribution/population of another species in the same indicator decreases by 50% in the same time period, and all other species in the indicator remain stable. If the number of species declining outweighs the number of species increasing at the same rate, the mean goes down, and vice versa. The geometric mean values were converted into annual indices with first year = 100. We included the uncertainty due to sampling error of species indices into the confidence limits of our indicator, thereby ensuring error propagation. Finally, we applied LOESS (locally weighted polynomial regression) to produce smoothed indices and confidence intervals (Van Strien et al., 2016).

Multispecies indicators were compiled for separate functional groups, for periods 1965–1984 and 1994–2013; for non-roadside ECM species an indicator was constructed for the period 1965–2013. Trends in individual species and in multispecies indicators are expressed as percentage annual change. Thus, a trend value of 0 means no trend, 1 means 1% increase per year, −1 means 1% decrease per year. Trends in the indicator were tested by estimating the percentage change between the smoothed index value of the last year and that of the first year. The percentage change is significant if its CI does not include 0. Differences in trends between periods were tested similarly.

3 | RESULTS

3.1 | National trends

The majority of ECM species declined significantly before 1985 (43 of 75; Table S2). In contrast, only five species increased in that period. The opposite is true after 1994: most species (46) increased and few (6) declined. Some declined both before 1985 and after 1994, e.g. *Xerocomus badius*; or increased in both periods, for example, *Laccaria laccata* (Table S2). But many ECM species (27) had both a significant decline before 1985 and a significant increase after 1994. The recovery of the 27 ECM species became apparent soon after 1994: the first years in which the upward trends were significant were 1997 for *Gomphidius roseus*, *Russula fellea* and *Suillus bovinus*; 1998 for *Cantharellus cibarius* and *Suillus grevillei*; 1999 for *Boletus edulis*, *Hydnellum concrescens*, *Russula paludosa* and *Suillus cavipes*; 2000 for *Amanita fulva*, *Boletus erythropus*, *Cantharellus tubaeformis*, *Cortinarius bolaris*, *Gyroporus cyanescens*, *Laccaria amethystina*, *Lactarius helvus*, *Lactifluus vellereus*, *Leotia lubrica*, *Russula claroflava*, *Russula sardonia* and *Suillus luteus*; and a later year for *Amanita porphyria*, *Cortinarius alboviolaceus*, *Cortinarius semisanguineus*, *Hygrophorus hypothejus*, *Tricholoma albobrunneum* and *Sarcodon scabrosus*. The multispecies indicator summarizing the trend for all ECM species showed a significant decline before 1985 (−3.3% ± 0.6; $n = 75$; $p < .01$) and an increase after 1994 (3.4% ± 0.6; $p < .01$; Figure 2). These trends were even stronger if only the nitrophobic species were combined in the indicator (−5.1% ± 1.0 and 5.1% ± 0.8 respectively; $p < .01$; $n = 45$; Figure 2). Nitrotolerant species showed no significant trend for both periods (0.4% ± 0.6 and 0.1% ± 0.4 respectively; $p > .05$; $n = 9$; Figure 2).

The analysis with non-roadside species confirmed the opposite trends before 1985 and after 1994. The indicator declined before 1985 (−5.0% ± 2.2; $p < .01$; $n = 14$; Figure 3), remained stable thereafter for some years and then increased (trend from 1994 4.5% ± 1.4; $p < .01$).

In general, trends of litter saprotrophic fungi differed from those of ECM species. There were only two (of 25) species (*Clitocybe clavipes* and *Gymnopus perforans*) with a decline before 1985 and an increase after 1994. In contrast, there were six species with an increase before 1985 and a decline after 1994 (Table S2). Some terrestrial saprotrophs increased in both periods, for example, *Clitocybe nebularis*, while *Rhodocollybia maculata* declined in both periods (Table S2). The multispecies indicators showed a slight and similar increase for both periods (1.0% ± 0.8; $p < .05$; 0.9% ± 0.6; $p < .01$ respectively; $n = 25$; Figure 2).

