ENVIRONMENTAL DNA METABARCODING AS A WAY TO IMPROVE BIODIVERSITY ASSESSMENT IN LCA FOR REGENERATIVE AGRICULTURE IN FINLAND

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ABSTRACT

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Biodiversity loss is strongly influenced by land use transformation on farms, overexploitation of resources and deterioration of soil and water health due to pollution. Regenerative agriculture (RA), which is one of the agroecological practices, has proven to be effective in terms of biodiversity conservation and restoration globally in general, and in Finland in particular. However, there is a lack of standardized methods that can comprehensively assess biodiversity of products systems or along product life cycles. This issue can be solved by environmental DNA (eDNA) metabarcoding and life cycle assessment (LCA). eDNA, which is a method with simultaneous determination of various taxa in different land use types, can provide more accurate and updated inventory data for LCIA. To find ways to integrate eDNA data into LCA models, an in-depth literature review was conducted to scrutinize various trends of correlation between eDNA-, LCA-, RA- and biodiversity-related studies. As a result, the tremendous gap in the combination of these studies was proved, suggesting possible reasons for this and ways of further elaboration. Based on literature analysis, an eDNA sampling strategy in RA was proposed, which is also applicable to Finland. Due to limitations with specificity of spatial distribution of biodiversity, the lack of RA standardization and absence of use of eDNA results in LCA models, ways to adjust characterization factors, reference state and baseline for more precise biodiversity assessment in LCA models were proposed. The following improvements were also presented to further enhance the approach.

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ABBREVIATIONS

AEP	AgroEcological Practice
BD	Biodiversity
CA	Conventional Agriculture
CE	Circular Economy
CF	Characterization factor
cSAR	Countryside Species-Area Relationship
DNA	DesoxyriboNucleic Acid
EBV	Essential Biodiversity Variable
eDNA	Environmental DNA
exDNA	Extracellular DNA
GEP	Global Extinction Probability
GHG	GreenHouse Gas
GLAM	Global Guidance for Life Cycle Impact Assessment Indicators and Methods
HTS	High-Throughput Sequencing
iDNA	Intracellular DNA
IW+	Impact World+
LCA	Life Cycle Assessment
LCIA	Life Cycle Impact Assessment
NGS	Next Generation Sequencing
OA	Organic Agriculture
PBF	Product Biodiversity Footprint
PDF	Potentially Disappeared Fraction

PNV	Potential Natural Vegetation
RA	Regenerative Agriculture
RS	Reference state/situation
SDG	Sustainable Development Goal
SOC	Soil Organic Carbon
TAL	Total Agricultural Land
UNEP	United Nation Environment Program
USDA	United States Department of Agriculture

Table of contents

Abstract

Acknowledgements

Abbreviations

1. Introduction

The problems of the biodiversity (BD) crisis are getting more significant every year, having exceeded safe operating space of both genetic and functional planetary boundaries (within "biosphere integrity") (Richardson et al., 2023). This problem was later recognized by IPBES as one of the most dangerous threats not only to the nature, but also to human well-being in particular (IPBES, 2019) due to rapid loss of BD abundance by 69% averagely and even by 94% in Latin America during last half century (WWF, 2022). Despite continued worldwide contributions to BD conservation, according to experts and their publications such as Global Biodiversity Outlook 5 and other scientific studies, almost none of the previously established Aichi Biodiversity Targets have been fully achieved by 2020 (Han et al., 2020; Maira et al., 2021; CBD, 2020). Thus, raising the question of the reality of full and inclusive implementation of the Sustainable Development Goals by 2030 (Leal Filho et al., 2020) at least from the BD perspective, and the subsequent realization of the Vision for Biodiversity targets by 2050 under The Kunming-Montreal Global Biodiversity Framework (CBD, 2020).

One of the sensitive areas where BD might be changed, simplified, or even declined is agriculture due to transformation of the natural habitat land to the pastures and croplands, contamination with overutilization of variety of fertilizers and pesticides, soil depletion and water deterioration (NAS, 2021). Meanwhile, BD decrease and the amount of crop production in agriculture have been shown to have a mutual influence on each other (Seppelt et al., 2016). As the number of people and their life expectancy grows, the need to provide enough food for everyone increases as well. Thereby raising the amount of land used for agriculture and boosting the production of crops, meat, and dairy products by 54%, 53% and 58% respectively over the last 20 years (FAO, 2023) with predicted growth of agricultural production by averagely 46% (maximum by 56%) from 2010 to 2050 (Dijk et al., 2021).

Since it's almost impossible for constant augmentation of the land area to cover all of humanity's food needs due to the already high utilization of the global land areas for food production, incorporating regenerative agriculture (RA) seems to be a perfect way for solving problems in food production. According to unified definition by Schreefel et al., (2020) RA is sustainable practices that prioritize soil conservation and restoration using different methods for food production. RA methods can include amplification of the efficiency of utilization of resources, minimization of leakages of nutrients and further looping of them inside the ecosystem, which enhance soil quality and conservation of BD in agricultural ecosystems (Velasco-Muñoz et al., 2021). RA practices also allow to combat climate crisis thanks to Greenhouse gas (GHG) emission mitigation through soil organic carbon (SOC) sequestration, which has better results (averagely by 17% higher SOC sequestration, depending on climate and soil type) in comparison with conventional agricultural (CA) practices. Implementing different RA practices contributing to accumulate averagely 4.7 Mg C/ha/year of SOC (Rehberger et al., 2023). Agroforestry (when trees or shrubs are planted on farms to improve environmental performance) being one of the RA practices shows higher results with an average SOC content of 35.2 Mg C/ha/year (De Stefano and Jacobson, 2018.)

For better understanding and proper assessing the quantified impacts of variety items and services through their lifespans on environment, scholars usually have been using the life cycle assessment (LCA) tools due to its commonly standardized methods, broad scopes of research and possibility to see implications on environment from different points of view. LCA has a wide scope of application, from the item or process design of almost any man-made entities to the development and evaluation of long-term sustainable strategies for entire companies or even country to reduce their adverse impact on nature and humanity. (Moutik et al., 2023.) BD assessment using LCA tools has been no exception and is becoming more and more relevant every year (e.g. Marques et al., 2021).

However, as emphasized by many researchers, integrated biodiversity LCA still faces difficulties for a several reasons, such as incomplete input data, high rates of species richness, different types of BD levels, divergent evaluation approaches and confusion with relevant indicators (Crenna et al., 2020; Marques et al., 2021; Winter et al., 2017). In order to clearly see the effects of BD change, including in agricultural systems, using different life cycle impact assessment (LCIA) methods, besides using data about the land itself (land use occupation and fragmentation) (Marques et al., 2021; Trottier et al., 2021), it is necessary to have a generalized and standardized indicator (Chaudhary et al., 2015). Such an indicator in the scientific community is called characterization factor (CF), thanks to which it is possible to know the quantitative damage to the BD of a particular area of ecosystem and calculate impacts from other indicators (e.g. Chaudhary and Brooks, 2018; Trottier et al., 2021). At the same time, the need for more systematized BD indicators for more accurate assessments of their impacts within agricultural systems has been frequently stated (Crenna et al., 2020; Damiani et al., 2023). A promising CF covers different land use types (from croplands and grasslands to urban lands and agroforestry) and intensity levels (intense, light and minimal usage) of BD (Chaudhary and Brooks, 2018). In order to indicate BD loss, the special unit Potentially Disappeared Fraction of species (PDF) is used (Verones et al., 2020). It has been also tested and proven to be effective in assessing BD change for comparing CA and organic agriculture (OA) (Knudsen et al., 2017). However, PDF being one of the examples of using with the CF (Scherer et al., 2023) it cannot comprehensively assess BD in different types of land use, thereby it cannot account and analyze well all impacts within agriculture (Knudsen et al., 2017; Quevedo-Cascante et al., 2023). Summarizing abovementioned, after several years of continuous improvement in BD assessment using LCA methods, current scientific studies agree that LCA is not sufficiently developed enough for a full and meaningful assessment of BD in general as well as in agricultural systems (Crenna et al., 2020). Despite of a lot of different methods and LCA tools (more than 64), none of them fully takes into account all aspects and parameters at the same time (Damiani et al., 2023).

Thanks to comprehensive and collaborative cooperation of researchers and experts from all over the world during last decade, there has been a shift in technologies towards more sustainable approaches to land and natural resources use and changing behavior patterns. Altogether can lead to better conservation of BD throughout the different ecosystems. (IPBES, 2019.) One of such technologies is environmental DNA (eDNA) metabarcoding, providing larger and more updated BD input data throughout combination with LCA tools, which can help to determine and quantify some impacts on environment in general (e.g. acidification, eutrophication) and BD in particular. eDNA can be defined as the simultaneous identification and detection of total DNA from different species of different taxonomic groups taken from environmental samples, which relatively recently has been developed and became revolutionized during last decade (Pawlowski et al., 2020; Taberlet et al., 2012a).

Many different advantages have been discovered through numerous studies for eDNA in comparison to conventional tools of BD assessment, such as i) broad widespread use (can be used in almost any ecosystems in any weather conditions and it’s possible to detect broader amount of taxa from only one sample), ii) can detect more hidden species such as cryptic, elusive, rare, nocturnal and even extinct species, iii) easier to use (less people are required to collect and analyze samples as there is no need for specialists in collecting data from different biomes, easier to interpret for the end-users), iv) molecular processes in laboratory can be fully automated, v) non-invasive method, vi) cost effective (due to widespread utilization and lower cost per each species) and there is a room for improvement (Holdaway et al., 2017; Thomsen and Willerslev, 2014). Also, it is possible to take eDNA samples from water, soil, and air, as well as from different parts of extant and extinct species (e.g. fur, epithelium, secretions and excretions, bones, crops and rotten bodies, vegetation parts of the plants etc.) (Ruppert et al., 2019).

However, despite the quick development and sophistication of eDNA metabarcoding technology and expansion to the new spheres of utilization, there is still a significant lack of systemized information about using this method in BD assessment in RA. Also, despite the claims that methods related with eDNA can benefit BD, nowadays there is a little knowledge on BD levels of RA (Kestel et al., 2022). Solving this problem about lack of clear standardize information of eDNA methods within RA, allows to facilitate better detection of different species and further improves BD assessment on RA farms both locally in Finland and, potentially, globally. Furthermore, deeper development of eDNA metabarcoding in RA might allow i) notify about health trends of ecosystem (e.g. thanks to demonstrable changes in the quantity and diversity of biota and soil quality) (Frøslev et al., 2022; Rishan et al., 2024) ii) detect and envisage threats to the ecosystem (e.g. pathogens, pests, etc.) (Brunner, 2020; Mehetre et al., 2021), iii) based on the results of BD changes might show the better management practices with usage of pesticides and fertilizers (Nam et al., 2023).

Despite of all benefits, eDNA metabarcoding has also some drawbacks due to the still not enough developed and researched knowledge base of eDNA metabarcoding sites (Kestel et al., 2022; Ruppert et al., 2019), problems with data interpretation and analyzing (Buxton et al., 2021; Darling et al., 2021), DNA degradation (Goldberg et al., 2018; Harrison et al., 2019) and others (Forstchen, 2020), which will be scrutinized later within this thesis work. However, eDNA metabarcoding is still a promising and robust method that, together with advancing science and combining with other complementary methods, can improve BD assessment.

Many researchers point to the lack of comprehensive data in LCA, combining all pressures on BD, fairly standardized groups of taxonomic diversity at each level (genetics, species and ecosystem) as well as impacts from some of the essential BD variable classes and other fundamental aspects (Damiani et al., 2023). That's why development of collaborative methods of using eDNA and LCA technologies is so crucial because it can enhance taxonomic capturing and characterizations for different agricultural practices. It also provides primary data collection from actual farms along the life cycle, making BD assessment much more accurate than average ecoregion level CFs (Järviö et. al., submitted).

Earlier literature reviews have been conducted by some authors on approaches to use eDNA in agricultural systems, including for BD assessment (e.g. Kestel et al., 2022). However, there is a large gap in research on the use of eDNA as a tool to improve BD assessment through LCA, especially in RA systems, which is proven within this thesis through the conducted literature review. Correspondingly, there is no standardized protocol for eDNA sampling strategy in RA systems and how eDNA can be used within LCA methods. This thesis contributes to filling the gaps in this knowledge and presents a plan how eDNA metabarcoding can improve BD assessment thanks to LCA in RA in Finland, which also has a big potential for spreading globally.

According to abovementioned, within this thesis work, I aimed:

i) Analyze current situation with opportunities and limitations about eDNA, LCA, RA and their correlations in order to find way how eDNA can help to enhance biodiversity LCA in RA;
ii) Develop and present improvements of eDNA sampling strategy plan for regenerative farms in Finland;
iii) Determine baseline and reference state for biodiversity assessment in LCA for RA;
iv) Define and propose ways how characterization factors can be adjusted to improve the LCIA of BD in RA;
v) Envise possible improvements in this method for the future.

This thesis consists of an introduction, background information, methods, results, discussion and conclusion parts with a total of 5 chapters and 16 subchapters.

2. Background information

This part briefly presents necessary aspects of LCA, eDNA metabarcoding and RA related to BD assessment, with a great emphasis on how they can complement each other and be used together.

