

The use of living labs to advance agro-ecological theory in the transition towards sustainable land use: A tale of two polders

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ABSTRACT

Agricultural ecosystems worldwide are on life support. A key challenge is the translation of global sustainability goals to local contexts, particularly those related to sustainable land use, climate and biodiversity at the landscape scale. Living labs, place-based, focal areas as pilots of change, have the potential to be instrumental in driving the development of local solutions. When used to their full potential, they can both enable the advancement of agro-ecological theory and aid the transition to sustainable agricultural land use. In this viewpoint paper we present two conceptual advancements culminating in their high potential: (1) a methodological approach with replicated modes of transition and reference sites, while proposed agricultural modes are co-created through stakeholder encounters, (2) a framework that enables long-term monitoring of the relation between ecosystem functioning (expressed as leakiness) and biodiversity (expressed as ecological interaction networks), taking into account the full scale of ecological interactions within the agro-ecosystem. We illustrate how these conceptual advances can be implemented in a living lab in the Netherlands. Here, we discuss how these advances can generate impact and accelerate the transition to planetary-scale sustainability in agricultural ecosystems.

1. Introduction

1.1. The need for transforming agricultural ecosystems

Land use change, intensified human land use, and agricultural intensification have affected ecosystems worldwide (Foley et al., 2005), resulting in e.g. precipitous drops in insect biodiversity (Raven and Wagner, 2021), soil community richness (de Graaff et al., 2019) and an increase in soil erosion (Montgomery, 2007). There is widespread consensus that large-scale system changes are needed to ensure the provisioning of ecosystem services by natural and agricultural systems (Rockström et al., 2017). Agro-ecosystems thus require a shift towards more sustainable practices, reducing their impact on water, soil, air, climate and biodiversity, while at the same time generating enough food

for the growing world population (Lanz et al., 2018; Erisman, 2021). These pivotal challenges are condensed into a number of sustainability goals, particularly those related to sustainable agricultural land use at the landscape scale including 1) a major reduction in greenhouse gas emissions from soil, moving towards a net uptake of greenhouse gases in soils, 2) limited emissions of nitrogen to air (ammonia (NH₃), nitrous oxide (N₂O) and nitrogen oxides (NO_x)) and leaching of nutrients to groundwater (phosphate, nitrate, NO₃), 3) limit pollution of adjacent ecosystems (pesticides, phosphate etc.), 4) maintaining or increasing (agro)biodiversity to address goals 1–3, and 5) economic sustainability: systems are sufficiently profitable, such that they can be managed sustainably and translated laterally to areas that are geographically similar^{1,2}. To enable this transition in land-use intensity to reach these goals, we urgently need a systemic approach, in which researchers

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¹ https://commission.europa.eu/strategy-and-policy/priorities-2019-2024/european-green-deal_en

² https://food.ec.europa.eu/horizontal-topics/farm-fork-strategy_en

collaborate with key stakeholders to co-create solutions drawing on an interdisciplinary body of literature from (agro-)ecology, economy and sociology (Bernstein, 2015).

Despite the emerging consensus on these goals, their actual implementation relies on their translation to locally applicable alternatives to conventional agriculture, because this is the scale at which agricultural management takes place. While experiments on such translations are happening at a broad range of sites (Hossain et al., 2019; Toniolo et al., 2023), we currently lack a robust framework to jointly evaluate the above-mentioned challenges. Living labs are collaborative platforms where a variety of actors work together to address complex societal issues (Bouma, 2022). The EU has placed a strong focus on the use of such living labs to facilitate a sustainable agriculture transition (Potters et al., 2022). Ultimately, living labs are expected to enable essential developments to drive the sustainable agriculture transition and allow for studies ranging from e.g. the development of alternative socio-economic business models, to real-time ethnographic studies on co-creation processes. Here, we specifically focus on the agro-ecological component of living labs and how living labs enable the advancement of agro-ecological theory while simultaneously accelerating the transition towards sustainable land use.

The required agro-ecological framework should answer the following questions: 1) what to measure and how to link (changes in) biodiversity to (changes in) ecosystem functioning to establish if we are moving in the right direction for each of the 5 goals? 2) how to assess reproducibility of results and insights in such a way that they can be translated laterally to areas that are geographically similar? First and foremost, this requires an in-depth understanding of the interactions between ecological communities and ecosystem functioning and how to track changes therein. Here, we propose a framework based on agro-ecological approaches underlying the transition, which adequately addresses the aforementioned questions, and we use a recently established living lab, *Polderlab Vrouwe Venne*, to illustrate how this framework can be implemented.

1.2. Conceptual framework to link ecological communities and ecosystem (dis)function in transitioning systems

We propose a conceptual ecological framework (Fig. 1) in which agriculturally-driven changes in species community structure are linked to changes in carbon and nutrient losses. This enables a better understanding of the associated ecological mechanisms, and allows for assessing the impacts of different agricultural practices on the agro-ecosystem and its surroundings. As such, our framework (Fig. 1) facilitates the linking of complex ecological interactions to the leakiness (i.e. loss of carbon and nutrients) of agricultural soils, which is key to achieving a great level of sustainability.

To assess how species communities change in response to agricultural practices and changes therein, we propose to apply ecological networks (Castro et al., 2021; Morriën et al., 2017; Ochoa-Hueso et al., 2021), a powerful approach that can be used to assess species co-occurrence of entire ecological communities and allows for the evaluation of temporal changes therein. Network analyses reduce the ecological complexity into several metrics, thereby allowing for linking species co-occurrence to ecosystem functions. Furthermore, network representations are sensitive metrics even when changes in species assemblages are small (Ochoa-Hueso et al., 2021). They present an intuitive representation of complex data, to e.g. local stakeholders, when compared to complex representations of changes in both community structure (i.e. using multivariate ordination) and alpha diversity of multiple groups of organisms.

To assess how ecosystem functioning changes in response to (changes in) agricultural practices, we consider losses in terms of “leakiness”; the ability of an agro-ecosystem to retain its organic and inorganic resources. Here, we focus on nitrogen (N) and carbon (C), but our framework can be easily extended to include other elements if

desired (e.g., P, K). We focus on both emissions to the atmosphere and leaching through soil, in the remainder of this paper referred to as greenhouse warming potential (GWP) and mineral nitrogen (N).

In summary, our framework (Fig. 1) implies that over time:

- 1) Agro-ecosystems can shift along the axes of emissions, depending on changes in absolute emissions of mineral nitrogen (NH₃ to air and NO₃, NH₄ to water) and greenhouse gases (CO₂, CH₄, N₂O), as they become more or less ‘leaky’, indicating a decrease or an increase in functioning (less or more balanced nutrient cycling).
- 2) Ecological interaction networks, as an expression of biodiversity and ecological connection, can become tightly connected (Ochoa-Hueso et al., 2021).
- 3) Based on ecological theory linking network connections and carbon and nitrogen cycling (Morriën et al., 2017), we expect that these changes in ecological interaction networks coincide with observed changes in leakiness/emissions.