Also trajectories of wood parasitic and wood saprotrophic fungi differed from those for ECM species. No single wood-inhabiting species declined before 1985 while showing an increase after 1994. Several species increased in both periods, among which were *Exidia truncata* and *Pycnoporus cinnabarinus* (Table S2); the latter was extremely rare before 1965. A few of the wood-inhabiting species declined in both periods, for example, *Tricholomopsis rutilans*: a species associated with decayed conifer wood. The multispecies indicator for wood-inhabiting species increased in both periods (2.8% ± 1.4; $p < .01$ and 1.1% ± 1.0; $p < .05$ respectively; $n = 30$; Figure 2).

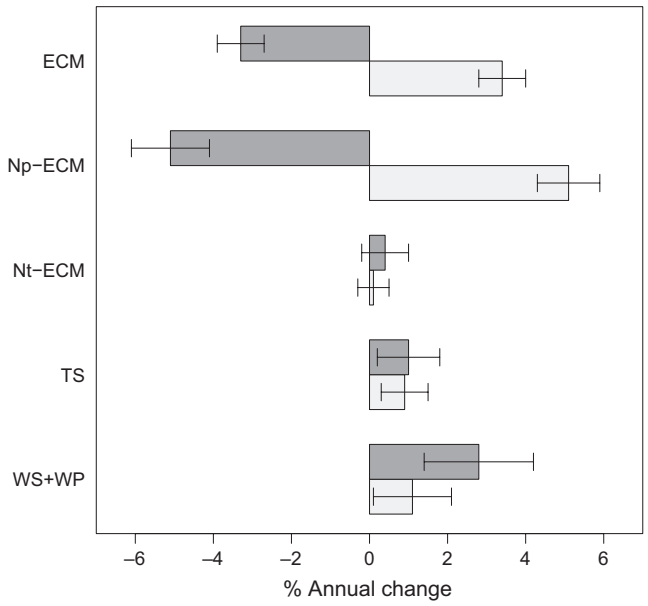

FIGURE 2 Trend in multispecies indicators (mean ± CI) for mycorrhizal species (ECM; $n = 75$), nitrophobic ECM species (Np-ECM; $n = 45$), nitrotolerant ECM species (Nt-ECM; $n = 9$), terrestrial saprotrophs (TS; $n = 25$) and wood saprotrophs and wood parasites (WP+WS; $n = 30$) before 1985 (dark grey) and after 1994 (light grey). A trend value of 0 means no trend. The trend is significant if its CI does not include 0

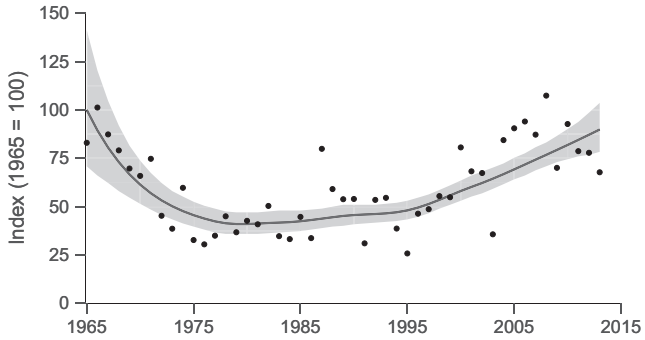

FIGURE 3 Multiple-species indicator based on non-roadside species, that is, nitrophobic ECM species (n = 14) that hardly occur in roadsides (see text for explanation). Species included are *Russula paludosa*, *Suillus bovinus*, *Tricholoma albobrunneum*, *Tricholoma equestre*, *Tricholoma terreum*, *Coltricia perennis*, *Cortinarius cinnamomeus*, *Cortinarius semisanguineus*, *Gomphidius roseus*, *Gyroporus cyanescens*, *Hygrophorus hypothejus*, *Inocybe subcarpta*, *Lactarius deliciosus* and *Lactarius vietus*. Grey area depicts the confidence interval

3.2 | Regional trends

Before 1985, ECM species as a group significantly declined in the central and southern region, but not in the north (Figure 4). After 1994, they significantly increased in the north and the central region, but not in the south. The number of increasing species after 1994 is highest in the north and lowest in the south, while the reverse is true for the number of declining species (Table S3). Nitrophobic ECM species declined significantly in all regions before 1985 and increased significantly in all regions after 1994 (Figure 5a; Table S3). Trends for nitrotolerant ECM species were non-significant for both periods for all regions (Figure 5b; Table S3). Terrestrial saprotrophic species increased significantly in all three regions before 1985, but a significant decline occurred in the central region and in the south after 1994 (Figure 5c). Wood-inhabiting species increased significantly in the central region in both periods and in the south before 1985 (Figure 5d).