2.1. Brief review of biodiversity assessment in LCA

According to the globally accepted definition by the ISO 14040 the Life Cycle Assessment (LCA) is "*compilation and evaluation of the inputs, outputs, and potential environmental impacts of a product system throughout its life cycle*" (Sala et al., 2016, 6). Since the utilization of LCA has a broad range of applications, this work mainly considers this method from a BD assessment perspective and with a big focus on agricultural systems.

Although BD assessment has not been included directly into the LCA from the beginning (Teillard et al., 2016; Winter et al., 2017), there has been a gradual enhancement in operational indicators and their integration into life cycle impact assessment (LCIA) of BD (Winter et al., 2017). Nowadays, LCA methods implemented for assessing environmental impacts on BD in horticulture (croplands (Fan et al., 2022), orchards (Van Der Meer et al., 2020) and vegetable gardens (Ordikhani et al., 2021)), aquaculture (Vélez-Henao et al., 2021), livestock pastures (Goglio et al., 2023), and agroforestry (Quevedo-Cascante et al., 2023) and other systems. Only about 35% (or 42 out of 119) of indicators can be conceivably utilize for LCIA of BD (Winter et al., 2017). The most popular indicators will be presented below. However, some of the most informative of LCA indicators related with BD assessment (e.g. land use intensification (Chaudhary and Brooks, 2018), landscape fragmentation (Larrey-Lassalle et al., 2018), species richness (Beckmann et al., 2019) and extinction rates (Rounsevell et al., 2020)) are recognized and included in the GLAM project led by UNEP (Scherer et al., 2023).

BD assessment through LCA in RA systems has the same four main steps as LCA of any other processes and products. It starts from determination of goal and scope; making inventory analysis with collecting all necessary data from inputs and outputs; then doing life cycle impact assessment (LCIA); and final interpretation of the results. Firstly, researchers define their goal and scope, which depend on the main objectives of conduction LCA of BD (conservation of species diversity or protection BD in global scale) (Winter et al., 2017). Having analyzed 64 existing LCA methods Damiani et al. (2023), presented that some methods mostly analyze some specific sector (only terrestrial, or only aquatic), when the focus of others depends on pressures (e.g. climate change) or ecosystems (e.g. freshwater). For more detailed BD analysis, the system boundaries should include the entire lifespan of some item, system, or service, but most of the LCA methods are limited to specify particular system boundaries due to various possibilities of utilization. According to study led by Damiani (2023) only one LCA method assesses and covers a whole life cycle throughout BD assessment (Woods et al., 2018), while other LCA methods may define system boundaries differently depending on the research objectives. Reference states (RS) and baselines are also different in research LCA methods due to the reasons, which will be discussed further, resulting in a lack of overarching coherence. Inventory data taken from existing LCA datasets or literature, results from the maps or manual expert surveys usually also differ. (Damiani et al., 2023.) The completeness, correctness, and trustfulness of the data (both inputs and outputs) depending on all pressures, ecosystem types, taxa, and BD levels varies across the LCA model. When compiling an inventory, it is also crucial to collect data for the same spatial site. (Winter et al., 2017.)

LCIA of BD consists of defining type and power of pressures on the specific ecosystems, which allow to use impact indicators and determine necessary methods and/or models for their assessment (Damiani et al., 2023). Due to divergence of indicators and metrics it is complicated comprehensively assess BD and various measurement methods (both LCA and beyond-LCA) suggest different approaches (Lammerant et al., 2021). One of the first attempts to standardize and combine integrated indicators for BD assessment was the creation and determination of the Essential Biodiversity Variables (EBVs). The eponymous article proposed 6 classes of EBVs including genetic and community composition, ecosystem structure and function, as well as species populations and traits. (Pereira et al., 2013.) They have subsequently been developed more precisely for specific ecosystems (Dias et al., 2023) or globally (Kissling et al., 2018). Later on, different EBVs classes were integrated into some LCA of BD (Crenna et al., 2020; Damiani et al., 2023). Nowadays, together with other indicators (e.g. different pressures on BD, ecosystem types, taxa, and fundamental aspects such as global species extinction and ecosystem multifunctionality), as far as I know, they have become the most exhaustive metrics for BD impact assessment in the context of LCA.

Such LCIA methods as ReCiPe 2016 (Huijbregts et al., 2017), LC-Impact (Verones et al., 2020), Impact World+ (IW+) (Bulle et al., 2019), Stepwise2006 (Weidema, 2008), Global Extinction Probability (GEP) method (Verones et al., 2022), have the diverse quantity of BD loss pressures (maximum 7). The leader in pressures coverage with 9 of pressures, which together with those, which are included into operational LCIA methods, has also pressures as invasive species and overexploitation is Product Biodiversity Footprint (PBF) method (Asselin et al., 2019). For their assessment operational (i.e. commonly used in LCA studies) and LCA-based methods (i.e. not too popular but based on LCA principles) are utilized (Crenna et al., 2020). Most common EBV class is community composition due to often implementation of PDF. Meanwhile, among all LCIA methods only ReCiPe 2016, LC-Impact, IW+, GEP and PBF are fully investigated impacts in all ecosystems (terrestrial, marine and freshwater). The most diverse methods for analyzing different taxa are IW+, LC-Impact and GEP methods, because they are taking into account a maximum of 15 different taxonomic groups. (Damiani et al., 2023.)

In spite of gradual enhancement and updates of LCA and beyond-LCA methods for assessing BD as well as the wide range of approaches, there are no fully independent and inclusive LCA method, which consider all aspects and parameters of BD simultaneously (Crenna et al., 2020; Damiani et al., 2023). Some of them are better from analyzing the pressure point of view (PBF) or fully covering different ecosystems (ReCiPe 2016, LC-Impact, IW+, GEP and PBF), while other are better to study taxa completeness (GEP) or cover maximum EBVs categories (Habitat Change Potential method) (Damiani et al., 2021). (Damiani et al., 2023.)

Due to the complexity (e.g. high species richness) and heterogeneity of BD (e.g. different type and intensity BD levels), many studies refer to the difficulties of assessing BD using a single and universal indicator (or group of indicators) in LCA (Crenna et al., 2020; Marques et al., 2021; Winter et al., 2017). For example, according to a study by Winter et al. (2017), just over one third of indicators evaluate species and ecosystem diversity (40 and 35% respectively), more than 20% of indicators do not assess BD directly, and the rest are relatively evenly split between assessing only genetic diversity and a combination of assessing the three levels of BD. Also, another study shows that there is significant prevalence of analyzing diversity from the taxonomic point of view, rather than from functional (92% and 8% correspondingly) (Mathieu et al., 2020).

In order to somehow cope with the myriad of various LCA indicators to properly assess BD alteration, an accurate standardization of LCA methods and determination of a universal measurable indicator (or group of them) is needed. One possible reason for the lack of such universal methods and indicators could be the controversial issue of whether BD impacts are considered as midpoint or endpoint impact categories. Within LCA, midpoint methods illustrate environmental ramifications of intermediary effects of specific environmental impacts (biosphere flows). (Crenna et al., 2020.) Mostly the main force of BD loss in such examples are human overexploitation of natural resources (e.g. overfishing) (Crenna et al., 2018; Woods et al., 2016) and subsequent changes on species habitat (e.g. land use) (Damiani et al., 2021) in specific areas. However, the impact of the midpoint can also be studied at the global level if key habitat types and BD status are taken into account (Winter et al., 2018). Endpoint impacts are much broader, being a direct or indirect consequence of midpoint impacts, and affect larger important areas of protection such as human health, ecosystem quality and scarcity of resources. In such case, BD depletion plays a significant role in ecosystem quality and that is why it can be considered as part of the endpoint impact. BD also can be studied in both categories simultaneously because species are equally natural resources and parts of the ecosystem. (Crenna et al., 2020.) For more profound analysis Winter et al. (2017) have proposed several new midpoint (sound and odor pressure, irradiance, behavior and physiological change and tissue damage) and endpoint (loss of genetic and ecosystem diversity) categories.

To quantify the loss of BD for any reason, a special indicator is used, which has a common name in LCA as characterization factor (CF), which shows the damage caused to BD in a particular area in units (De Schryver et al., 2009). CFs are actively used in both terrestrial (e.g. Chaudhary and Brooks, 2018) and aquatic ecosystems (Trottier et al., 2021) within LCIAs. Inconsistencies in BD assessment approaches also arise when considering the same factor at different levels, which can lead to different results. For example, one of the CF could be the Potentially Disappeared Fraction of species (PDF) for determining the relative BD loss in richness of species mostly due to anthropogenic impacts for assessing ecosystem quality. In some studies, PDF is used as an indicator of midpoint effects (Ahlgren et al., 2022) and in others at the endpoint level (Crenna et al., 2020). In the study led by Scherer

(2023), authors used PDF, land use CF, integrated allocation factor and regeneration time for calculation impacts to species abundance in specific regional scale area. They also presented that if there are shifts from localized relative extirpation of species leading to global extinction, then regional PDF metrics can be converted to global metrics, showing long-term consequences. Moreover, the PDF unit can be applied to BD assessments of both animals (e.g. mammals, amphibians) and plants (Scherer et al., 2023). However, due to its relative simplicity and clear results, this has not constrained researchers from using the PDF extensively for BD assessment in CA and OA (Quevedo-Cascante et al., 2023).

Quite often ubiquitous CFs also have some flaws. Firstly, what important to keep in mind that CFs show not the real time BD changes due to the different land use management practices but provide potential change due to the delay (i.e. extinction debt). If pressure on an ecosystem is kept for a long time, the probability of BD change increases, so the temporal aspect is also important to consider. For example, a PDF/m2 unit should be used when measuring occupational CF, which is used in inventory data reporting of some system over time, and a PDF·yr/m2 unit is used to estimate transformation CFs. Both CFs measure PDF within a year, and changes between the results of the two CFs can be interpreted as the time it takes for the biota to regenerate. (Scherer et al., 2023.)

Another small drawback was emphasized by Ahlgren et al. (2022). They asserted that invented by Chaudhary and Brooks (2018) CFs, despite the well-described various research ecoregions (over 800) for different taxonomic group and their correlation with 3 levels of BD intensity, these CFs are pretty similar between the levels of intensity. That leads to non-significant differences in the final outputs comparing OA and CA practices. (Ahlgren et al., 2022.) A possible reason could be the different choice of a reference situation/state (RS) within one comparison group. They chose baseline and re-naturalization RSs, i.e. undisturbed/unmanaged natural habitat and natural (semi-natural) states after human interventions (mostly croplands and pastures) correspondingly (Chaudhary and Brooks, 2018). Ultimately, their work shows only adverse BD impacts, without covering some possible improvements (Ahlgren et al., 2022). In general, the selection of an accurate RS (i.e. example of environmental integral ecosystem to which BD is compared) or baseline (i.e. starting point against which altered BD will be quantitatively compared) is a complex process that affects clear conclusions (Damiani et al., 2023). This is due to the great range of approaches and different interpretations of terms, targets, and objects of research in BD conservation, temporality of baselines, presence/absence of threatened species, dynamism of biomes changes and illusion of “naturalness” (i.e. when an initially seemingly pristine nature has already been subjected to human influence on some rate). (Vrasdonk et al., 2019.)

The most popular pressure within LCA methods, which assess BD in agricultural terrestrial systems, is land use. Land use is included in around 83% of all methods, which consider maximum 8 various land use classes such as croplands and grasslands, pastures, and forest (both natural and plantations). (Damiani et al., 2023.) This popularity is explained by the fact that land use is recognized as one of the strongest drivers of species depletion in terrestrial ecosystems due to the alteration of a homogeneous natural environment into often heterogeneous, fragmented, and degraded mosaic parts with different levels of intensity (Jaureguiberry et al., 2022; Semenchuk et al., 2022).

In contrast to previously mentioned criticisms (Ahlgren et al., 2022) of the CFs developed by Chaudhary and Brooks (2018) that the results of BD change are almost indistinguishable from the level of intensity of land management, study led by Semenchuk (2022) proves the opposite. They quantitatively showed that, due to different types of land use intensity, the average global loss of vertebrate species could be about 15% in human-altered landscapes. That puts more than 550 species at risk of extinction, with a higher risk for species with smaller habitats. Different alterations with diversity of microorganisms are also mostly negatively affected by different land use practices (Burton et al., 2022). Cropland and pasture contribute most to BD depletion (5.7% and 4.2% totally, correspondingly) even more aggressively than urban areas (less than 1.6% totally) (Semenchuk et al., 2022). Furthermore, without applying any action to regulate land use, which otherwise land will be used more intensively, may lead, according to the researchers' calculations, to the BD loss of up to 26%. Meanwhile, the authors observed almost similar rates of BD loss between vertebrates (where amphibians were less affected (12.2% on average), but reptiles were more severely affected (15.2% on average). The highest species losses were observed in the regions of Asia and Europe in dry tropical and temperate broadleaf forests, as well as in Mediterranean forests with maximum species depletion rates of even more than 50%. (Semenchuk et al., 2022.)

Moreover, Semenchuk et al. (2022) determined that about 3.6% of the global 15% BD loss is caused by land use intensity effect (the rest part is driven by conversion natural areas to any land use type). Even low intensity land use is responsible for about one quarter of all BD loss globally due to large used territories. Untransformed ecosystems on which humans do their activities anyway (e.g. grazing on natural lands or harvesting timber and mushrooms/berries in the forest) were calculated separately, and the sum of all anthropogenic actions on natural sites contributes about 3.6% of BD loss.