Overall, our expectation is that certain alterations in agro-ecosystems (particularly those that result in a transition towards less intensive agricultural practices): 1) result in ecological communities to become more connected and 2) that this coincides with a decrease of the systems’ leakiness.

1.3. Living labs and the conceptual framework

Here, we regard living labs as learning spaces where changes in biodiversity and agricultural emissions can be assessed over time to allow for exploring the effects of different land use practices on ecological communities and ecosystem functioning, while strongly considering their embedding in the local landscape through stakeholder encounters enabling co-creation of novel agricultural practices, in line with the views of Ceseracciu et al. (2023), who regards living labs both as a living system as well as a laboratory. When used to their full potential, we believe they can play a crucial role in the transition towards sustainable agricultural land use. A recent analysis of the literature has underscored the need for appropriate evaluation criteria of the effectiveness of living labs, including the operational, social, methodological, and environmental aspects (Cascone et al., 2024), which is where our agro-ecological framework comes in. While we fully acknowledge that the process of co-creation through active participation of a diverse set of stakeholders constitutes an essential ingredient to the success of a living lab (McPhee et al., 2021; Cascone et al., 2024; Gardezi et al., 2024), we specifically focus on the analysis of the environmental component. As such, our living lab, in combination with small-scale lab experiments, modeling and field experiments enables the discovery of mechanistic changes over time as a result of land use transitions and the role of stakeholders therein. This places our living lab in the third ideal-type, as categorized by Toffolini et al. (2023), focusing on experimentation as a catalyst for long-term local collective action. We believe that our conceptual framework, illustrated by how it is implemented in a true living lab setting helps stakeholders to transition the agricultural system together with other enabling conditions such as financing, capacity, policies and behavioural change, nonetheless acknowledging that each component is essential for the successfulness of living labs to truly transform agriculture.

In the remainder of this viewpoint paper, we illustrate how the above-presented framework can be implemented in the context of a local living lab and its reference site. As such, this demonstrates the potential of living labs for (1) facilitating a methodological approach with replicated modes of transition and reference sites while proposed agricultural modes of transition co-created with local stakeholders and (2) enabling long-term monitoring of the relation between ecosystem functioning (expressed in terms of ‘leakiness’) and biodiversity (expressed as ecological interaction networks), taking into account the interactions within the food web.

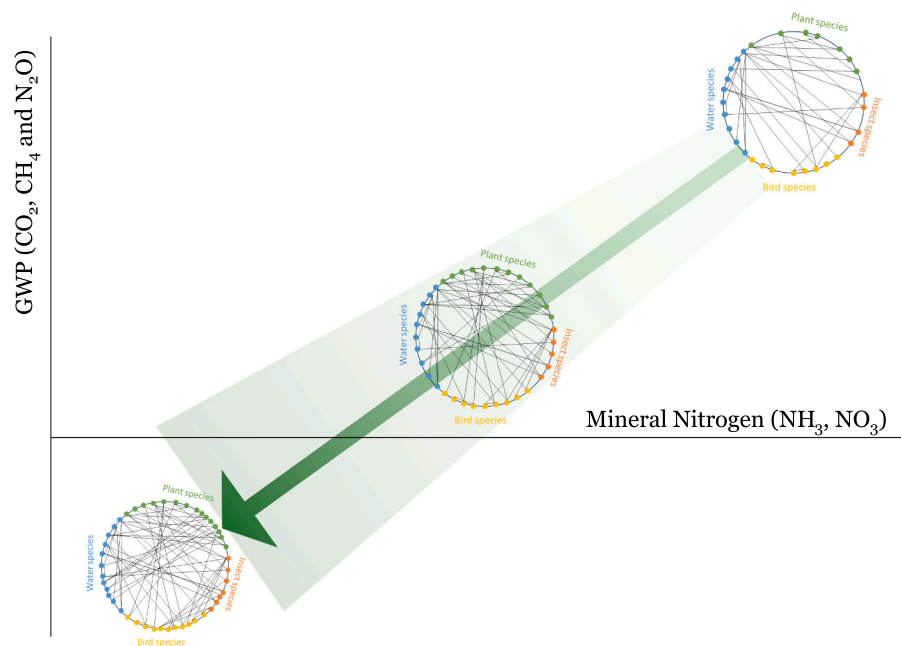


Fig. 1. Visualization of the conceptual framework. Indicated are the expected relation between changes in connectedness of the ecological networks (circular diagrams, different colors indicating different species groups, lines indicating co-occurrence of species, also refer to chapter 2.4) with the expected variation in connectedness visualized as green gradient arrow indicating low to high connectedness. Changes in greenhouse warming potential (GWP) and mineral nitrogen, together expressing the 'leakiness' of the system, are indicated on the y and x axes. Over time we expect the GWP and the mineral nitrogen emissions to fall as a result of the changes towards more sustainable agricultural practices, coinciding with more well-connected ecological communities. Note that GWP can become negative (net GHG fixing) while mineral nitrogen emissions cannot. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

2. Studying the agricultural transition in a living lab

2.1. Polderlab Vrouwe Venne: Contrasting focal and reference sites

Here, we introduce the *Polderlab Vrouwe Venne*, a living lab in the western part of the Netherlands, composed of two adjacent, contrasting polders, the Vrouwe Vennepolder, and the Boterhuispolder, both are typical polders in the agricultural landscape of the Western part of the Netherlands (52°2' N, 4°5' E). Both the Vrouwe Vennepolder (VV, 33 ha) and Boterhuispolder (BP, 28 ha) are hydrologically isolated pieces of agricultural land, located in the archetypical Dutch peatland landscape in the Western part of The Netherlands and are currently dominated by agricultural grassland (Fig. 2). In both polders, the aforementioned challenges apply: high greenhouse gas emissions (largely related to soil subsidence as a result of peat decomposition and the application of manure), eutrophication and pollution of freshwater systems, and an agricultural model that is highly dependent on draining the organic peatlands (van de Ven, 2004). While the pedogenesis of these polders is very similar, management in these polders has been markedly different in the past decade. The VV has been used as a highly productive grassland until the end of 2020, i.e. an intensive management regime with regular mowing (4–5 times a year) and fertilization by injection of liquid slurry (150–250 kg N per ha), groundwater tables of 40 (summer) and 60 cm (winter) below the soil surface and no grazing. The BP has historically also been used as high-productivity grassland, but around 2010 the owner decided to switch to more extensive practices, with mowing taking place 2–3 times a year, fertilization through disk slurry tanks ~100 kg N/ha, groundwater tables varying between 30 (summer) and 50 cm (winter) below the surface, the inclusion of Agri-Environmental Schemes including late mowing (after June 15th), meadow bird protection, vegetated river banks, and grazing of sheep and cattle. The agricultural management in the BP is expected to remain the same for the coming decade. As such, these two polders provide an ideal starting point for contrasting agricultural land use to test the conceptual

framework of changes in leakiness and accompanying changes in ecological communities.