4 | DISCUSSION

4.1 | Trends in ECM species

We report a highly significant recovery of ECM species in the Netherlands since 1994. This increase is in strong contrast with the strong decline before 1985. The fall before 1985 and rise after 1994 were more conspicuous for nitrophobic species and contrasted with changes in two other functional groups (litter-inhabiting saprotrophic and wood-inhabiting saprotrophic and parasitic species) with a generally lower sensitivity to N deposition. Contrary to the nitrophobic species, nitrotolerant ECM fungi as a group appear not to respond much to the decrease in nitrogen deposition after 1994; the overall trend was not significant for both periods. These results are in line with our expectation, and they seem to confirm that the found trends for nitrophobic ECM species are indeed nitrogen driven.

The analysis with non-roadside species showed that the upward trend for ECM species began in the late 1990s (Figure 3), thus a few years after the reduction in nitrogen deposition started. The decline before 1985 was slightest in the north and the increase after 1994 slightest in the south, in line with the hypothesis that the (cumulative) effects of nitrogen were the least pronounced in the north and the strongest in the south (Figures 4 and 5a).

Our analyses provide evidence that ECM species have benefitted from the nation-wide measures to reduce nitrogen deposition. The detected recovery is not an artefact of a change in searching behaviour because we controlled for this. It is further unlikely that the recovery is a result of the reduction in SO_2 because this process began already in the early 1980s. Local changes in forest management are also not likely causes, because recovery occurred in woodlands of all considered regions. Local management practices, as part of the Survival Plan Forest and Nature, that were specifically geared towards restoration of ECM fungi were successful, but were implemented only very locally and would not have impacted the large-scale trends that we report here (Baar & Kuyper, 1998). Changes in the management of roadsides, after their high mycological value was noted, cannot explain this positive trend either, because recovery has happened in the forest as well. Also increased precipitation is not a likely cause of the observed recovery. Annual and summer precipitation have increased over the last decades (CBS, PBL, Wageningen UR, 2016c) and generally higher rainfall in summer leads to more mushrooms (Boddy et al., 2014). But the recovery observed after 1994 occurred in a relatively dry period; except for 1998, summer precipitation was lower than in 1994 for all years between 1995 and 2000.

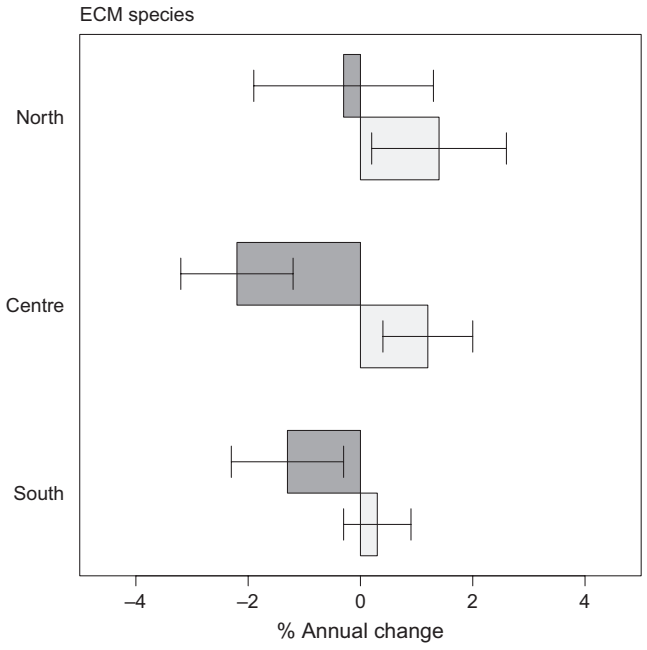

FIGURE 4 Trend in multispecies indicators (mean ± CI) for ECM species (n = 35) for each of the three regions (north, centre, south) and for each of the two study periods (1965–1985: dark grey; 1994–2013: light grey). A trend value of 0 means no trend. The trend is significant if its CI does not include 0