For solving problem with heterogenous impacts on BD and quantify species loss, researchers often used previously developed countryside species-area relationship (cSAR) approach and expanded its application (Semenchuk et al., 2022). CSAR is a gradually refining model for more accurate BD assessment that uses functional classification of certain species and their affinities to different habitat types (Pereira et al., 2014). The cSAR was later updated by adding vulnerability score of species and reflection of land use intensity levels, as was previously mentioned (Chaudhary and Brooks, 2018). Subsequently, other researchers have increased the use of BD assessment methods where cSAR models are also applied, from animals to plants as well (Scherer et al., 2023). In recent article led by Semenchuk (2022), researchers improved cSAR by increasing the spatial resolution and more precisely including intensity land use types. Hence, in their BD assessment they used 2 different integrated sets of metrics, where one of them represented indicator for comprehensive net primary production, and another combined various independently derived metrics of relevant inputs and outputs. The authors clarify that data about BD from field experiments around the world may differ from their model because they are looking at the problem from a global perspective and not local. The authors call for the incorporation and improvement of more sustainable types of land use intensity (Semenchuk et al., 2022). These two statements suggest that eDNA metabarcoding techniques can be used to better define BD on the RA, which are presented further in this thesis.

2.2. Brief review of eDNA metabarcoding

DNA research has long interested scientists since the early years of its discovery, and many different studies have emerged in its long history (e.g. DeSalle and Goldstein, 2019; Ruppert et al., 2019). With further delving into the study of DNA, researchers became interested in detecting traces of RNA and DNA of microorganisms from the environment, but initially there was no accurate definition of eDNA (Ogram et al., 1987; Olsen et al., 1986). Later, with the development of technology and the enlargement of areas, objects and methods of research, abundant number of papers appeared (Ruppert et al., 2019), where authors interpreted the definition of eDNA differently (Nagler et al., 2022). There was even confusion due to the same eDNA terms (i.e. Environmental DNA and Extracellular DNA), making it difficult to generally perceive and analyze the literature, which however was later rectified (Cristescu and Hebert, 2018). For unifying and standardizing the multitude of definitions that had already appeared, a classified terminology was proposed (Pawlowski et al., 2020; Taberlet et al., 2012a). Therefore, according to Taberlet et al. (2018, 2012a.), environmental DNA is a mix of DNA traces from various species, which can be simultaneously detected from the samples which had been taken from environment. Also, they stated that the term “DNA (or eDNA) metabarcoding” is more suitable among other high-throughput sequencing (HTS), if the research target related with determination of different taxonomic groups. There are variety of HTS, for example, metagenomics, metatranscriptomics, environmental barcoding (Ríos-Castro et al., 2021; Shendure and Ji, 2008). Some scholars use the term next generation sequencing (NGS) instead of (Satam et al., 2023) or together (Ruppert et al., 2019) with HTS, but in a general sense it is the same (Creer et al., 2016).

In order to avoid confusion in terms, which exists in the scientific community, and for a slight simplification due to the rather wide scope of this work, according to the recommendations of experts, in this thesis the total eDNA is considered (Cristescu and Hebert, 2018; Nagler et al., 2022). Thus, there was no delving into the definition of structure and/or taxonomic composition when eDNA can be extracted from outside of destroyed cell (e.g. due to lysis or extrusion) such as extracellular DNA (exDNA), or inside of the unharmed cell such as intracellular DNA (iDNA). According to study conducted by Nagler et al. (2022) they proved that variety types of DNA (e.g. exDNA and iDNA) can affect differently on quality of the final results of research. Also, this work considers different taxonomic groups, micro-, meso-, and macroorganisms, without a general specialization on the two-level definition based on (1) the origin of environmental samples and (2) the target taxa proposed by Pawlowski et al. (2020). Because of the broader scope of this thesis, the work specializes on eDNA metabarcoding, which differs from DNA barcoding or species-specific method by a wider identifying of species from single environment sample (Cristescu and Hebert, 2018; Riaz et al., 2011; Thomsen and Willerslev, 2014).

Due to the invention and development of polymerase chain reaction (PCR) (Kaunitz, 2015) and relative straightforwardness of extraction and further determination, initially the main objects of eDNA research were various microorganisms (Bass and Cavalier-Smith, 2004; Ogram et al., 1987). Only in the beginning of the XXI century technologies for studying eDNA from meio- (e.g. Brannock and Halanych, 2015; Taberlet et al., 2012b) and macroorganisms (e.g. Willerslev et al., 2003) became available and they have been actively further developed (Pawlowski et al., 2020). Nowadays, there are plenty eDNA related studies that have examined different taxa (plants, fungi, microorganisms, invertebrates, fish, and extant tetrapods such as amphibians, reptiles, birds, mammals) in different habitats (air, terrestrial (both above and below ground), marine (both surface and sediments), freshwater, ice and snow, inside other organisms), which were detailed presented in many reviews (e.g. Bohmann et al., 2014; Deiner et al., 2017; Kestel et al., 2022; Ruppert et al., 2019). Moreover, thanks to eDNA it is possible to analyze results both in temporal and spatial scale, however more researchers (68% of reviewed studies) conduct eDNA studies in the spatial scale (Mathieu et al., 2020). All of this has been made possible through the development and standardization of detailed user-friendly protocols for each phase (from sample collection from the site to taxonomic identification in laboratory) for both aquatic (e.g. Goldberg et al., 2016) and terrestrial ecosystems (e.g. Taberlet et al., 2018).

The large number of publications is explained by the distinctive advantages of eDNA metabarcoding over other methods (i.e. traditional monitoring methods) which are useful for BD assessments, including in RA (Deiner et al., 2017; Fediajevaite et al., 2021). Thanks to universal primers utilization, eDNA metabarcoding has a broader taxa detection coverage in any ecosystems, which allows it to find millions of tiniest traces of DNA, assess BD efficiently qualitatively, and quantitively. BD can be assessed through eDNA in terms of species richness and/or species abundance, although correct determination of species abundance might be challenging due to false results from dead species or local physical features of specific area. (Tillotson et al., 2018.) However, the feasibility of utilizing eDNA metabarcoding to quantify BD is still debatable. For instance, Fonseca (2018) studied fungi and plants using eDNA methods has concluded, that it is impossible to determine species abundance quantitively in soils due to statistically vague and unspecified data and biases which might occur during sequestration. On the other hand, there is opposite clear results that thanks to eDNA metabarcoding is possible to accurately quantify plants and fungal abundance in soils (Pauvert et al., 2019). However, the results of species abundance in soil might differ due to different applied bioinformatic approaches showing, for example, overestimated richness of species and often depends on specific taxa or research area.

(Pauvert et al., 2019.) Beside that, eDNA methods can show the accurate quantitative results of species abundance of microorganisms (e.g. Smets et al., 2016), fish (e.g. Hänfling et al., 2016) or amphibian (e.g. Li et al., 2021). Also, often there are no any specific weather or even season requirements for eDNA metabarcoding (Thomsen and Willerslev, 2014).

Scope of eDNA applications is so broad that it is possible to detect cryptic and rare (Ruppert et al., 2022), nocturnal (Sakata et al., 2022) and even extinct (Willerslev et al., 2014) species which is highly beneficial for RA systems. Another advantage of eDNA metabarcoding is its relative ease of use because there is no need for highly skilled experts for sampling and determining species due to automated processes (Puillandre et al., 2021) and massive datasets (Beng and Corlett, 2020; Rodríguez-Ezpeleta et al., 2021). Since during eDNA methods scientists mainly take small samples of soil, water or air that may contain traces of DNA from different organisms without directly interacting with them, this method is considered relatively non-invasive and non-destructive (Banerjee et al., 2022; Farrell et al., 2022). The exception is the use of traps or clipping of plant parts for further eDNA analysis of insects (Jusino et al., 2019; McPherson et al., 2022). eDNA is also widely recognized as a relatively cheap and fast method (Fediajevaite et al., 2021). Herewith, the price could differ in 10 times (both in absolute value and in relation to 1 hour of work of an employee) in comparison with traditional methods (Biggs et al., 2015; Fu et al., 2021).

Due to the previously mentioned benefits, eDNA is more convenient (required less efforts) (Johnson et al., 2021), and most importantly more sensitive for BD determination compared to conventional methods (Banerjee et al., 2022; Fediajevaite et al., 2021). The superiority of eDNA over traditional methods in the literature is most often observed for studies on fish (e.g. were founded 11 undiscovered species (Evans et al., 2017)), amphibians (e.g. at least in 300% higher (Wineland et al., 2019)) and fungi (e.g. at least in 1080% higher (Banchi et al., 2018)). It is possible to use eDNA metabarcoding for agricultural conservation purposes as detection and control of invasive species (Coghlan et al., 2021; Piggott et al., 2021). However, traditional methods of BD assessment should not be completely abandoned, as in some cases they may be even more successful than eDNA metabarcoding, as it was proved in the study led by (Ulibarri et al., 2017), where authors compared conventional and eDNA-related techniques for detection of some rare fish species. Moreover, combined use of eDNA metabarcoding and conventional methods may partially overlap with the disadvantages of both and show better results (Banerjee et al., 2022; Brys et al., 2021). For example, in the article written and led by Ji (2021), the authors compared how well traditional and eDNA-related methods assessed aquatic plant BD. Even though eDNA metabarcoding presented better results than traditional methods of species identification per site (e.g. abundance in hydrophytes), but traditional methods showed more broad and accurate aquatic plant assemblage in different study sites. Nevertheless, it is important to keep in mind that it is quite likely that the scientific community does not know the full picture of the comparison between eDNA metabarcoding and conventional methods due to reluctance to publish negative results of eDNA analysis (Beng and Corlett, 2020).

Besides that, eDNA metabarcoding is not an ideal method, because it still has several drawbacks which might affect on BD assessment. Difficulties might occur at every stage. For instance, during sample collection, due to inaccurate perceptions of the species being studied from a biological perspective, the location of the study and its characteristics (Beng and Corlett, 2020; Stewart et al., 2017), and quite often the accidental taking of already dead DNA (Pochon et al., 2017), incorrect samples may be obtained. Sample transportation is also crucial, because eDNA can start to degrade due to physical (e.g. time and temperature (Lamb et al., 2022; Qian et al., 2022), weather conditions and rate of ultraviolet light (Valentin et al., 2021)) or environmental reasons (e.g. soil, water, or air conditions as well as due to different biotic factors or biological interactions (Ariza et al., 2023; van der Heyde et al., 2020)). That can lead to false negative or positive results, which can cause repercussions with further analysis (Burian et al., 2021). For example, in amphibian eDNA detection experiments in streams, the effects of the first eDNA decay were detectable after 3 days and disappeared completely after 8 sunny days or 11 cloudy days, depending also on the species density rate (Pilliod et al., 2014). DNA degradation under natural conditions in soil occurs in less than a week on average, while in artificial conditions the detection of DNA integrity can take up to 80 days (Sirois and Buckley, 2019). To prevent DNA degradation during transportation, it is important to follow protocol standards (e.g., retrieve the material as soon as possible and deliver by stopping DNA degradation with cold, special substrate (Taberlet et al., 2018), or even desiccation with specific filter (Thomas et al., 2019)). When samples are delivered, care should be taken to prevent contamination (Forstchen, 2020) as well as following protocols accurately during multiplication of eDNA in laboratories to avoid errors with PCR amplification, such as incorrect amplicon measures, degeneration of specific primers, and variables of PCR efficiency (Nester et al., 2020). This is essential to avoid misinterpretation of the final data (Darling et al., 2021; Goldberg et al., 2016). Another flaw a researcher may encounter when working in the lab with already collected eDNA samples is inaccurate reference databases due to massive data and biases, as well as final data interpretation (Beng and Corlett, 2020; Kestel et al., 2022).

Despite of all disadvantages, benefits of eDNA mainly outperform drawbacks, which make eDNA metabarcoding quite useful tool for many research purposes. Many of them have been presented in various articles with a deep focus on BD assessment, including in RA systems (Beng and Corlett, 2020; Cristescu and Hebert, 2018; Kestel et al., 2022; Ruppert et al., 2019), but scientists are still finding applications for eDNA that facilitate human life (e.g. for identification COVID-19) (Rishan et al., 2024). One of the most relevant and crucial application of eDNA methods for agriculture is detection different pathogens and pests, especially when the threat is in its incipient stage and the number of pests is difficult to determine due to low abundance (Manfrin et al., 2019). Awareness about pests allow to prepare to such threats as vegetation degradation and reduction of crop production in advance, which enable to develop better management practices related to the sustainable use of various pesticides and fertilizers (Nam et al., 2023) or to human health preservation (Rishan et al., 2024), which can improve RA practices. Also, indirectly it allows to understand the soil health quality and hence develop more sophisticated methods for tillage conservation and agriculture practices (Frøslev et al., 2022; Kestel et al., 2022). Moreover, eDNA technologies are applicable for pollinators’ assessment for preventing degradation of cultivated ecosystems (Evans and Kitson, 2020), or for understanding trophic interactions between plants and animals, which allow to keep and save environmental health (Hassan et al., 2023). More examples of how eDNA can be used in RA are presented in Chapter 4.1.