A citizen's cooperation named '*Land van Ons*' (English translation: '*Our land*') acquired the Vrouwe Vennepolder in 2020. Their primary vision is to increase biodiversity on agricultural land.³ This requires the involvement of different stakeholders and the development of new business models, local knowledge and new innovations, co-created by all those involved. Therefore, they reached out to scientists at Leiden University and VU Amsterdam (MS, KB, SM, MB, all authors of this manuscript) to develop a Living Lab with the aim to initiate a biodiversity-inclusive agricultural transition on peat during a transition period of 10 years. Stakeholders were approached, primarily including local farmers, new farmers following agro-ecological principles, the 13 surrounding municipalities, the Ministry of Agriculture, Nature and Food Quality and the local water board. A co-creation process was initiated to develop the vision for VV. First, extensification of current land use took place in VV, before the implementation of novel agricultural modes (see 2.4). Starting in 2021, manure application has been reduced to 90 kg N/h/year while management at the BP reference site was kept constant at the same grazing intensity. Baseline data on ecological communities were collected in 2020 and 2021, and data on nitrogen and carbon losses were collected in 2020. This data allows for a first assessment of the structure of the ecological communities and the 'leakiness' of the areas, and as such will serve as a first assessment of our framework.

2.2. Baseline relations between leakiness and ecological communities

Details on methods of data collection and computing ecological networks can be found in Appendices A and B. During the null measurements (t_0 and t_1) we found that the VV had much higher GWP and

³ <https://landvanons.nl/>

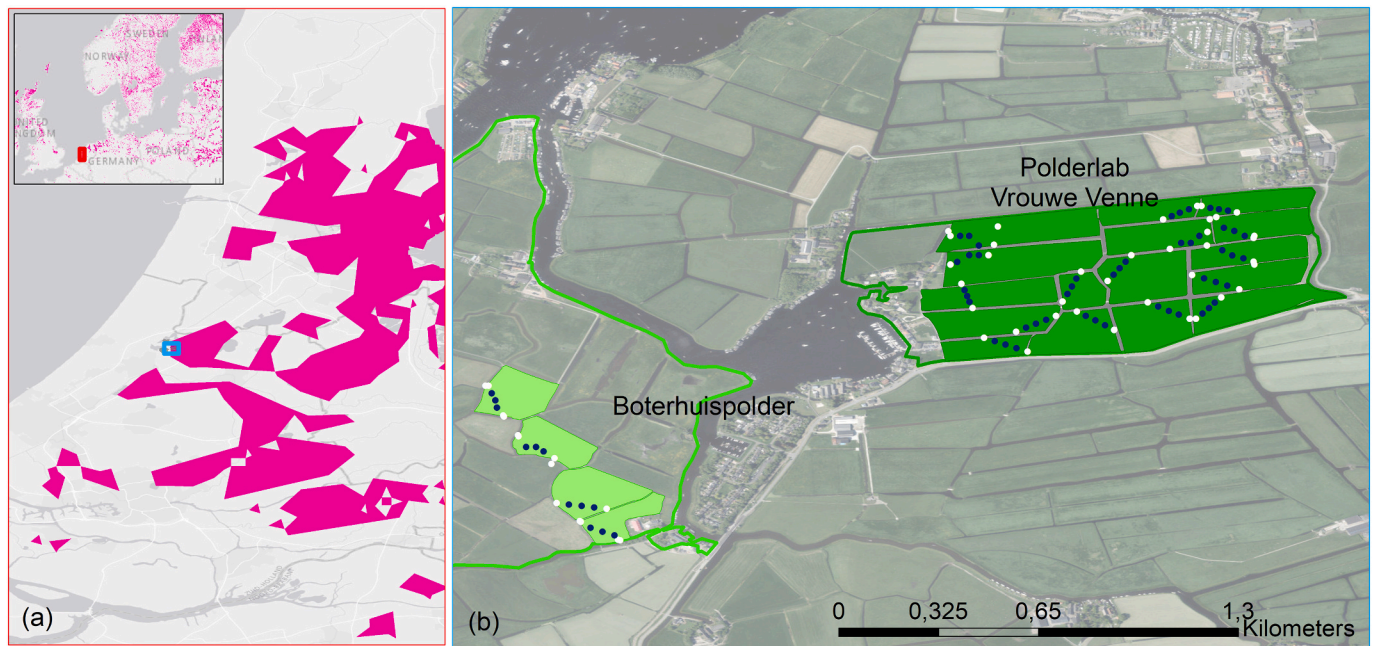


Fig. 2. Peatland areas stretch from the UK along the northern part of Europe (indicated with pink shades, Xu et al., 2017). The experimental (dark green) and reference polder (light green) are located in the western part of the Netherlands. Terrestrial sampling points are indicated with blue markers, white markers indicate the location of a 2-fold sampling point: terrestrial (ditch bank) and aquatic. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

mineral N content than the BP (Fig. 3). This can likely be attributed to the different management styles; where BP was managed with low livestock numbers (~1.5 heads/ha), little manure and less frequent mowing, the VV was managed at high intensity up until September 2020, including the frequent use of manure injections and frequent mowing in combination with a lower water table. These differences in emissions coincided with different co-occurrence networks: the VV had less connected networks and higher GWP and increased NO_3 concentrations in surface water while BP had more connected networks and a lower level of leakiness (Fig. 4). More connected networks (measured as

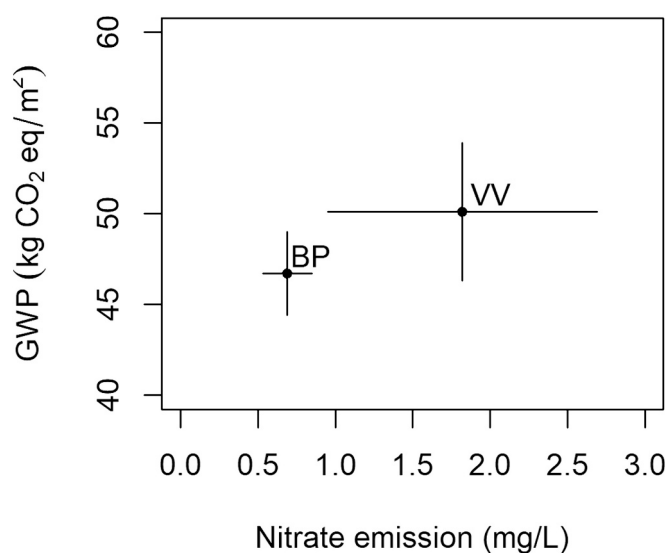


Fig. 3. t0 (2020) measurements of leakiness in the BP and VV expressed with NO_3 concentrations in surface water (x-axis) and GWP (global warming potential) based on fluxes of CO_2 , CH_4 and N_2O (y-axis). Bars indicate range of measurements across all measured plots. Nitrate emissions significantly different between the two polders.