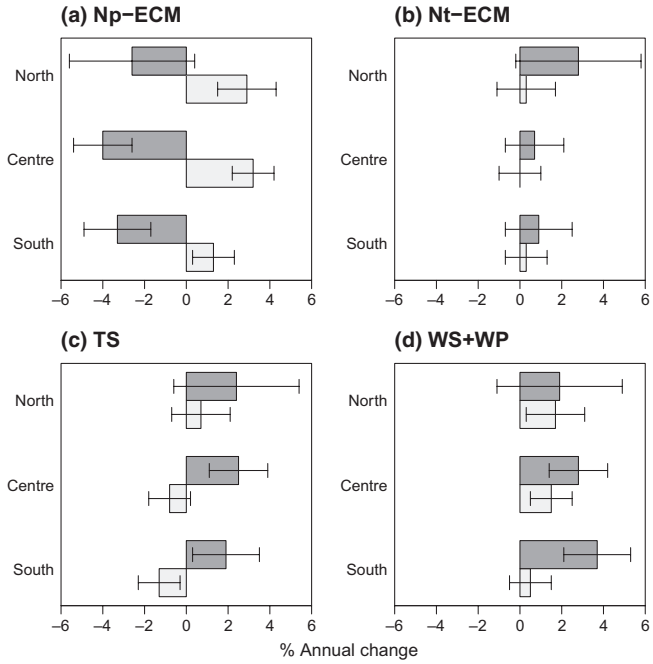

FIGURE 5 Trend in multispecies indicators (mean ± CI) for (a) nitrophobic ECM species (Np-ECM; *n* = 12), (b) nitrotolerant ECM species (Nt-ECM; *n* = 9), (c) terrestrial saprotrophs (TS; *n* = 20) and (d) wood saprotrophs and wood parasitic (WS+WP; *n* = 20) in three regions before 1985 (dark grey) and after 1994 (light grey). A trend value of 0 means no trend. The trend is significant if its CI does not include 0

In experimental studies, significant recovery of ECM species occurred within 6–15 years after termination of nitrogen loading (Boxman et al., 1998; Hasselquist & Högberg, 2014; Högberg et al., 2010). Some studies, however, reported much longer recovery times (Strengbom et al., 2001). Long delays are expected because species respond to accumulated levels of nitrogen in the soil over many years rather than to current deposition levels (Dise et al., 2011; Kuyper, 2013). Reduction in nitrogen deposition will initially have only a very limited impact on high cycling nitrogen rates in the, often thick, organic layers (Lucas & Casper, 2008). Surprisingly, our findings suggest that ECM mycorrhizal species responded already after 4–6 years. Possibly this is because our study quadrats have sizes of 1 km × 1 km and within such large quadrats there may be patches with less litter and hence less nitrogen accumulation, that respond more rapidly to lower nitrogen input.

Our results confirm the large-scale decline of ECM species in the Netherlands reported previously (Arnolds, 1988, 1991; Jansen & Van Dobben, 1987; Nauta & Vellinga, 1995). Our results also confirm the positive trends described by Arnolds and Veerkamp (2008). Arnolds (2010) reported the increase in a number of rare hydnoid fungi after 1995, and here we show that recovery has occurred for many more ECM species.

4.2 | Trends in other functional groups

In general, terrestrial saprotrophic species have slightly increased over the entire study period (1965–2013). Recent trends in some regions, especially in the southern and central region, however, were negative. The causes of this decline need further study. Trends in wood-inhabiting species are more easily explained. Dutch woodland is relatively young, with an average tree age of 60 years (CBS, PBL & Wageningen UR, 2014). The maturation of the forest contributes to the increase in several wood species (Arnolds, 1988; Nauta & Vellinga, 1995). Many wood parasites have profited from the aging of the trees, especially in forests that were not well managed. Furthermore, forest management practices have changed since the 1970s; dead trees and torn down branches are left on the forest floor rather than being removed (CBS, PBL & Wageningen UR, 2014). Wood-inhabiting saprotrophic species have taken advantage from that.