Due to high perspective of usage eDNA methods for assessing impacts on BD and preserving biota, eDNA metabarcoding has been chosen as one of technology for national monitoring program within “Vision and Action Plan for 2022-2025” in Finland as a part of “European Biodiversity Strategy for 2030”. Stakeholders involved in this project not only evaluated global current eDNA technologies with all opportunities, challenges, and their possible enhancements, but also, they have developed concrete plan for conceivable implementation of BD conservation and preservation activities in Finland before 2030. According to this plan eDNA metabarcoding is going to be integrated into the national monitoring system of agriculture and forestry. Thus, it promotes also international coordination and collaboration as well as cross-organizational interaction in practice among research organizations related with eDNA and BD across Europe. (Norros et al., 2022.) The joint partnership of this project with the International eDNA Standardization Task Force (iESTF) (2024), Horizon Europe (2024) and Biodiversa+ (2024) contributes to the development and enhancement of the best eDNA practices. For instance, nowadays, the special technology related to eDNA sampling from the air is being developed and perfected within the SPORELIFE collaborative project.

The "Vision and Action Plan for 2022-2025" is a piloted roadmap, which has not been implemented before for comprehensive BD monitoring in Finland. There were mostly specific cases, with a theoretical description of the situation (Elbrecht et al., 2017; Iso-Touru et al., 2021; Kahlert et al., 2021; Turunen et al., 2021a), but without applicable implementation of BD conservation and improvement at the national level. At least one of them analyzed methods for BD improvements but focused only on some invertebrates (Turunen et al., 2021b). As assured by one of the authors of this roadmap by Tiina Laamanen in the interview, there were several national efforts within molecular environmental monitoring methods, but they are scattered and most of them in the testing stages. For example, there is research work under extensive metabarcoding detection of phyto- and zooplankton, benthic invertebrates, fungi, and diatoms. Since this thesis fits well within the aims of this roadmap, it is expected that thesis can serve as a supplement to optimize this roadmap even more. In addition, the eDNA sampling strategy for RA systems presented in Chapter 4.2 can also be useful to refine this roadmap.

2.3. Brief review of regenerative agriculture

The large number of people and the amount of food needed for all, has long-term impact on agriculture, leading to large losses of BD (WWF, 2022). That induced researchers to find methods to prevent BD depletion and keep agriculture as sustainable as possible, which entailed that they have started to apply regenerative agriculture (RA) techniques. Although the first attempts of defining and implementing of RA appeared in 1980s (Francis et al., 1986; Sampson, 1982), there has been no standardized definition of RA due to heterogeneity in the interpretation of that term. For example, there were terms such as "regenerative farming", "organic agriculture", "sustainable agriculture" and their variations. The idea of utilizing such practices was ahead of the time and capabilities of humanity at that moment, which led to a decrease in citation about RA. However, since 2016, both in the news media and in scientific literature, RA has once again become widely discussed and the number of publications has been growing every year. (Giller et al., 2021.)

Relatively recently, working simultaneously, but in parallel, two groups of researchers have tried to analyze the trends in the use of the RA concept in the scientific community and give a definition to it (Newton et al., 2020; Schreefel et al., 2020). To obtain the results, both groups analyzed more than 200 scientific publications. The first of them emphasized the reasons for the use of certain related RA concepts, highlighting definitions based on processes, outcomes, or their combination (Newton et al., 2020). Another group analyzed the concept of RA in terms of the objectives of the use of such practices, including socio-economic dimensions' point of view. Based on that work, Schreefel et al. (2020, 5) provided a unified, but temporary definition of RA, which can be interpreted as "*an approach to farming that uses soil conservation as the entry point to regenerate and contribute to multiple provisioning, regulating and supporting ecosystem services, with the objective that this will enhance not only the environmental, but also the social and economic dimensions of sustainable food production*". Later, the desire to bring their own more refined definition of RA arose among researchers, but the initial definition was either restated or expanded only by particular cases (Sands et al., 2023). For instance, article of Velasco-Muñoz and his partners (2021) has added into original term part about regeneration of BD, implying BD in general. It is important to keep in mind that conventional agricultural (CA) practices are quite often used as a counterpoise against RA practices. CA, which is the opposite of alternative agriculture, being the target of abusive discussions as mostly outdated or even insufficient for the environment. Although in most situations this is false. (Sumberg and Giller, 2022.) As outlined in the Global Land Outlook by UNCCD (2022) the combined use of CA and alternative agricultural practices can cease to be the main reason of land degradation and become the main driver for its restoration. This master's thesis adheres to the definition of RA by (Schreefel et al., 2020), but other combinations of words (see chapter 3.2) used that refer to RA were also considered to address the objectives of this thesis.

According to UNCCD (2022) and statistics from Ritchie and Roser (2024) agriculture takes the largest part (44%) from all habitable land in the world and cause about 70% of BD depletion in terrestrial ecosystems (UNCCD, 2022). This encourages researchers to focus mainly on developing methods to restore soil health and contribute to improving BD rates (or reverse BD loss). Among the various practices applied in RA, most of them referred to improving soil quality (implied relatively equally for enhancing BD (both under and above ground), physical characteristics and soil organic carbon (SOC) capturing), amplify the efficiency of utilization of natural resources and reduce harmful effects from climate change. (Schreefel et al., 2020.) To be more precise, the most popular practices of RA are reduction or fully stopping tillage, diverse crop rotation, SOC sequestration, circular principles with using biological nutrients and water, excluding synthetic fertilizers and rotational livestock grazing (Giller et al., 2021; Khangura et al., 2023). Meanwhile some of these practices is useful for mostly restoration soil quality (e.g. minimization tillage), some is better from BD improvement point of view (e.g. circumvention of synthetic pesticides), and some are both beneficial for these objectives (e.g. SOC sequestration or diversification of crops) (Giller et al., 2021). RA's promising SOC sequestration practice has shown good results in retaining and mitigating GHG emissions in the soil, which helps to counter rapid climate change. For instance, Rehberger et al. (2023) have proved that following "no-tillage" strategy allow to increase SOC level on 17% averagely in comparison with CA practices and can accumulate averagely 4.7 Mg C/ha/year of SOC in agriculture (results depends on climate and soil type, time passed after inception and method effectiveness). The best results with SOC capturing showed agroforestry allows binding averagely 35.2 Mg C/ha/year if CA would be converted to agroforestry (the maximum results were 66% better if the transition from CA to silvopasture no deeper than 60 cm was realized) (De Stefano and Jacobson, 2018). Agroforestry is one of the types of RA when trees and/or shrubs are planted on agricultural system to improve environment in general and land use management in particular (Ramachandran Nair et al., 2010).

A promising method for enhancing BD within ecosystems with crops could be agricultural diversification, which was confirmed by plenty of studies. This approach not only improve BD generally, but also responsible for pollinator richness and nutrient cycling, combat pests and generally impact positively on soil quality and fertility, herewith without detrimental effect on the crops themselves (Tamburini et al., 2020). For example, diversification has a positive impact on rice yields and production in 80% of cases (He et al., 2023). It is also crucial to consider such implementations from long-term perspective for elimination long-lasting pernicious effects for both nature, farmers and economic (Carof et al., 2022). Moreover, integrated joint implementation of different approaches leads to more beneficial improvements in BD. For instance, collaborative land management and agricultural diversification (related to both crops and livestock) stimulates the expansion of soil microbial communities (quantitatively and qualitatively), which in turn amplifies and accelerates nutrient circulation. Ultimately, this allows to substitute synthetic chemicals with natural fertilizers, which brings positive environment and businesses advantages. (Voisin et al., 2024.) Besides that, the abundance diversity of invertebrates is improving for the same reason (Fenster et al., 2021b).

A qualitative assessment of RA practices was conducted by a group of researchers led by Fenster (2021a), where they not only analyzed various RA practices, but also ranked them and proved advantaged of RA techniques. Authors reviewed the most popular RA approaches for crops and pastures and rated them from 0 (for traditional practices) to 1 or 2 (depending on approach, intensity, or site). Their evaluations were based on 60 sites in the USA (farms, orchards, ranches, and pastures), where RA practices had been implemented for more than 3 years and some of the farms were recognized as the best in their area for the application of RA methods. They assessed soil quality; water infiltration; diversity and structure of microbial, insect (also pests, but separately) and plant communities, as well as yield and profit from each site. In all cases, RAs were found to be the best in terms of BD and soil quality, which also contributed to increased profitability. The only exception was corn production, where traditional methods were beneficial from the yield rate and profitability point of view, which can be explained due to recent starting of using RA practices in that particular studied farm. It is noteworthy that through study conducted by (Fenster et al., 2021a), it is clear evident that there is not one single RA technique that will improve BD, as the positive aspects of each method are important.

Such method of evaluation presented by Fenster et al. (2021a) can be used to attribute some farm being RA or CA. Based on the data from this study, if 3 and 5 points are scored in pasture and farming respectively, and these practices have been applied for at least 3 years, such a farm can be classified as RA. More information on the status of RA awarded to the farm and the number of years passed when RA practices were applied is explained in Chapter 4.3. As it was mentioned earlier, the process of standardization and attributing a particular agriculture to RA is a complex process due to the influence of many factors. There are independent programs for certification and classification of RA (e.g. A Greener World, 2024; ROA, 2023), as well as examples of strategies with policy recommendations at the European level (EASAC, 2022; Nyssens-James et al., 2023). For example, in Finland, many agriculture farmers adhere to the BSAG’s definition, criteria and principles of RA (BSAG, 2024). Although the BSAG's definition differs from Schreefel et al. (2020) only in that in BSAG's definition the main objectives are aimed at improving the environment and do not affect directly the social or economic aspect, for example. However, until an international standardized classification principle is implemented, the awarding of RA status to a crop or pasture farm will remain blurred.

3. Methods

In this part, a comprehensive literature review is conducted to find articles on how LCA, eDNA metabarcoding and RA are related to each other for BD evaluation in order to find out how eDNA can help to improve biodiversity LCA for RA. The literature review also allowed to collect best recommendations and practices for the joint adoption of LCA and eDNA methods to update eDNA sampling strategy for RA systems and to propose improved baseline, RS and CFs for BD assessment through LCA (see Chapter 4).

3.1. Correlation between LCA, eDNA, RA and their contribution to BD

As was mentioned before, there were conducted and presented a plenty of separate scientific articles about LCA, eDNA and RA (e.g. Damiani et al., 2023; Kestel et al., 2022; Khangura et al., 2023). Each of these can be studied independently to a greater or lesser extent. However, all three of these terms can be bound by BD and since BD loss has become a prominent topic, I decided to analyze their joint combination in terms of achieving a unified solution for proper BD assessment in RA systems. All of these methods are unique and have their own benefits. For example, LCA is a convenient and all-encompassing tool for assessing BD and detecting the scale of human influence on it through entire lifespan of some product or service. At the same time eDNA metabarcoding method allows researchers to get vast and broad data of species, which could supplement inventory analysis in LCA. Moreover, integrating methods to obtain taxa data using eDNA techniques can fulfill part of the most important best practice recommendation proposed by Curran et al. (2016), complementing the multidimensional approach in LCAs. RA practices provide an auspicious basis for BD improvement because they involve the implementation of protective and conservative actions for the environment, which stimulate reduction of BD loss with further restoration. A more detailed study and comparison of BD changes between CA and RA can also contribute to the 4th recommendation of Curran et al. (2016) to improve LCA.

All of them have some flaws in terms of BD assessment as was mentioned in Chapters 2.1-2.3. For instance, LCA hasn’t got globally unified indicator(s) for BD assessment and there are some difficulties with CFs, RSs and data fragmentation. Meanwhile, if the researchers would not carefully follow protocols during eDNA analysis it may lead to biased results. Due to the relative innovativeness of RA practices and the consequent confusion in defining and standardizing of it, these agricultural practices are not perfect either. However, by complementing each other LCA, eDNA and RA can offset common shortcomings and together achieve BD restoration and conservation in the future. For example, accurate extensive simultaneous detection of different species in eDNA metabarcoding can fill gaps in incorrect inputs related to LCA, (see Chapters 2.1-2.2) and better understand BD impacts through LCA approaches. In addition, new automated technologies can provide near-instant species assessments right on the field using eDNA (Tsuji et al., 2018). Combined with the use of relatively fast-analyzing LCA methods (da Costa et al., 2024) will not only speed up the process, but also allow to apply sustainable measures in almost real time. With near-instant species identification using eDNA metabarcoding, it would be much easier to detect invasive species and some pathogens (Hashizume et al., 2023), which would be beneficial to agricultural systems and implementation of RA practices could reduce their detrimental impact on the environment. A future clear understanding of standardized RA principles will allow narrowing and refining BD assessment methods for any particular area, reducing the number of possible imperfections (Beacham et al., 2023). Moreover, further in-depth integration of new technologies (e.g. the internet of things, big data and machine learning, wireless sensor networks) in RA will allow to fully automate agricultural management processes, increasing their efficiency and productivity while remaining safe for nature (O’Donoghue et al., 2024). That’s why it is so essential to their further development and collaborative integration of one method into the other.

As was discovered by team of the researchers led by Järviö (submitted) there is a significant gap in the collaborative study and simultaneous use of eDNA metabarcoding, LCA and RA principles in terms of BD assessment. In order to prove this, I conducted an extensive literature review analyzing the possible interactions of the above-mentioned terms for joint use for BD assessment.

3.2. Screening and selection of data

In order to broadly evaluate correlations between LCA, eDNA, RA and BD the literature review has been conducted. The literature search has been done on the Scopus because, according to several analyses, this database has a wider coverage in articles in Life Sciences and Biomedical, and Technological disciplines in English than Web of Science (e.g.Pranckutė, 2021). The search was conducted in 29th of April 2024 in English. The literature review was completed adhering to the guidelines and review protocol for LCA (Zumsteg et al., 2012) and Environmental studies (Mengist et al., 2020).