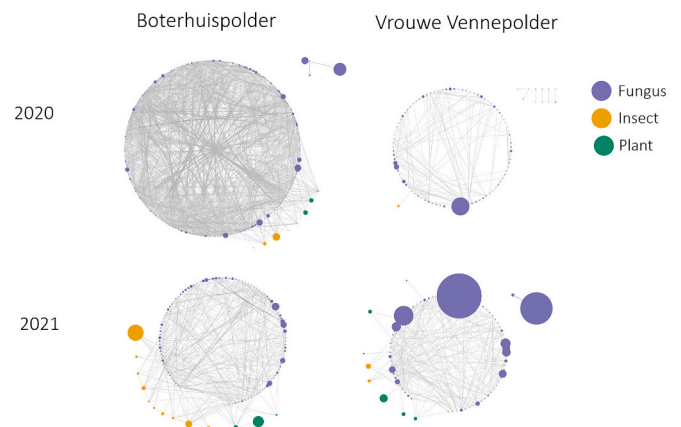


Fig. 4. Connectedness of species networks in the in the Boterhuispolder (reference site) and the Vrouwe Venne polder (focal site, in transition) for two years. The total number of taxa determines the size of the network and the size of the nodes marks the average abundance of the organisms across the plots in that polder and the color the identity of the organism. Purple marks fungi identified as amplicon sequence variants (ASV)s, green color marks plants, yellow indicates insects. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

number of edges and their centrality; Table 1) as observed in BP (3069 edges in BP vs 163 in VV in 2020) show that more species co-occur with each other at the landscape level which is a sign of a functional community. Further, more species co-occurred (number of nodes) in BP compared to VV (335 vs. 102) and more cross-kingdom connections were found, indicating more established structures of communities (for more information on network interpretation, refer to S1). In 2021 the connectedness of the network in BP had decreased, while the connectedness of the network in VV had increased (Fig. 4). Yet, in 2021 the network of BP was still more connected than the network of VV which was reflected by all network parameters (Table 1). The number of cross-

Table 1
Most important network parameters measured.

	BP 2020	VV 2020	BP 2021	VV 2021
Nodes	335	102	120	85
Edges	3069	163	524	270
Average degree (avgK)	18.322	3.196	8.733	6.353
Node with max betweenness	<i>Absidia cylindrospora</i>	<i>Rhizophydiales</i> sp.	<i>Mortierella</i> sp.	<i>Microdochium bolleyi</i>

kingdom co-occurrences had increased in both polders: in VV from 1 to 8 and in BP from 6 to 13.

2.3. Upcoming agricultural experiments in the Polderlab

In 2021, *Land van Ons* organized a series of meetings with aspiring new farmers to co-create the agricultural vision for the Vrouwe Vennepolder. From the visions of eight local farmers, four farmers were selected to carry out their vision in the living lab. During 2021 and 2022, the details of the implementation of the four farming practices were further developed and discussed with a wider group of local farmers, local governments and a group of scientists from various universities in five workshops to discuss how the requirements of a robust well-replicated long-term field experiment could be combined with a necessary long-term prospect for the respective farmers. In the end, this resulted in the four different agricultural visions which were co-designed by local farmers, the citizen cooperation *Land van Ons* and the involved researchers.

VV consists of 32 ha of agricultural land divided over 16 fields of roughly 2 ha each. Each of the agricultural practices will be replicated four-fold, randomly distributed across the polder (Fig. 5). As of mid-2023, implementation of the four practices started. The water table in the Vrouwe Vennepolder will be increased relative to those of the surrounding polders, reducing soil subsidence and greenhouse gas emissions (van Hardeveld et al., 2017), and the area will undergo a complete land use transformation to the aforementioned four types of agriculture.

All four proposed practices: extensive herb-rich grazing grassland, wet species-rich mowing land, cranberry peat moss and perennial agriculture on ridges (Fig. 5) will be closely monitored on all environmental aspects by researchers in close collaboration with farmers during a 10-year time period (2023–2033). This monitoring effort is financed entirely by the knowledge institutes involved in this Living Lab. During the time period 2023–2033, changes in leakiness of the system and shifts in ecological communities will be closely monitored according to methodologies mentioned above, supplemented with high frequency measures on gas fluxes and nutrient losses, again paid for and carried out entirely by the involved knowledge institutes. Given that changes in (soil) ecosystems are slow we will maintain the implemented practices for the entire 10 year period.

Over time, on an annual basis, the findings on ecological communities and nutrients and carbon losses will be shared with the involved stakeholders at the end of the growing season, allowing for iterative learning. This will be followed by a co-creative design session, which will take place before the start of the next growing season, during which the stakeholders discuss and agree upon the details regarding system management for the following growing season. Changes to management can include, but are not limited to, small adaptations to the current implementation scheme (in that case implemented in all four fields of a treatment) or in extenuating circumstances the cancellation of a mode of transition that is performing very poorly. This type of interaction through workshops is commonly used for the co-creation process in agricultural living labs (Cascone et al., 2024).