4.3 | Potential biases

We used a combination of data filtering and statistical modelling to deal with sources of bias in our data. Isaac et al. (2014) demonstrated that the LL model that looked most similar to ours (referred to as "RR + SF + LL + Site" in their paper) is robust against changes in sampling effort. Yet, Isaac et al. (2014) also pointed out that this model delivers biased trend estimates for a study species when the number of species per site changes over time (see also Szabo et al., 2010). Suppose that the longer lists length we found (Table 1), were not due to higher sampling effort but to a "real" average increase in species after 1994. The LL model interprets longer lists as higher sampling effort and consequently would estimate reduced year effects in later years. This may lead to false declines after 1994. However, we found the opposite for ECM species. Consequently, this drawback of the LL model does not undermine our conclusion of the increase in ECM species after 1994. On the contrary, if many species in reality have increased since 1994 and have led to longer lists, we rather underestimated the recovery. In the same vein, the estimated declines before 1985 may be overestimated by the model if the number of species per quadrat has increased in 1965–1984, but we think such an increase is unlikely. Isaac et al. (2014) further demonstrated that the LL model is vulnerable to changes in detection probability for particular species (Mackenzie et al. 2006; Szabo et al., 2010). The shift of observers towards an increased tendency to include roadside verges in their surveys may cause such a change in detection for species. Another reason may be selective reporting of particular species, but the majority of observers reported all species they could reliably identify. The detection of species may also change if observer skill to recognize a species has increased. The increase in *Russula betularum*, for example, may partially be explained by improved field recognition, although this species has previously been reported to increase in moderately high nitrogen areas in a nitrogen deposition gradient study (Lilleskov et al., 2001). Although we cannot exclude the possibility of biased trends caused by changes in detection, changes in observer skill cannot explain the overall recovery we found for ECM species, because almost all of these species are easily recognizable by field mycologists.

We have shown that this type of study is possible even with unstandardized data that suffer from several biases. In earlier studies the same data were analysed by aggregating all observations into

5 km × 5 km grid cells, whereafter the number of occupied grid cells was compared between 5-year periods (Arnolds, 2010; Nauta & Vellinga, 1995) or much longer periods (e.g. Arnolds, 1988; Arnolds & Veerkamp, 2008). Such broad aggregations over space and time suppresse variation in observer effort, but at the same time reduce sensitivity to detect short-term changes. By examining potential biases and finding solutions to control for them, through data filtering and statistical modelling, we were able to assess annual changes rather than changes between longer periods and to estimate trends in the number of occupied 1 km × 1 km quadrats rather than in 5 km × 5 km quadrats.

4.4 | Perspectives

The negative impact of nitrogen on terrestrial ecosystems has prompted many studies in the last decades (e.g. Bobbink et al., 2010; Dise et al., 2011; Kuyper, 2013). Yet, it remained largely unknown whether large-scale reduction in nitrogen deposition has any rapid positive effects or whether long-term legacies of previous deposition would frustrate the rate of ecosystem recovery. This study is one of the few scientific studies to date documenting evidence of success of large-scale (nation-wide) environmental measures in terrestrial ecosystems in the Netherlands and elsewhere in Europe. Another example is Van Herk, Aptroot, and Van Dobben (2002) who reported positive effects of the large-scale reduction in SO_2 deposition on lichens in the Netherlands. Not only common ECM species have benefitted from the nitrogen load reduction but also several Red-List species (Table S1). Yet, recovery is only partial and many woodland ECM species are still on the recent Red-List of macrofungi species (Arnolds, 2010; Arnolds & Veerkamp, 2008). That may be because current regional nitrogen levels (21–35 kg N ha^{-1} $year^{-1}$ in 2015) still exceed the critical loads (10–20 kg N ha^{-1} $year^{-1}$) for eutrophication for the nutrient-poor forest ecosystems in which most of these ECM species occur (Bobbink & Hettelingh, 2011; Suz et al., 2014).

Our study indicates that, despite continuing high nitrogen deposition levels, abatement policies already lead to partial recovery of ECM communities soon after instalment.

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AUTHORS' CONTRIBUTIONS

A.S., R.V., M.B., M.N. and T.K. conceived ideas for the manuscript, M.B., M.N. and T.K. provided specific mycological expertise; A.S. designed methodology and analysed the data; A.S. and R.V. led the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

DATA ACCESSIBILITY

All data analysed for this study are available from Dryad Digital Repository https://doi.org/10.5061/dryad.bp096 (Van Strien, Boomsluiter, Noordeloos, Verweij, & Kuyper, 2017).

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