The purpose of this literature review is not to thoroughly analyze each research object (LCA, eDNA, RA and BD), as such reviews have already been conducted (Damiani et al., 2023; Kestel et al., 2022; Maxwell et al., 2020; Rehberger et al., 2023). The main idea of this literature review is to find out how eDNA can help to improve biodiversity LCA for RA. The final results of the literature review can show ways to further improve this technology, which has great potential both for Finland and worldwide. In addition, final results allow to collect current recommendations and update the eDNA sampling strategy specifically for RA systems.

The literature review was conducted in three broad phases. First, it was necessary to understand the total number of literature sources about LCA, eDNA, RA and BD related topics in order to see the broad picture. Then it was necessary to find those literature sources where the use of at least two research objects was considered, but great attention was paid to the works mentioning 3 and 4 research objects. The third step consisted of a detailed analysis of the literature that mentioned the combined use of at least two study objects under consideration (LCA, RA or eDNA) with BD assessment (see Table 1).

Table 1. Phases of literature review with study objects.

Phase	Study objects
1. Screening the total number of relevant literature sources	LCA, eDNA, RA, BD
2. Find, screen, and briefly analyze study objects from 2nd phase	LCA + eDNA, LCA + RA, LCA + BD, eDNA + RA, eDNA + BD, RA + BD
3. Find, screen, and deeply analyze study objects from 3rd phase	LCA + eDNA + RA, LCA + eDNA + BD, LCA + RA + BD, eDNA + RA + BD, LCA + eDNA + RA + BD

To get an initial list of references for each study object, the following keywords and their variations were used (see Table 2). The keywords search included "Author keywords" (if available) and "Index keywords" (suggested by Scopus). Each search string topic has wider search terms to cover more topics and increase the chances to find relevant articles that use eDNA methods for biodiversity LCA on RA farms. Consequently, if one of the search string topics is mentioned, e.g. “RA”, it is not necessarily RA, but can be e.g. OA. The literature review considered some (and not always all) RA practices on farms (e.g., no tillage). If other practices (e.g. OA) were not mentioned, for aims of the thesis, it was assumed that these farms were categorized as “RA”. However, in Chapter 4.1. all such cases as well as particular eDNA-related methods for assessing BD using LCA on RA are separately emphasized. For a more accurate search for abbreviations, all possible articles with other abbreviation decipherments were first found and then excluded from the search by combining queries.

Table 2. Search strings with terms and logical operators.

Search string topic	Search terms
Life Cycle Assessment (LCA)	“LCA” OR “Life Cycle Assessment*” OR "Life Cycle Impact Assessment*" OR “LCIA” OR “Environment Impact Assessment*” OR “LCA Method*” OR "Life Cycle Method*" OR “Life Cycle Analys*” OR "Life Cycle Impact Analys*" OR "Life Cycle Approach*" OR “Life Cycle Perspective*” OR “Carbon Footprint” OR “CO2 Footprint” OR “Environmental Footprint” OR “Impact Model*”
Environmental DNA (eDNA)	"Environment* DNA" OR "eDNA" OR "Extracellular DNA" OR "exDNA" OR "Intracellular DNA" OR "iDNA" OR "Total DNA" OR "DNA Barcod*" OR "DNA Metabarcod*" OR "eDNA Barcod*" OR "eDNA Metabarcod*" OR "Environment* High-Throughput Sequencing" OR "Environment* High-Throughput Screening” OR "Environment* HTS" OR "HTS" OR "Environment* Next Generation Sequencing" OR "Environment* NGS" OR "NGS" OR "Metagenomics"
Regenerative Agriculture (RA)	"Regenerative agricultur*" OR "Regenerative farm*" OR "Regenerative aquacultur*" OR "Regenerative horticultur*" OR "Regenerative practic*" OR "Regenerative agricultur* practic*" OR "Organic

	agricultur*" OR "Organic farm*" OR "Organic aquacultur*" OR "Organic horticultur*" OR "Organic practic*" OR "Organic agricultur* practic*" OR "Organic Regenerative Agricultur*" OR "Organic Regenerative farm*" OR "Organic Regenerative aquacultur*" OR "Organic Regenerative Horticultur*" OR "Organic Regenerative Practic*" OR "Organic Regenerative Agricultur* Practic*" OR "Alternative agricultur*" OR "Alternative farm*" OR "Alternative aquacultur*" OR "Alternative Horticultur*" OR "Alternative practic*" OR "Alternative agricultur* practic*" OR "Circular agricultur*" OR "Circular farm*" OR "Circular aquacultur*" OR "Circular horticultur*" OR "Circular practic*" OR "Circular agricultur* practic*" OR "Circular Economy agricultur*" OR "Circular Economy farm*" OR "Circular Economy aquacultur*" OR "Circular Economy Horticultur*" OR "Circular Economy practic*" OR "Circular Economy agricultur* practic*" OR "Sustainable agricultur*" OR "Sustainable farm*" OR "Sustainable aquacultur*" OR "Sustainable horticultur*" OR "Sustainable practic*" OR "Sustainable agricultur* practic*" OR "Conservation agricultur*" OR "Conservation farm*" OR "Conservation aquacultur*" OR "Conservation Horicultur*" OR "Conservation practic*" OR "Conservation agricultur* practic*" OR "Agroecological agricultur*" OR "Agroecological farm*" OR "Agroecological aquacultur*" OR "Agroecological Horticultur*" OR "Agroecological practic*" OR "Agroecological agricultur* practic*" OR "RA" OR "Agroecolog*" OR "Agro Ecolog*" OR "Agrosilvicultur*" OR "Agrosilvopastur*" OR "Silvopastur*" OR "Agroforestry" OR "Improved Fallow*" OR "Agricultural Efficiency" OR "Agricultural Sustainability" OR "Sustainable Intensification" OR "Agricultural Practic*”
Biodiversity (BD)	“Biodiversity” OR “Biodiversity Assess*” OR “Biodiversity Impact Assess*” OR “Biodiversity Monitoring” OR “Biodiversity Los*” OR “Biodiversity Depletion” OR “Biodiversity Deterioration” OR “Biodiversity Damage” OR “Species Assess*” OR “Species Impact Assess*” OR “Species Monitoring” OR “Species Los*” OR “Species

	Depletion" OR "Species Deterioration" OR "Species Diversity" OR "Population Assess*" OR "Population Impact Assess*" OR "Population Monitoring" OR "Population Los*" OR "Population Depletion" OR "Population Deterioration" OR "Population Damage" OR "Population Diversity" OR "Biological diversity"

General exclusion and inclusion were applied to refine the search boundaries in Scopus. For example, the general exclusion was limited to research papers written in English and which had open access. The year 2015 was chosen as the starting point of the study as a year of rapid increase of number of scientific articles about RA (Giller et al., 2021). General inclusion were peer-reviewed articles that assessed BD using one (or more) of the mentioned study objects. Due to the extensive scope of LCA, eDNA and RA (both individually and jointly), all disciplines and journals were included in the literature review.

All received data was thoroughly collected and manually screened and sorted. Firstly, according to Table 1, a broad search has been implemented (phase 1). Due to the huge number of existing scientific articles on these topics and their heterogeneous detailed analysis by other specialists, the search for the first phase was limited. It was performed only by field code "TITLE-ABS-KEY", considering the more in-depth goals of this thesis. As stated earlier, phase 1 is necessary to form a superficial idea of what the general trends in the study of these topics are, and to further compare them with the particular cases from phases 2-3. In order to refine the search results and find suitable articles from the thousands of initially retrieved articles from phase 1, further narrowing was performed. The extraction of suitable articles for phase 2 and 3 was done by combining all the relevant search terms from the corresponding category of search strings (see Table 2). This action retrieved relevant articles that contained combinations of search string topics for phase 2 and 3. Once relevant articles corresponding to phase 3 were found, manual selection was conducted. Articles were selected that specifically explored 3 or 4 search string topics, rather than just mentioning one word (e.g., "biodiversity") without studying it in the article. For articles in phase 3 that had the search string topic "RA", an additional manual sorting was done to sort those articles that had specifically RA practices (or some of them) and those articles that had all other types of alternative agricultural practices (e.g., OA). Then, once relevant articles for phase 3 were selected, they were analyzed in detail.

4. Results and discussion

This chapter presents the findings and results of the conducted extensive literature review and based on it, proposed guidelines for eDNA sampling for RA farms, as well as suggested improvements to baseline, RSs and CFs for biodiversity LCA.

4.1. Literature analysis

The literary review was conducted in several stages according to Table 1. All phases are presented in this chapter with summary part at the end.

4.1.1. Phase 1

In order to understand the big picture, various literature sources were searched using search strings with specific terms and logical operators (see Table 2). According to the first search in Scopus, there were 44367 articles on "LCA", 53539 articles on “eDNA”, and 55678 articles on “RA” related studies. However, not all of them are related to BD assessment, because some of them can be used for other purposes (see paragraphs 2.1-2.4). Studies related to “Biodiversity” were almost as many (147038 articles) as the sum of articles of the three search string topics “LCA”, “eDNA” and “RA”.

4.1.2. Phase 2 with generalized data focused on biodiversity

Among all articles on LCA-based studies, only 742 papers (1.67%) were found, where “BD” was mentioned. These results can be explained that BD assessment is not the main objective of LCA research as most of the studies focus on the SDGs and CE principles, engineering, and energy fields (Moutik et al., 2023). Moutik et al. (2023) in their review discovered a great number of articles in Agricultural and Biological Sciences fields written between 1993 to 2022, when the percentage of using LCA methods remains low (2.88%). Also, there were 4798 articles (8.63% of total “RA” papers) found that were related to both “RA” and “BD”. The percentage is above that of LCA studies, because BD is one of the popular research subjects in most RA-related studies, but not the main one. The highest number of results (6207 studies or 10.85% of total "eDNA" papers) showed the combination of studies related to "eDNA" and "BD". This is probably because eDNA is a great method for simultaneous BD assessment improving each year. The results may be higher since the search included generic terms rather than names of species that can be studied using eDNA metabarcoding.

The number of articles retrieved during phase 2 and mentioned in this subchapter does not imply that they are all directly relevant to BD assessment, as more search terms (e.g. "species loss" or the single word "biodiversity") were used in the search queries to cover different articles more broadly. There were several articles which correspond to a broad search query but used "eDNA" methods for other purposes than assessing BD. For example, in the new study (Tuomikoski et al., 2024), authors used DNA metabarcoding to confirm the diet of predators and their overall threat to the local ecosystem).

4.1.3. Phase 2 and 3 with LCA-related studies

For further in-depth analysis, it is necessary to consider possible variations in the use of the concepts of "LCA", "eDNA", "RA" in terms of BD assessment. A search query with LCA and eDNA collaboration resulted in 58 articles. After their manual screening and selection, only 29 papers (50%) have studied relevant aspects from Table 2, with only 2 studies using the exact terms "Life Cycle Assessment" and "Environmental DNA" (Sniffen et al., 2017; Yadav and Goyal, 2022). The remaining manually screened articles had their synonyms or specific cases (e.g. metagenomic) (see Table 2). Only 3 of them can be classified into a group from the combinations of "LCA", "eDNA" and "BD", which will be presented further.

Sixteen of the 29 research papers (55%) examined the use of eDNA-related methods to find microorganisms to predict consumption or production of CO2 eq. in different study areas (from natural (e.g. Yang et al., 2023) to anthropogenic sites (e.g. Grégoire et al., 2023). Despite the large emphasis on CO2 eq. emissions, many of these articles do not mention the use of LCA tools to estimate emissions due to their other objectives and research questions. The integration of this method could allow a more accurate understanding and analysis of emissions and other factors affecting nature, occurring during microbial life. The study within this search, carried out by Guillardin and MacKay (2023), shows a comparison of eDNA sequencing methods from a CO2 eq. perspective. Three studies mentioned the production of CO2 eq. during different bioinformatics software utilized in the eDNA metabarcoding process, allowing the selection of the best options with less CO2 emission (Armstrong et al., 2021; Grealey et al., 2022; Lee et al., 2022). Another work led by Yadav suggests using separately LCA, eDNA monitoring techniques with a several other new technologies to conserve and restore the pond ecosystem in India (Yadav and Goyal, 2022).

George et al. (2021), Morais et al. (2016) and Yang et al. (2021) showed conflation between the use of eDNA methods and estimation of microbial carbon emission production and SOC (although without explicitly mentioning LCA methods). In addition, 7 articles were identified in which the methods considered could be potentially applied to agriculture. 3 of them addressed the role of microorganisms in retention or production of CO2 eq. gases in symbiosis with rice or reed (Bhattacharyya et al., 2016; Liu et al., 2019; Padhy et al., 2021). This might be useful for RA farmers, because microorganisms can reduce the amount of GHGs and their negative effects on the environment. For example, it has been shown that annual harvesting of reeds decreased the total amount of CH4, but the soil temperature increased (Liu et al., 2019), while conventional rice cultivation might lead to a significant increase in CO2 eq. (Bhattacharyya et al., 2016; Padhy et al., 2021). The remaining 3 articles focus on the application of eDNA methods for BD assessment on livestock farming (Ren et al., 2021; Roehe et al., 2016; Wang et al., 2017). Ren et al. (2021) studied the improvement of pig manure composting through accurate assessment of the microbial community. Two different papers argue that not only methanogenic microorganisms contribute to CH4 production (Wang et al., 2017), but that bovines themselves through the fodder are influenced by these microorganisms (Roehe et al., 2016). The authors agree on the idea that accurate analysis of the microbiota using metabarcoding methods will improve the selection of animals with those microorganisms that produce less methane.