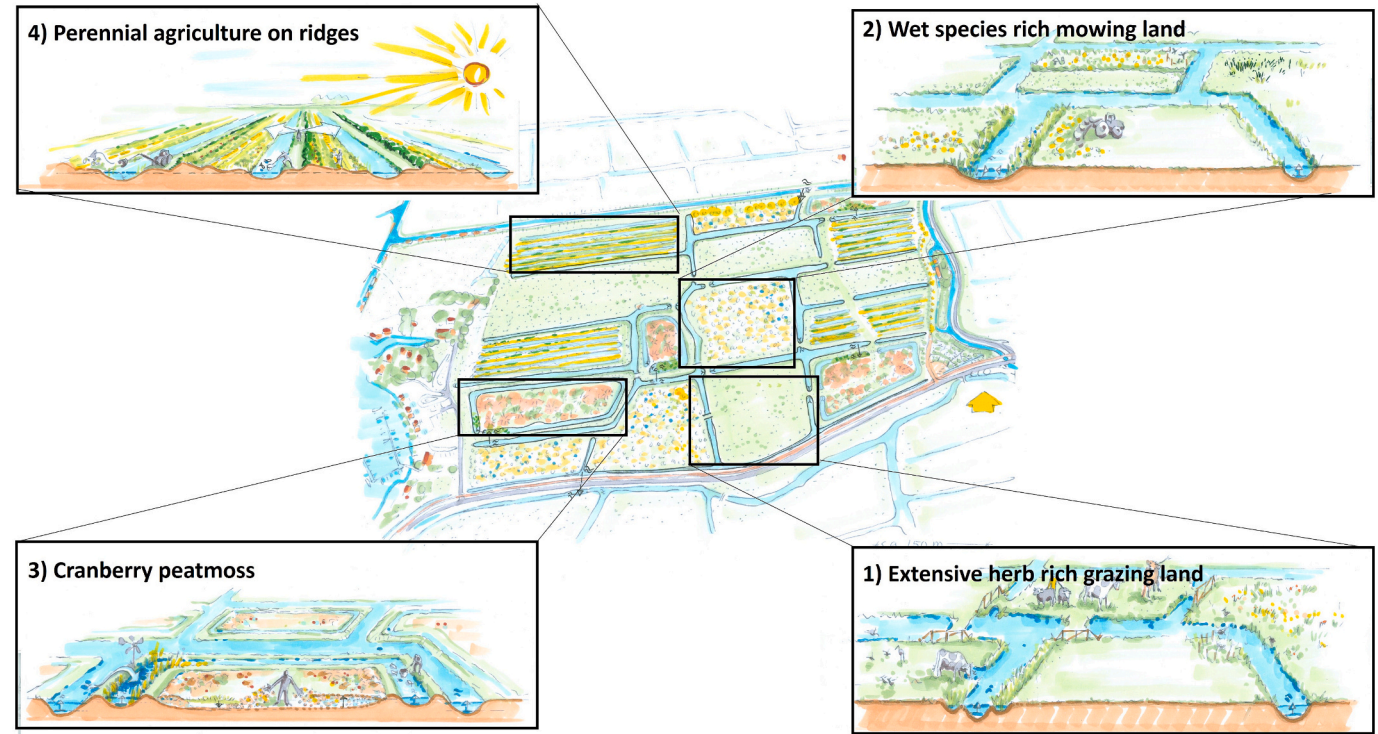


Fig. 5. A visual interpretation of future changes to the landscape in the Vrouwe Vennepolder, indicating the randomized location of four agricultural modes, each replicated four times.

2.4. Hypotheses for the interrelated changes in leakiness and connectedness

We expect to find that the implementation of the (partly radically) different agricultural practices will lead to pronounced changes in biodiversity as well as C and N cycling of soils (Figs. 1 and 6). As such, we expect that proposed framework can be used to closely evaluate/monitor if the changes in land use indeed result in the expected environmental effects. It is important to mention that the direct emissions from cattle of N (mainly NH_3) and C (mainly CH_4) in the treatments where cattle form an integral part are not (yet) included in this framework, neither are the emissions travel and machinery as they do not pertain to the functioning of the local ecosystems. For each of the modes of transition, the expectation is that the profound changes in biodiversity will not just coincide with the changes in land use, but that they are also instrumental in reducing leakiness of soils, by guiding or coinciding with a decrease in leakage of mineral N, emissions of greenhouse gases and at the same time sequestering of the elements involved. The four novel agricultural practices are expected to lower N and/or C emissions as follows (Fig. 6): increasing the water table will lead to a reduction of GWP across the polder (Hardeveld et al. 2017). Extensive herb-rich grassland and moist, species-rich mowing land are expected to have the lowest reduction in emissions, given that those management practices are comparable to the current management of the BP. The leaching of nitrogen and phosphorus is expected to lower in cranberry peat moss because of the reduction of nitrogen inputs and the fixing of C in root systems. GWP emissions from soils are expected to decrease given that soil subsidence - the biggest source of carbon emissions which occurs as the decomposition of soil organic matter - will reverse due to the peat moss cultivation. A larger change in emissions is also expected for the perennial cultivation on ridges, which is expected to become a net fixer

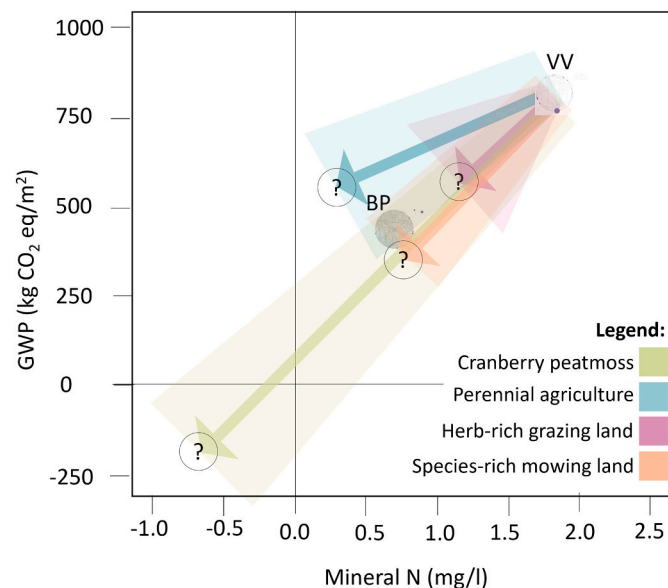


Fig. 6. The current GWP and mineral N in the conventionally, intensively managed VV are much higher than in the extensively managed BP. We expect that these emissions will reach a new stable state once the alternative agricultural practices are implemented and fully developed. The image shows the hypothesized endpoint 10 years from now. The expected trajectory of these emissions, and the speed of change, differs per agricultural management type and ranges from a reduction in mostly N emissions (perennial wet cultivation), to a limited reduction in both N and C emissions (herb rich grassland and damp hay land), to a high reduction in both GWP and mineral N (peat moss). The expected trajectories of these emission changes are indicated with the shaded color bands, these bands indicate a range as it is yet to be discovered what the exact trajectories will be. The circles with question marks indicate expected changes in the ecological network for the different agricultural practices.

of CO_2 and mineral nitrogen.

Ultimately, only the peat moss agricultural practice is expected to significantly lower both GWP and mineral N; the cultivation of peat moss is expected to lead towards soil rising (instead of subsidence) hereby taking C from the atmosphere and fixing N. The trajectories from current emissions to expected emissions after 10 years may vary (indicated by the shaded bands in Fig. 6). We expect the emissions to reach a new 'dynamic equilibrium' after the novel agricultural practices are fully implemented and matured. The speed at which this new stable state will be reached is yet to be discovered, but we expect that it may depend on, among others, the changes required in the landscape (e.g. moving to herb-rich grassland requires less adaptation than transitioning to cranberry farming in peat moss).