Among all analyzed articles, Xavier et al. (2019) causes special interest and can be attributed with a large assumption to the unification of 3 methods: “LCA”, “eDNA” and “RA”. “With a large assumption” because it does not directly mention LCA method due to other research questions, although the paper itself provides a lot of calculations with GHG emissions. This paper is useful because it compares the long-term (15 years) use of some individual RA practices (specifically no-tillage and crop rotation) with CA practices. It also focuses on eDNA-related assessment of microbial BD and its role in the production of CO2 eq. emissions and their accumulation in soils. The study clearly showed that the application of some individual RA techniques significantly reduces the amount of CO2 eq. emissions in soil, especially in the upper surface layer. Organic matter, enzyme activity levels, and biomass carbon made a great contribution. Xavier (2019) also emphasizes the need for further development of soil management assessment methods, with a strong emphasis on the assessment of soil CO2 eq. emissions (which could be possible thanks to LCA methods) and the use of eDNA-related methods for a more accurate assessment of microbial BD.

Due to the great incentive to achieve the SDGs, prove that one's farm or their product is sustainable, and show their great contribution to the environment, there is a big trend (over 6000 articles) of applying LCA in agriculture (van der Werf et al., 2020). That's why search query with "LCA" and "RA" related terms gave 1252 articles. Since the characteristics of agricultural products (e.g., kg of meat) and ecosystem services that use a set of metrics represented per unit area (e.g., ha of farmland) can be measured, they are more easily defined as a functional unit for LCIA (van der Werf et al., 2020). Despite the comprehensive LCA of various farms and food products, there is a strong lack of LCA methods which assess BD on farms with AEPs. There were found only 77 (6.2% of total amount of "LCA" and "RA" related papers) articles with combination of various terms of "LCA", "RA" and "BD". However, among them there are only 18 (23% from search with "LCA", "RA" and "BD" related terms and only 1.4% from search query with "LCA" and "RA" related terms) where researchers used LCA methods for assessing BD on farms with AEPs. 7 of these articles used particular principles of RA and not of OA, three of them related to crop farms, 3 with livestock and one has considered integration RA principles into crop and livestock farm.

In Lago-Olveira et al.' study (2023), the main driver for BD depletion was land transformation, while land use intensity mainly affected soil quality, even though other impact were taken into account (e.g. fertilization, climate change or overexploitation). The inventory analysis was based on primary data collected from farmers and secondary data taken from Ecoinvent database (Lago-Olveira et al., 2023). It is crucial to consider that sometimes some databases can have unreliable data due to farmer bias and outdated databases (Järviö et. al., submitted). The PDF was chosen as the CF unit, which due to the similar land use pressures showed only a slight improvement in BD in farms with RA practices in comparison with those farms, which without RA practices (Lago-Olveira et al., 2023). This suggests that in combination with other RA practices (not just crop rotation and minimum tillage) the level of BD can be improved. The authors emphasized that more accurate BD indicators that also include unrecorded taxa such as invertebrates, bacteria or fungi are needed. That could be possible if in-depth eDNA metabarcoding analysis were integrated into biodiversity LCA (Järviö et. al., submitted).

Two other studies used special system SALCA for biodiversity (i.e. species richness) LCA in agricultural farms with different land use types, some of which applied practices also found in RA (Lüscher et al., 2017; Prechsl et al., 2017). The SALCA model, based on the analysis of empirical data in a small agricultural area (i.e. a field), evaluates agricultural practices based on different land use types and their correlation with local BD. The authors used this model to assess the impact on BD at the midpoint level caused by agricultural activities and land occupation. Using more than 130 land areas with different farming practices from 8 various European regions as a functional unit, Lüscher et al. (2017) evaluated groups of indicator species (e.g. arable croplands and grasslands flora, spiders and bees) as well as their richness, distribution and habitats. For LCIA, the authors ranked the suitability of different land use types (e.g. croplands, grasslands and seminatural areas) for species, sensitivity and impact effects from agricultural practices on species. Even though SALCA method showed accurate correlation between different land use type and species richness, it does not include land transformational impacts on BD and cannot present adequate results in larger than field scale. (Lüscher et al., 2017.) In another study, Prechsl et al. (2017) thanks to LCA and SALCA model analyzed how different cropping systems (OA and CA), fertilizer application (and subsequent possible emissions), tillage types (intense, reduced or no tillage) and crop coverages (with or without legumes, mixtures and control) affect the BD of wheat, maize and field beans. In their on-farm study authors found that minimized tillage played almost a tiny role for BD (only no tillage had a visible positive effect) but cover crops and more efficient fertilizer usage (e.g., reducing mineral fertilizers and increasing natural fertilizers) were more beneficial. It can be explained by the fact that in their study authors did not estimate belowground diversity, which is more influenced by tillage.

Another method that was used to improve biodiversity LCA, including for agricultural systems, was the interaction of BioImpact indicators with the LCA software (Turner et al., 2019). The BioImpact method incorporates different impact metrics related to BD components (genetic, species and population composition, diversity, function, and disturbance) of 12 various species taxonomic groups in LCA framework. These species groups cover all main kingdom expect *Bacteria*, such as *Fungus* (e.g. fungi and lichens), *Plantae* (e.g. mosses and vascular plants) and *Animalia* (invertebrates and underground extant tetrapods). In study conducted by Turner et al. (2019), authors compared richness of different species (except belowground species) in 4 systems (crop- and grazelands, natural and humanely planted forest for timber production) and their net primary productivity. The visible advantage of BioImpact method is that it has gradation of BD impacts, compiled from a comprehensive literature review and a survey of environmental experts. This method can be used in conjunction with LCA for a faster and more accurate preliminary assessment of key impacts on global BD from land use and land transformation. It also can be integrated with eDNA-related methods for covering also belowground organisms.

Only one founded article, which assess BD in specifically RA system through LCA was paper led to (Colley et al., 2020). Authors compared RA with CA in sheep farms and showed that RA practices have better environmental performance (including BD rate) than CA. However, BD was superficially evaluated and did not cover underground species communities, which also played a significant role in agriculture, and can be well considered through eDNA metabarcoding.

4.1.4. Phase 2 and 3 without LCA-related studies

According to the search query there were 309 articles where eDNA-related methods have been used within AEPs, which is about 0.6% of all eDNA-related studies. According to Kestel et al. (2022) only about 4% of all studies that used eDNA-based analyzing methods in agriculture, where more than half of them studied soil invertebrates, microorganisms and plants. According to my search there were only 32 already sorted articles (initially were 72 articles), which used eDNA-based methods and focused on BD assessment in farms with AEPs. At the same time, 29 of them (91% of sorted articles) were devoted to the study of microorganisms (e.g. bacteria, fungi, invertebrates or viruses). A possible reason for the predominance of studies focusing on microorganisms could be that, as Hermans et al. (2023) have shown that "healthy" microbial diversity can be an indicator of properly implemented RA practices with good environmental quality. Only 8 of the initially founded articles were related to farms with some RA practices (described below). Authors compared how different land use (crops, livestock or forests), vegetation type (e.g. Navarro-Noya et al., 2022) and

RA practices (no-tillage or minimization of tillage, biofertilization, crop rotation and covering) affected on BD in comparison with CA practices. Most of the researchers studied microbial species richness (e.g. Frøslev et al., 2022), while only two studies led by Goss-Souza (2019) and Krishnamoorthy (2021) evaluated functional diversity of bacteria in farms with RA practices. Notably, depending on research case, in some articles, the species diversity was higher in farms with RA practices (Navarro-Noya et al., 2022), in another it was lower than in CA (Herren et al., 2020), but undoubtedly species diversity was bigger in natural biomes (Goss-Souza et al., 2019). For example, compost made from crop residues and woodchips caused a negative effect on nematodes diversity (Herren et al., 2020). However, in another study, researchers introduced an innovative biofertilizer with a major phosphorus component that not only increased soil microbial diversity, but also improved soil quality and environmental performance (Mącik et al., 2023). Authors also emphasized importance to consider the tillage type, location where the sample was taken (from the center or from the edge of the field) (Frøslev et al., 2022) and is there any natural sites, which can bring false results. Also, it has been proved that there is a good potential to increase BD for those farms that are not located near with diverse natural habitat, if crop diversification is implemented (Hutchins et al., 2023).

A new approach was proposed by Dyson et al. (2024a), where they combined eDNA-based metabarcoding and remote sensing assessment in sustainable farms with RA practices. They used arthropods as target primers for eDNA analysis and their own system TerraBio with 12 different indicators of BD assessment. TerraBio is a personally developed methodological platform that helps to assess products from agricultural and forest systems in terms of environment and profit. It allows annually assessing BD depending on land use and its transformation through a combination of remote sensing and eDNA analysis approaches. BD was assessed in cacao plantations with and without AEPs, and restored forest as a control in Brazil. The authors used 12 indicators to assess BD, divided into 3 types: 1) related to land cover and use, 2) BD indicators, and 3) landscape integrity indicator as number of ha of essential habitat area conserved. The first type includes the number of ha indirectly conserved and directly restored and total carbon sequestration. The researchers used number, abundance, species richness, BD indices of key species, alpha and beta diversity as BD indicators. (Dyson et al., 2024a.) Alpha diversity shows species richness within functional community on a local scale, while beta diversity presents the amount of variation between species communities (Andermann et al., 2022). After evaluating 150 cocoa plantations, they found that those with individual RA practices were closer resembling natural sites in terms of BD and had better SOC characteristics. This method also helps to assess land use and forest transformation and its regeneration over time. (Dyson et al., 2024a.)

4.1.5 Summary of the literature review

Having done the literature review, I proved that there is a tremendous gap in the scientific community with biodiversity LCIA using eDNA methods (see Figure 1), which was discovered by (Järviö et. al., submitted). There is only in one article authors mentioned LCA and eDNA as separate ways which can be implemented for improving environmental performance including BD (Yadav and Goyal, 2022). Among all the articles using the broad search query there was only one article that showed all 4 search words and their variants (“LCA”, “eDNA”, “RA” and “Biodiversity”) (Bhattacharyya et al., 2016), but after manual analysis of it, it would be incorrect to claim that it covers all these topics, since authors did not use LCA methods on RA, but just referred to them.

The only exception that considers the use of eDNA methods and LCA for BD assessment on farms using agroecological principles (authors studied OA) is the recent article written by (Simona et al., 2024), which could be useful for integrating eDNA methods to improve biodiversity LCA in RA in the future. The main study species were soil microorganisms (oomycetes, bacteria, and fungi). Researchers assessed their richness, distribution and phylogenetic diversity using specific indices. Study results that applying OA practices brough positive effect on bacterial and fungal diversity but did not show any effects on oomycetes. Having analyzed impacts within the LCA (e.g., carbon, water, and nitrogen footprints), the authors ranked “microbial BD” in last place due to the lack of accurate soil health RS in correlation with microbial diversity. They also considered the implications of agriculture at planetary boundaries, only briefly emphasized its importance, but did not take a deeper consideration, as did Uusitalo et al. (2019) on a sheep farm with OA practices. Uusitalo et al. (2019) proposed a framework that first used LCA method to assess different environmental impacts (with a big focus on BD) on OA farm, and then normalized the LCA results and presented within planetary boundaries showing positive impacts on genetic sheep diversity and negative effects on climate, land and water use.

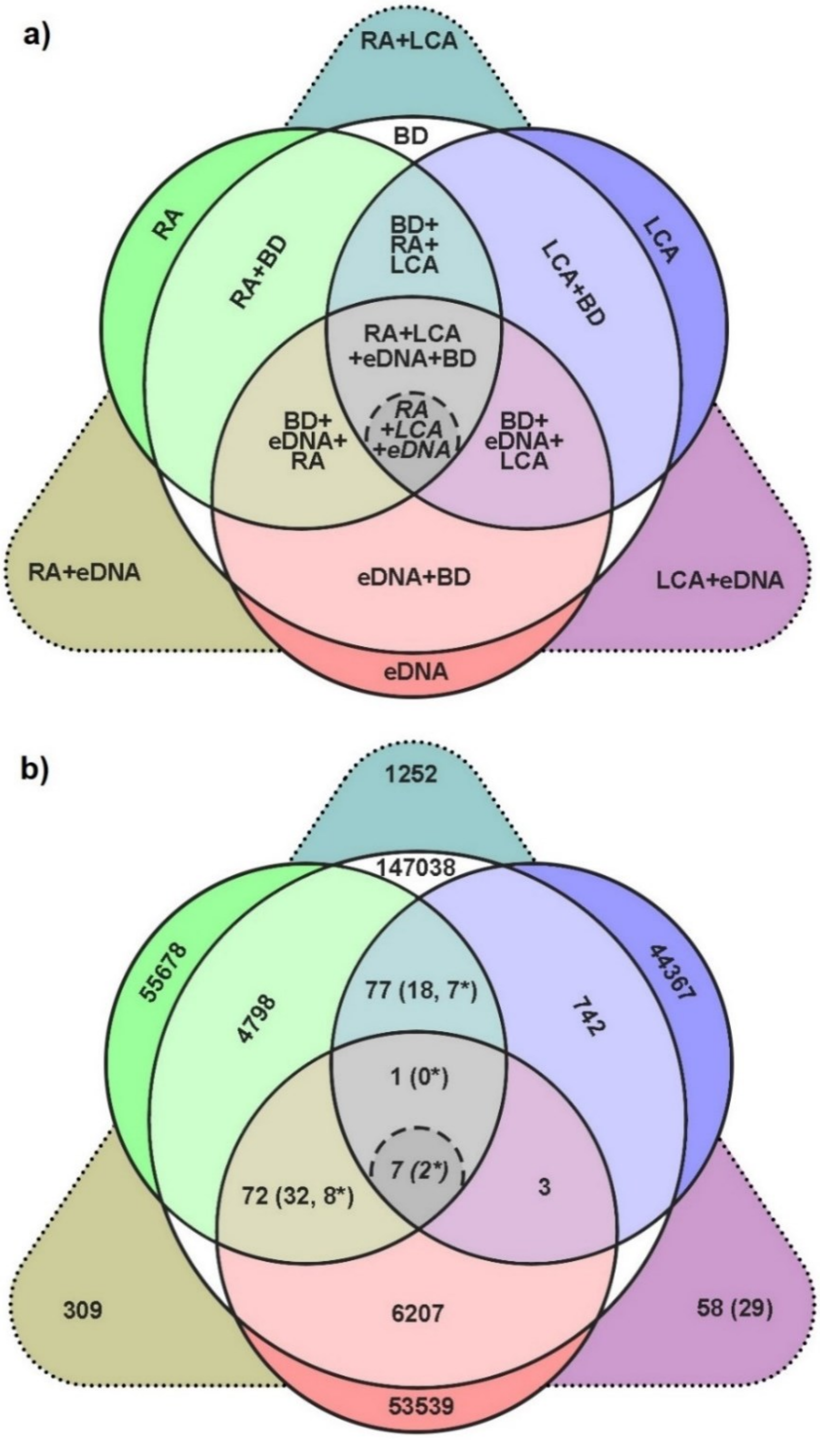

Figure 1. All possible combinations of the terms, which were considered within literature review (LCA is Life Cycle Assessment, eDNA is environmental DNA, RA is Regenerative agriculture, BD is Biodiversity). Dotted line means articles combining search terms without "Biodiversity". Parentheses indicate manually selected articles with agroecological practices on farms. * indicates articles where farms with RA practices were studied. Colors have no semantic load and are created for clarity of combining articles with certain terms. The figures do not show the proportional distribution of articles, but only schematically shows the possible research options in different areas.

Another crucial exception, which was not found within my query because it has not been published yet, was the article written and led by Järviö (submitted), which they kindly provided to this thesis. In their work, the authors suggest how modernizing current characterization factors in LCA by integrating data from eDNA methods would allow for better assessment, conservation, and preservation of BD in RA. They stated that eDNA, as a tool for more exhaustive actual BD assessment (e.g. covering functional diversity), has the potential to improve and extend the CF that is used in LCA in agroecological farms. That also leads to improvement of assessment of other impacts on BD and the environment in general, as well as estimate more accurately how well sustainable farming practices are implemented. Other authors asserted that they need more precise and at the same time broader indicators of biodiversity LCIA on farms with AEPs (Teillard et al., 2016; van der Werf et al., 2020), where eDNA methods could be one of them. Thanks to possibility of eDNA metabarcoding cover and harmlessly detect more species diversity of various taxa in different ecosystems (Taberlet et al., 2018), it might be integrated into biodiversity LCA for more profound and precise analysis. It would be notably beneficial for evaluation and further sophistication of RA practices.

4.2. Improvements for eDNA sampling strategy for biodiversity LCA in RA

Having done a literature review and found several articles where eDNA-related methods have been used to assess BD on RA farms, as well as having done additional searches, there were no any protocols or comprehensive guidelines for various taxa identification on farms where agroecological principles are applied. There might be several reasons for this. Firstly, it can be explained by the general lack of eDNA studies on BD assessment in agricultural systems in comparison with all eDNA-related studies (Kestel et al., 2022). Secondly, due to divergent objectives of each study, which may differ in terms of type of the environment (e.g. terrestrial or aquatic), ecosystem (e.g. tropical forests or grasslands), coverage of various taxa in different levels of BD as well as time frames or financial aspects, there is no universal eDNA sampling protocol (Taberlet et al., 2018). Another reason is that most often (about 95% of all "eDNA" related articles), researchers conduct eDNA studies without specifying which eDNA sampling protocol they used, inventing their own sometimes subjective sampling strategy, or using unsuitable sampling methods (Dickie et al., 2018). Only some of them specified brief description about characteristics of study area and/or sample technique (e.g Guerrieri et al., 2021). The more detailed the sampling design process is presented, the more reliable the results can be considered. Thus, standardized eDNA sampling protocol for terrestrial farms with AEPs, such as RA, are needed.

There are already several well-described sampling eDNA protocols (e.g. Lear et al., 2018), guidelines (e.g. Taberlet et al., 2018), or supplements for them (e.g. Dickie et al., 2018), most of them related to BD assessment in general, without giving any recommendations for eDNA samplings in RA. Within this thesis, I have presented an improved strategy for eDNA sampling for terrestrial RA, compiled mainly from Dickie et al.' (2018) work and improved thanks to the papers of researchers, related to determining the BD of RA systems, using eDNA metabarcoding, founded through literature review. This guideline is not specialized for any particular farm, thus the recommendations for eDNA sampling can be applied to different RA systems. This updated guideline is more suitable for identifying different belowground microorganisms (e.g. fungi, bacteria, invertebrates, and plants) due to their big role for the environment, which was discussed in Chapter 2.2 and 4.1. Although advice on what can be done and how to take DNA samples from the environment of larger aboveground vertebrates and plants is also presented.

Through literature review of articles using combinations from Table 1, most of the studies did not specify what protocol or guideline for sampling they used. It was found only one exception from recent study (Dyson et al., 2024a) which has a brief separate guideline of sample collection from soils in nature sites, CA and sustainable agricultural cocoa farms using eDNA metabarcoding (Dyson et al., 2024b). However, researchers estimated only richness and abundance of diversity of arthropods and richness of mammals, and no other taxa. In their guidelines, authors mentioned basic sampling tips to avoid contamination and indicated which sampling kits/tools they used. They also presented their sampling strategy, describing relevant aspects of the sampling plot (where and when samples have to be taken, soil and ecosystem type, presence/absence of RA practices at the sites) and properties of the taken samples and subsamples (number, weight, depth of samples, how they extracted them and how they prevented degradation) (Dyson et al., 2024b). The main article presents the study areas with all site characteristics (geographical location, climate features, main taxa of the target study and other non-target species, time and scale of implementation of RA principles) (Dyson et al., 2024a).

Unless the researcher has already been given specific locations for eDNA metabarcoding prescribed by other stakeholders, the broader boundaries of the study area, or so-called sampling universe, must be clearly defined before proceeding with sample collection. This includes considering the boundaries of the study and excluding some areas where appropriate, and equally crucial is explaining why this particular location was chosen as the study site (same for control sites). The use of background information from scientific reference books on habitats and/or biological and physiological characteristics of species can greatly improve the results of species capture using e-DNA techniques, especially if the primary objective is to search for rare species (Hassan et al., 2023). Planning in advanced the sampling strategy is necessary for providing the opportunity to extrapolate similar studies to other sites with similar conditions and/or larger scale and to avoid biased manipulative studies where desired results are fitted to the study site. (Dickie et al., 2018.) Defining the study boundaries in advance also allows for better planning and thus reduces the number and volume of unnecessary eDNA samplings (Lear et al., 2018). It is important to take into account that species appearance can vary due to different geographic location of farms with different AEPs in north and south, which relevant to Finland. It can be explained due to different climate and soil conditions, leading to lower number of crops and soil fauna in the northern regions. (Hagner et al., 2023.) To properly define eDNA sampling boundaries in agricultural system in Nordic country such as Finland, it is crucial to correctly stratify eDNA sampling according to abiotic (e.g. soil physical characteristics, humidity, temperature, light intensity) and biotic (e.g. characteristics of taxa from natural sites) natural features, which are well described in a study conducted across Denmark (Brunbjerg et al., 2019).

More often for farms with agroecological principles, the study boundaries are limited to the size of the field or farm itself or area where different RA practices are applied. Clear indication of which, where, how, for how long and to what extent RA practices have been implemented on a particular farm is an important aspect when studying the conservation or recovery of a species community in RA and/or comparison with CA systems. If a farm has not been awarded a RA status from any organization awarding certification, researchers can grant such status for study purposes only, based on recommendations of Fenster et al. (2021a). As discussed in Chapter 2.3, due to the gap with standardization of RA and lack of research on related topics, there are no rules and correlations between the success of implemented RA practices for BD and the time when such practices have been applied. However, based on the conducted literature review, researchers have studied farms that have been applying RA practices for a maximum of 20 years. According to review by Dickie et al.' (2018) and the findings from Fenster et al.'s article (2021a) at least 3 years of implementation of RA practices is required to confirm some positive impact on BD in RA using eDNA-related methods. List of RA farms in Finland can be taken, for example, by contacting BSAG (2024).

If the area where the agricultural activity is applied is large and/or heterogenous and/or includes natural habitats as a control, it is worth clarifying the borders of the particular study area. For example, it is possible to have bigger study area boundaries, which can include agricultural and natural sites, and within these boundaries it is important to have particular borders of study area. In some farms it is difficult to objectively define the sample universe as part of the sampling strategy, since it is essential to strive to obtain the most accurate BD results with minimal errors and efforts (Taberlet et al., 2018). A pilot experiment is needed to tackle this issue. A pilot experiment can be useful to representatively check the characteristics of single sample, species richness of the taxa under study and their spatial distribution. Conducting a pilot will also allow determining where to take samples and how many samples to take. (Taberlet et al., 2018.) However, everything depends on the specific project. For example, if the timeline is only one year and/or budget for eDNA analysis hinders pilot experiments, it can be neglected.

The next step is to identify the plots where samples will be taken. It is important to follow the principles of objective sampling to show the most representative data and to allow other researchers to replicate similar actions. However, most authors (about 90% of total "eDNA" related papers) used subjective eDNA sampling methods, thereby compromising the scientific validity of the articles and causing possibility of false or unexhaustive results (Dickie et al., 2018). There are at least 4 options for choosing the sampling location, described in-depth in paper led by Taberlet (2018). The most accurate objective sampling designs are true randomization, regular grid (uniform distribution with equal spacing), clearly defined conditions (e.g. the deepest or northernmost point) or alternatives methods (e.g. using remote mapping). The application of one or another option for determining sampling location depends on the aims of particular study and conditions of study area, for example, regular grid is more appropriate for homogenous environment conditions, and true randomization for heterogenous study area. Meanwhile, true randomization should have a description of how it was carried out, excluding the element of "convenience" inherent in subjective haphazard sampling, when, for example, sampling is done along a walkway. (Dickie et al., 2018.) Determining where to sample in an RA system requires a clear specification of where RA practices apply and where they do not (if any). In cases with RA practices, it is crucial to take into account that BD (at least species richness of microorganisms) might be different depending on the location where it was taken, showing significantly higher results at edges (at least in 2 meters from natural perennial vegetation) than in the center (Frøslev et al., 2022). Quite often greater impact on BD (at least the species richness and composition of arthropods was increased) can come from natural habitats (e.g. forest) located from 800 m to 2 km from the farm, which should be also taken into account (Frøslev et al., 2022; Hutchins et al., 2023). In addition, the proximity of water bodies (e.g. river or lakes/ponds) also can change the final interpretation of eDNA results, because they mutually affect on each other's BD. For instance, rivers near with terrestrial land use systems can transport eDNA traces of microorganisms, invertebrates and fish from at least 12 km, showing incorrect data (Deiner et al., 2016; Littlefair et al., 2023). A river can alter terrestrial BD nearby by about 400 m or maximum 2 km (Zhang et al., 2023), thus the selection of such close farms should be avoided to get more accurate results. Also, terrestrial lands due to seepage of DNA parts with groundwater can strongly affect nearshore BD and often depends on the season (Ionescu et al., 2022). This should be taken into account and avoid samplings at least from 5 m from ponds. Reducing false results could potentially be achieved by conducting pilot experiments to find the optimal distance with fewer unexpected results. Another method could be to pool and mix samples into one and then subsample (sampling from already collected samples) by determining average species richness. However, this method can skew research results and might be applicable to predominantly homogeneous or large-scale areas or for detecting larger species (e.g. vertebrates) (Lear et al., 2018).

For particular purposes (e.g. for simplification of sampling or for finding average eDNA) and clarification of objectives of study, some researchers use subsamples (e.g. Drummond et al., 2015). However, it is necessary to carefully choose the sample location of subsamples especially in RA. In cases where the location of subsamples was chosen based on different environmental variables, this may lead to incorrect sampling results. For example, if a farm has implemented some RA practices, such as utilizing biofertilizers from manure, some subsamples may have no trace of the effects of such practices, potentially leading to false BD results. However, generally subsampling has several advantages. For example, it is possible to analyze sample again (i.e. resample) in the same area, which allows to see changes in the plot over time. (Dickie et al., 2018.) That might be beneficial for observing alterations in the systems with RA practices in which BD recovers over time.