Whether the reduction in GWP and mineral N will occur in a linear fashion is to be determined as well. We expect a general reduction over time, but there might still be highs and lows in the transition period before a new stable state is reached. Aside from the changes in the emissions we also expect changes in ecological network connectivity. We currently observe a large difference between the current ecological networks of the BP and the VV (Fig. 4), and we expect the ecological networks of the extensive herb-rich grazing grassland and wet species-rich mowing land in the VV to become more similar to those in the BP, while we expect them to become even more dense and well-connected for the cranberry peat moss and perennial agricultural ridges. We expect, in line with Morriën et al., 2017, that restoration/de-intensification will lead to tightening of the networks and to more efficient nutrient cycling. The long-term monitoring and replicated setup of this living lab will enable studying the consequentiality of the events; do changes coincide or do changes in one indicator follow the other?

We recognize four important limitations in our vision and we realize that the transition of agricultural systems needs much more and broader developments in terms of stakeholder involvement and co-creation. In Appendix C the limitation are discussed in more detail, and include: (1) the assumption that both the focal as well as the reference sites provide a good representation of the region, with modus operandi remaining as-is in BP and the currently committed farmers staying involved for the next 10 years. (2) the agro-ecological focus of the framework does not take into account the stakeholder involvement, wider societal impact and to what extent the knowledge and experience can be translated to other areas, as the implementation of novel agricultural practices relies heavily on the social acceptance of these practices (Gaba and Bretagnolle, 2020). (3) Currently, with the project still in the start-up phase, we have been able to test our framework for two years and we can only report the first results. Moreover, our measurement setup for t0 and t1 was just once a year, as such we are unable to capture differences on smaller timescales. In the future, we will expand this measurement scheme, including continuous monitoring of emissions and more frequent monitoring of ecological communities. (4) For assessing the leakiness of systems, we focus on GWP and nutrients from soils, and not on the emissions from farm animals (e.g. CH_4 , NH_3 , antibiotics). We focused on ecological communities as they demonstrate (potential) interactions between different taxa and allow for easy interpretation by farmers, citizens and other stakeholders involved in the project. Whether they accurately capture the changes in biodiversity resulting from the different agricultural practices, will have to be more closely evaluated in the future.

3. The potential of the living lab approach

Real-life living lab experiments have gained a lot of traction over the last few years, visible in the steep increase in publications on living labs and the initiation of the European Network of Living labs (Hossain et al., 2019). Living lab approaches have been argued to be well-suited to support the agri-food sustainability transition because they promote, among others, capacity building, empowerment through action, and iterative learning (Gamache et al., 2020). Such agro-ecosystem living

labs are becoming increasingly abundant and may address sustainability in the environmental, social and economic domains (McPhee et al., 2021). Living lab theory primarily focuses on the definition of living labs (Hossain et al., 2019; MCPhee et al., 2021) and discusses the potential impact of living labs (Beaudoin et al., 2022). However, generalization of knowledge and measuring specific impacts resulting from living labs, and strategies to achieve these, have been lagging behind (Beaudoin et al., 2022; Bronson et al., 2021), as has the development of knowledge on the use of living labs for advancing agro-ecological theory.

Here, we discuss: (1) the role of living labs in agro-ecological theory and (2) how impact from living labs can drive the transition towards sustainable farming.

3.1. The role of living labs in agro-ecological theory

Temporal agro-ecological processes are difficult to assess, as that requires long-term monitoring of changes in a controlled environment with replicated modes of transition. The living lab approach as proposed here, with a long-term research horizon (ten years for VV) allows for the observation of both short- and long-term changes in biodiversity and ecosystem functioning without relying on chrono-sequence methods that use a space-for-time substitute. Further, this setup allows for a temporal assessment of changes in ecological communities – at the full scale of interactions within the food web – and ecosystem functioning. The use of a reference site with business-as-usual agricultural practices ensures a control, an essential element often missing in chrono-sequence alternatives. The fully replicated experimental setup for each mode of transition (replicated 4 times in VV, and distributed randomly throughout the polder), provides a minimal level of replication required to ensure the validity of ecological findings. As such, the insights resulting from our findings will advance our understanding of these ecological interactions over time.

Here, we specifically publish the ecological framework in which we place the *Polderlab Vrouw Venne*. By clearly stating the ecological framework in which we place our experiments and findings, and by clearly communicating our hypotheses on the expected changes related to the transition towards alternative agricultural practices, we ensure that the results from our experiments provide insight into the interaction between ecosystem functioning and ecological communities, which will enable the translation of these findings to geographically similar areas elsewhere. The further integration of our ecological framework and findings in a socio-economic understanding of the changing agricultural practices is made possible because of the living lab approach that allows for inter- and transdisciplinary collaboration. This aspect requires further attention but currently falls outside the scope of this paper (also see 3.2).

Placing the findings in ecological theory furthermore enables a better understanding of the potential mechanisms that underpin the observed changes. Potential causal and consequential relations between the changes in biodiversity and ecosystem functioning can be assessed and discussed. In this way, the findings are more easily generalized, and larger-scale connections between different ecological mechanisms can be uncovered. This deeper understanding makes it possible to create expectations on the effects of the tested methods to be translated to other areas - an important prerequisite to ensure the successful translation of the investigated agricultural practices to areas with comparable environmental circumstances regionally and (inter)nationally in order to reach the five goals.

3.2. The role of living labs in sustainable agricultural transitions

Living labs constitute experimental spaces which can serve to co-create new agricultural practices and agro-ecological methods, beyond the realm of current possibilities, by including their impact on the environment and considering the societal context in which these agricultural methods will be placed. However, the transition towards

sustainable agricultural systems does not solely rely on the implementation of new agricultural methods. Stakeholders play a key role in enhancing sustainability transitions, especially when stakeholder involvement is viewed as an iterative relational and collaborative process in which stakeholders are engaged throughout the entire process (Gonzalez-Porras et al., 2021). This involvement should be reflected in all decisions made along the process; for example, in consultation with all stakeholders involved we chose to express ecological communities as ecological networks because changes therein, in combination with changes in emissions, can be clearly visualized for a variety of stakeholders, which is essential for effective communication (de Oliveira et al., 2023).

Here, we considered primarily the agro-ecological component of the living lab. However, integration of ecological findings with economic and social information is essential to further ensure that the tested agricultural methods can find their place within the local social-historical landscape. Drastic agricultural changes at the landscape scale require a willingness on the part of various stakeholders to adopt new systems, but also new visions of the landscape. This willingness is influenced by perceptions of the risks involved, stakeholders' valuation of, and attachment to, the landscape, including their position in the wider socio-economic and cultural context (Barnaud et al., 2018; Daneri et al., 2021; Zimmermann et al., 2022). Careful analysis of these values, attachments and positions is needed, in combination with an explicit engagement with a diversity of visions, interests and needs, to ensure that the changes follow a just and responsible path (Cuppen, 2018).

In other words, for living labs to be successful contributors to the much-needed transition towards sustainable agriculture and to have real impact, a wider experimental vision embedded in ecological theory is required, but needs to be combined with a strong societal embedding in which a large variety of stakeholders is committed to a shared vision.

4. Summary

Living labs have the potential to advance agro-ecological theory and accelerate the much-needed sustainable agriculture transition. Here, we propose a theoretical framework providing the ecological foundation needed to ensure that the change is going in the right direction. Such a framework is essential for successful transferability of the novel agricultural concepts that are co-created through stakeholder encounters in the local environment. A long-term investment in living labs can enable real-time monitoring of changes and relations in biodiversity and ecosystem functioning, giving important insights into agro-ecological theory as well as contributing towards the generation of new agricultural practices embedded in their societal context, while also opening possibilities to study e.g. the socio-economic aspects of the agricultural transition, constituting essential components that require further study.

Author statement

The authors declare that this work is original and not published elsewhere.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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We want to express our sincere gratitude to the cooperation *Land van*

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Appendix A

A.1. Baseline data collection of ecological communities and leakiness

Data collection in both study areas took place in September 2020 (t_0 , starting point) and September 2021 (t_1). Species composition of the ecological communities was assessed using a series of standardized, well-replicated measurements on plant, fungal and terrestrial insect diversity and carbon and nitrogen emissions (Appendix B).

The 2020 data provide information on the baseline leakage of both the BP and the VV, and the baseline ecological communities. The 2021 data provide the first result on the changes in ecological communities after the first changes in agricultural management in the Polderlab took place. Because of logistical and financial constraints, the baseline measurements on leakiness were not repeated in 2021.

A.2. Baseline ecological networks

The data on plants, terrestrial insects and fungal amplicon sequence variants were used to create co-occurrence networks (Friedman and Alm, 2012; Morriën et al., 2017). In short, data from different species groups was combined by assessing at their co-occurrences across samples. Co-occurring species were assumed to be either directly interacting with each other (mutualistic or antagonistic relationships) or responding similarly to environmental cues. Separate networks were calculated using FastSpar in R (Watts et al., 2019) relying on SparCC (Friedman and Alm, 2012) for both polders and both years resulting in four networks (Fig. 3). In brief, networks were inferred based on centered log-ratio transformed read counts and neighborhood selection. We removed spurious connections using the iDirect method (Xiao et al., 2022). The cut-off value for each network was calculated using random matrix theory using Poisson distribution at the level of $p < 0.001$. The networks were visualized in Cytoscape (Shannon et al., 2003).

Both positive and negative edges were included in the analyses and given similar importance in the analysis for network parameters as we were not trying to separate between mutualistic/antagonistic relationships and hence direction does not matter. The node sizes were scaled to the average (relative) abundance of the species within that polder and year (Table 1). The co-occurrence networks revealed that in 2020 the community in BP was considerably more connected than in VV (Fig. 4). This was reflected in all network parameters including the number of nodes, edges, and in average degree between edges (Table 1). There were furthermore more cross-kingdom (plant-insect, insect-fungus or fungus-plant) linkages in BP compared to VV. In VV there was only one connected insect taxa (Miridae), while in BP four insect groups (Coleoptera, Thymbiidae, Ichneumonidae and category 'others') were connected both with plants and fungi. Further, two plant species (i.e., *Ranunculus repens* and *Taraxacum officinale*) were significantly co-occurring with observed fungal species. The most connected species in the networks were both fungi, i.e. *Absidia cylindrospora* (saprotroph) in BP and *Rhizophydiales* sp. (saprotroph or parasite) in VV. In 2021 the connectedness of the network in BP had decreased slightly, while the connectedness of the network in VV had increased (Fig. 4). Yet, in 2021 the network of BP was still more connected than the network of VV which was reflected by all network parameters (Table 1). The number of cross-kingdom co-occurrences had increased in both polders: in VV from 1 to 8 and in BP from 6 to 13.

Table 1
Most important network parameters measured.

	BP 2020	VV 2020	BP 2021	VV 2021
Nodes	335	102	120	85
Edges	3069	163	524	270
Average degree (avgK)	18.322	3.196	8.733	6.353
Node with max betweenness	<i>Absidia cylindrospora</i>	<i>Rhizophydiales</i> sp.	<i>Mortierella</i> sp.	<i>Microdochium bolleyi</i>

A.3. Baseline leakiness

The measurements of leakiness showed similar nitrogen water concentrations for both years in the BP (Fig. 3). The GWP, measured only in 2020, was >3.5 times higher in the VV than in the BP (Fig. 3).

Appendix B

Data collection in both study areas took place in September 2020 (t_0 , starting point) and 2021 (t_1). Within each polder, multiple transects were laid out for sampling (Fig. 2). Each transect followed a straight line of 100 m from ditch to ditch through a field. Every transect contained 5 sampling locations: 2 on the ditch banks (Fig. 2b, white dots) and 3 in the field (Fig. 2b, blue dots). Sampling plots were spaced 25 m apart. For the analyses in this study only the field sampling locations (3 per field) were used. This sampling strategy totaled up to 4 transects (12 samples) for the BP and 16 (48 samples) in 2020 and 2021 for the VV. Plant, terrestrial insect and fungal diversity were sampled in 2020 and 2021 at these sampling locations.

B.1. Data collection on ecological communities

Plant diversity was sampled within 1 by 1 m plots which were constructed using rope and pins. All plants within these 1 by 1 m plots were

identified to a species level.

Terrestrial insects were collected at the field sampling locations in a 1 by 1 m plot, using an inverted leaf blower with a stocking duck taped on the nozzle. After insect collection the insides of the stocking were emptied in a glass jar and brought back to the laboratory for subsequent identification. Samples were emptied into a tray where the insects were separated from the plant and soil debris, using a suction device for a maximum duration of 5 min. The insects were subsequently transferred into a Petri dish, identified to a family level and individuals were counted. After use the trays were emptied and suction devices were cleaned out before subsequent use.

Fungal diversity was measured by removing the top vegetation at the field sampling locations and sampling the top 10 cm of the soil by using a soil core with a diameter of 3 cm. Five subcore samples around a field location were amalgamated forming one sample per field location. Of every sample approximately 250 mg was used for DNA extraction using a QIAGEN Power Soil Pro Kit following the manufacturer's instructions. The internal transcribed spacer 2 (ITS2) of the nuclear ribosomal DNA was used as a DNA barcode. Baseclear BV generated PCR-amplicons using the universal primers ITS3 and ITS4 (White et al., 1990), and created a ready to run library, which was sequenced on an Illumina MiSeq (v3 Kit, 2 × 300 paired-end). Sequencing data were processed in Linux and R. The ITS2 rRNA region was extracted with ITSxpress from fungal sequences (Rivers et al., 2018). After that, the DADA2 was used for quality filtering (maxEE = 2, truncQ = 2), to join paired-end reads, to remove chimeric sequences, for modeling sequencing errors and identifying amplicon sequence variants (ASVs) by the DADA2 algorithm (Callahan et al., 2016). Taxonomy was assigned by using the RDP classifier based on the UNITE v2020 database (Abarenkov et al., 2010). All sequences from non-fungal origin were removed from the dataset. The species data were filtered to include only species present in approximately one third of the samples in that year and polder (species present in <4 samples out of 12 in Boterhuispolder and species present in <10 samples out of 29 samples in Vrouwe Venne polder were removed from the dataset). The same number of samples was obtained in 2020 and 2021, hence the same filtering was done.

B.2. Data collection on emissions

Greenhouse gas (GHG) fluxes were measured September 2020 according to (Drost, 2022) on three consecutive days. GHGs were measured in static opaque PVC chambers, equipped with a battery driven internal ventilator for 1.5 h. Chambers with a diameter of 30 and 40 cm high were used (volume 28 l). PVC rings were inserted 5–10 cm into the soil and chambers were mounted on the rings, closed off by an internal rubber seal ensuring airtightness of the chamber system. The chambers were covered with isolation foil to prevent temperature increases inside the chamber during measurements. After closing the chamber, headspace samples (60 ml) were collected after 0, 20, 40, 60, 75 and 90 min using a disposable syringe equipped with a needle to penetrate the septum in the chamber sampling port. Roughly, 54 ml of sample was used to flush a 6 ml exetainer vial (Labco, UK). The last 6 ml of sample was introduced in the vial after removing the outlet needle, thereby creating an overpressure of 1 bar in the vial. The vials were stored at room temperature until analysis. CO₂, N₂O and CH₄ were measured simultaneously in the same sample. Samples from the exetainers were introduced into a GC using an autosampler (TriPlus RSH, Thermo Fisher Scientific, Bleiswijk, The Netherlands) connected to a gas chromatograph (GC1300, Thermo Fisher Scientific) equipped with a Methanizer and a Flame Ionization Detector (FID) to detect CO₂ and CH₄ and an electron capture detector (ECD) for detection of N₂O. The gas chromatograph contained two sets of a pair Rt-Q-Bond capillary columns (L; 15 m and 30 m, ID; 0.53 mm, Restek, Interscience, Breda, The Netherlands). Nitrogen was used as a carrier gas, and oven temperature was set at 80 °C. Chromeleon™ Chromatography Data System 7.1 (CDS, Thermo Fisher Scientific) software was used to analyze the obtained gas chromatograms from the GC. Fluxes were calculated based on the accumulation or reduction over the 1.5 h measured. Gas concentrations were calculated in ppm values by comparing with calibration curves, which were generated by dilution of a certified gas mixture (1 ppm N₂O, 2 ppm CH₄ and 2000 ppm CO₂; Linde Gas, The Netherlands). The concentrations (ppm) were converted into absolute amounts (mmol) using the ideal gas law: $pV = nRT$ in which p is the pressure in the flux chamber (assuming equal to outside pressure), V is the volume, n is the amount of gas in mol, R is the gas constant (8.31 J·K⁻¹ mol⁻¹) and T is the temperature. Afterwards we used the measured GHG fluxes to determine fluxes per m² (surface area of the chamber was 0.071 m²). We calculated GWP using Eq. (1).

$$GWP = 28 \times CH_4 + CO_2 + N_2O \times 265 \quad (1)$$

Appendix C

We believe that the proposed framework can become very important to further stimulate the translation of global sustainability goals to local contexts, as it allows comparing to a starting situation and a business-as-usual scenario on a set of indicators critical to the much-anticipated agricultural transition. However, there are a number of inherent limitations to the proposed approach, some of them with important ramifications for other living labs, four of which are shortly discussed below. First, our framework uses only one study and one reference area (in our case two hydrologically isolated polder areas), which is a fundamentally different approach compared to a chrono-sequence studies in which a reference site is virtually impossible. That hinges on the assumption that both the focal as well as the reference provide a good representation of the region, which we believe is the case in our setup, but is something to be cautious about if this approach is to be used in other study areas, for example, if this approach is to be used in small catchments under transition. We also rely on the various farmers, including those in the BP-reference site, to continue their current practices for the 10-year duration of this study. To overcome this practical hurdle, all farmers have been offered a 10-year lease contract, which intends to provide maximum security and minimize the financial risk on the farmer's side. This leads to a second limitation: the strong ecological focus of the current framework. In its current form, our framework does not take into account the stakeholder involvement, wider societal impact and to what extent these can be scaled up, although it is well known that the implement of novel agricultural practices relies heavily on the social acceptance of these practices (Gaba and Bretagnolle, 2020). Ultimately, this will be an important step to actual implementation, which will require a lot of additional work on the cross section of natural and social sciences. Having said that, we are convinced that without a solid ecological foundation (i.e. do transitions actually lead to increase in biodiversity and associated decrease in GWP and losses of nutrients?) there is no need to move towards acceptance of such models. The third limitation refers to the limited time scale that we have been able to test our framework. At this moment, with the project still in the start-up phase, and we can only report the first results. However promising they may seem, this may change over the course of time. Moreover, our measurement setup for the t_0 and t_1 measurements was just once a year, this is a limitation as we are unable to capture differences in emission across smaller timescales. In the (near) future, when the new agricultural practices will be implemented, we will greatly expand this measurement scheme, including continuous monitoring of emissions and more frequent monitoring of the ecological communities. This will greatly

enhance the data and allow us to make much more rigorous assessments of the observed changes and how they relate to one another. To our knowledge no other currently existing setups or living labs allow for a test of our framework. However, despite the project being in its early phase, it does provide valuable information on the starting point and early changes in both polders in terms of their ecosystem functioning and the ecological communities. The fourth and last limitation refers to the ecological parameters of choice. When assessing the leakiness of systems, we chose to focus on GWP and nutrients from soils, and not on the emissions from farm animals (CH₄, NH₃, antibiotics, etc) as these are often unconnected to processes taking place in the soil and will require additional work. Furthermore, we use ecological networks to express the ecological communities as an indication of the available biota, the biodiversity informing us also of species co-occurring across samples. We focused on ecological communities as they demonstrate (potential) interactions between different taxa and allow for easy interpretation by non-scientific stakeholders in the project. Whether they accurately capture the changes in biodiversity resulting from the different agricultural practices, compared to perhaps more conventional measures of biodiversity, will have to be more closely evaluated in the future. Despite these limitations, we are convinced that the presented framework can be instrumental in providing an essential step towards a much-needed transition in agro-ecosystems.

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