Once the sampling locations have been selected, the sample plots size needs to be determined. The larger the size of the sampling plot, the more representative the results can be, and the more BD can be covered (Brunbjerg et al., 2019; Dickie et al., 2018). The size of the sampling area depends on the objectives of the research but is most commonly an area of 20 by 20 meters (Dickie et al., 2018). For example, Dyson et al. (2024b) had a size plot 50 by 50 meters (20-25 meters between each plot), which also corresponds to recommendations made by Taberlet et al. (2012b). According to my literature review the minimum size plot was 10 by 15 meters and 6 meters was interval between them (Mącik et al., 2023). No recommendations were found on what the optimal interval between sampling plots should be, but most often in the literature reviewed, the interval corresponded to about half the size of the study plot (e.g. Dyson et al., 2024b). One example for eDNA sampling location on RA with all abovementioned recommendation is depicted on the Figure 2.

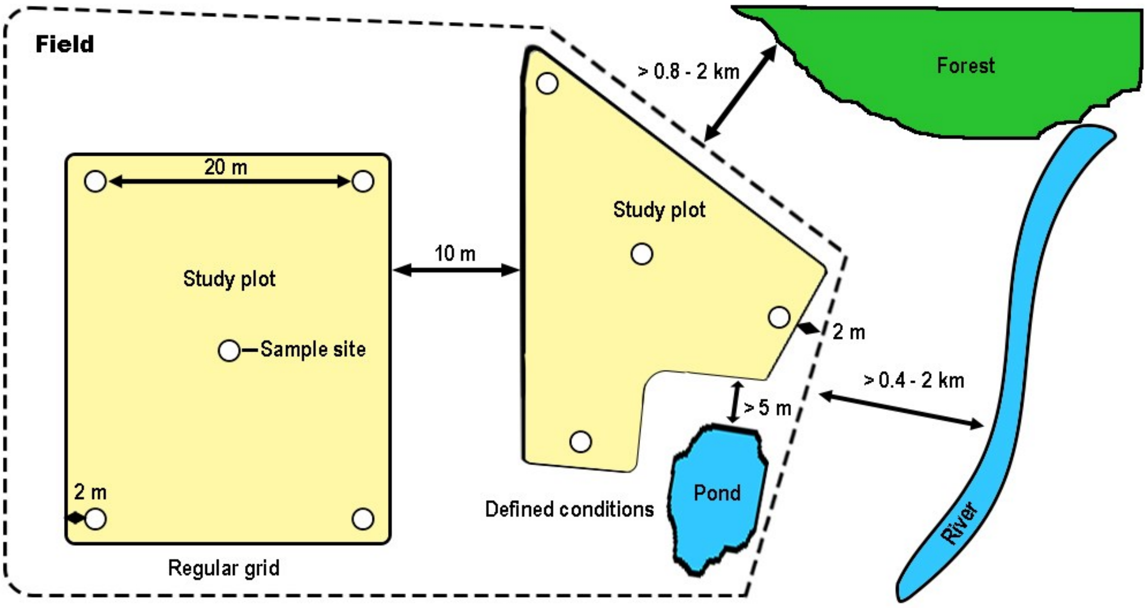

Figure 2. Schematic eDNA sampling strategy of random field with limitations which could be caused by natural sites (e.g. forest, ponds and river). Sampling on the left study plot (5 sampling spots) was based on a regular grid sampling design and on the right study plot (4 sampling spots) was based on defined conditions (e.g. extremes) depending on characteristics of plot. In the right example, the upper sampling extreme is based on the most northern point, the lower 2 are based on the average distance between the southern and eastern points separately and one sampling spot in the center.

The amount of eDNA samples per plot also depends on the objective of study as well as the financial situation. According to the work led by Dickie (2018), besides the fact in most studies the authors did not specify how many samples/replicates they took, but those which had data, more often had less than 6 samples. Depending on the properties of the sample and objectives of study, a good qualitative BD assessment might be done using from 1 to 15 samples (Erickson et al., 2019). For instance, in Dyson et al.' paper (2024b) authors took 4 samples by 30 g of soil each, pooled them and then extracted 2 g of subsamples of soil from them. Authors depicted on their protocol, that they took samples only from the corners of farms, which contradicts to recommendations by Frøslev et al. (2022), which has been discussed earlier. If the aim of study to find rare or invasive species, the recommended number of eDNA samples could be from 45 to 90 replicates (Erickson et al., 2019). For BD assessment in RA and other land use types, the relatively equal distribution and amount of eDNA samples are needed in order to provide more equal in terms of quality results, however, if RA system is the main study area (without control sites), number of samples can be determined based on objectives of study. If the number of samples is limited by the size and/or costs of the study, using only two dissimilar plots also provides distinguishable results when comparing BD metrics on farms with different agricultural treatment practices (Navarro-Noya et al., 2022).

The volume of sample soil in the plot and the depth at which the sample will be taken also plays an important role. The various tools which depend on the objectives of the study, which researchers use to extract eDNA from soil, determines how much soil volume is needed (Lear et al., 2018). It is more common to take a larger amount of soil first (e.g., 30 g) and then extract mixed subsamples of smaller amounts (e.g. 2 g) (e.g. Dyson et al., 2024b). The basic recommendations (if it is not contradicted to study aims) include removing manually or through sieving/filtering (He et al., 2021) objects which are outside of the targets (e.g. leaf litter, roots or branches, stones, etc.) (Lear et al., 2018). More advanced study might have sampling from different soil horizons (Dickie et al., 2018), but usually samples taken from upper level of soil from maximum 20 cm depth (10 cm is also acceptable) (e.g. Foucher et al., 2020). If researchers need to identify microorganismal soil diversity, it is also essential to consider microorganismal spatial vertical and horizontal distribution in order to take proper taxa. Using mixed subsamples from different soil layers allows to see the broader picture in terms of species richness through different soil layers.

All eDNA-related methods would not be possible without the main object of the study – species. As was discussed in Chapter 2.2, there is a possibility to detect almost any taxonomic groups using eDNA techniques. The abovementioned updated guideline for BD assessment in agricultural systems through eDNA methods mainly focused on belowground species (mostly microorganisms, such as fungi, bacteria, invertebrates, and plants) due to their absolute dominance in species diversity in the agroecosystem (Drummond et al., 2015). Another reason for the lack of studies assessing aboveground species diversity might be the faster time for decaying of eDNA traces of aboveground species (Newton et al., 2022; Valentin et al., 2021) and thus more challenging conditions for sampling and further analysis. As also discussed, the more divergent and broader the taxa included in eDNA analysis, the more representative results of ecosystem BD can be seen. Based on the objectives of the study and its budget, aboveground species diversity can be included. For instance, Valentin et al. (2021) presented comprehensive analysis of which conditions, depending on their degree (e.g. presence/absence or with different intensities), affect arthropods aboveground diversity, which could be considered within eDNA sampling guidelines. Depending on the species, they compared sampling techniques with different filters and even a special vacuum catcher. One of the crucial aspects of their work is that weather conditions must also be considered before sampling. This is because even after a light rain or fog, traces of terrestrial eDNA on vegetation are swept away and seep into the lower layers of soil, where can mix with eDNA from belowground species, leading to increased false negative results (Valentin et al., 2021).

To include vertebrates in the BD assessment through eDNA, researchers used different methods comprehensively reviewed in Deiner et al.' (2017) article. According to another article, various sampling methods in different substrates bring divergent species composition and combination of several methods is needed to cover all possible species in particular habitat (van der Heyde et al., 2020). Nowadays, scientific capabilities even allow identify fungi (Banchi et al., 2018), plant (Johnson et al., 2021) and animals (Clare et al., 2021) from eDNA samples taken from the air. It is noteworthy that due to the lack of literature that has explored the potential for using airborne DNA samples to assess BR in specifically RA systems, this guideline does not make any recommendations how to do so but shows that this could be a promising future direction. More often, abundances of aboveground species (e.g. invertebrates, reptiles, amphibians, and mammals) are detected using traditional methods by catching them thanks to special pitfall traps and then conduct further eDNA in-depth studies (e.g. Drummond et al., 2015). Birds also can be identified by their DNA traces they left in the soil (Drummond et al., 2015), but more often for identification of birds, scholars use specialized sound sampling methods, when researchers can record their sounds from relatively big distance and decode them later (Zhang et al., 2023). There are also several ways to identify plants using eDNA methods, comprehensively analyzed by Johnson et al. (2023), where their traces in terrestrial systems can be taken directly from different plant tissues (e.g. pollen, leaf, flower, seeds, roots) or indirectly from organismal derived sample (e.g. gut content, feces, honey, etc.). Meanwhile, the extracting eDNA plant samples is more often studied from pollinators or animal feces (Johnson et al., 2023).

In addition, it is important take into account that various taxa require different storage (i.e. eDNA fixation) approaches for their transportation to the laboratory. The main rule for eDNA preservation of any taxa is to stop all biological activities and extract DNA as soon as possible (Taberlet et al., 2018). The choice of the method of blocking DNA activity depends in most cases on the taxonomic group and researcher's capabilities, including availability of one or another technology, distance from laboratory and financial situation. For example, belowground microorganisms can be frozen down to -80 °C (depends on using methods and tools available), aboveground insects can be stored in 100% ethanol or glycol, and plants can be dried and stored in paper bags (Lear et al., 2018; Taberlet et al., 2018).

Moreover, it is essential to take into account the life cycle of different insects in a particular ecoregion, because depending on seasonal period and life cycle of arthropods their BD can be significantly differ. It was proven, for example, for tropical case study that during summertime diversity of aboveground insects was higher, but during winter BD was bigger in the soil. (Kirse et al., 2021.) However, Kirse et al. (2021) conducted their study in a tropical region, and the same pattern has not been tested in Finland. eDNA metabarcoding also can show various results for species diversity due to different plant vegetation stages, which can be a key aspect for RA systems (Navarro-Noya et al., 2022). All this should be taken into account for eDNA sampling on RA in Finland, as the vegetation is usually later than in Central Europe.

A summarized checklist of how to conduct eDNA sampling on farms where RA practices are applied and what should be considered during eDNA sampling is presented in Table 3.

Table 3. Summarized checklist for universal eDNA sampling in RA systems. Normal font implies obligatory steps, Italic font implies optional but recommended steps, depending on objectives of particular study.

#	Steps and recommendations
1.	Define the sampling universe/ boundaries of the study area. Could be supplemented by using background information from reference books about habitat (geographical, soil and climate features) and/or biological characteristics of species.
2.	Check validation of RA practices on farm (if available). If RA status has not been provided, researchers can grant it within their study following recommendations of (Fenster et al., 2021a).
3.	Clarify the borders of study area within the sampling universe if the study area is large and/or heterogenous and/or includes natural habitats as a control.
4.	*Conduct a pilot experiment to test the characteristics of single sample, species richness of the taxa under study and their spatial distribution*.
5.	Determine the sampling location, taking into account applied (if any) RA practices, field characteristics and proximity of natural sites and vegetation.
6.	*Decide about subsamples if needed (location, number, volume).*
7.	Determine the sample plot size. The recommended size is 20 by 20 m with half the distance between sample plots. The size may vary depends on aims of particular study.
8.	Define the number of eDNA samples per plot. Depending on the aims of particular study, the optimal number of eDNA samples per plot is 4 - 6.
9.	Determine the volume of the sample and the depth at which the sample will be taken. The volume and depth depend on the objectives of the study and taxa covered, but usually not more than 30 g for a sample and 2 g for a subsample taken from maximum soil depth of 20 cm.
10.	Indicate the taxa covered and how their DNA traces will be collected. There are some detailed recommendations that can be followed for macroorganisms (e.g. vertebrate extant tetrapods and plants) by Deiner et al. (2017) or Drummond et al. (2015), for aboveground arthropods by Valentin et al. (2021), for plants by Johnson et al. (2023).
11.	Storage of collected eDNA samples. The main rule is to stop all biological activities and extract the DNA as soon as possible. The choice of the method depends on taxa taken, researcher's capabilities, distance from laboratory and financial situation. Good recommendations in different situations for storage can be seen in guidelines by Lear et al. (2018) and Taberlet et al. (2018).

4.3. Baseline and reference state for biodiversity LCA in RA

This chapter proposes possible improvements to the baseline and reference states for estimating BD in LCA for RA systems, compiled mainly through literature review.

4.3.1. Reference states for biodiversity LCA in RA

As was discussed in Chapter 2.1 there are several reference states (RS) with different definitions which used in different LCA methods (Damiani et al., 2023). Although there are a lot of proposed RSs, there is a lack of explanations for why some particular RS have been chosen, which are crucial for CF in LCA. More often RSs may be based on the structure of a particular LCA and may differ from a BD conservation perspective (Vrasdonk et al., 2019), but it is critical to have the same RS definitions for proper comparison of farms with different land use types. Moreover, it is still a big issue to fairly estimate what is a correct RS for biodiversity LCA in RA. As suggested by Curran et al. (2016), developing alternative RSs for different spatial scale systems which can consider environmental, social and economic consequences can be one of the best-recommended practices for enhancing LCA.

The studied area can be compared with RS differently, depending on which method is chosen. If researchers use assessment methods with multiple impacts, various RSs definitions might be in that method, which may subsequently show different results (Damiani et al., 2023). For land use more often, RS is potential (semi-) natural vegetation (PNV). (Curran et al., 2016). Since, the PNV as one of RS for LCA has been exhaustively reviewed by other researchers (e.g. Vrasdonk et al., 2019) this chapter suggests another metric for RS.

After reviewing the literature where soil microorganisms were the main object of study, it was noticed that a healthy soil, supported mainly by microbial diversity, can also be used as a RS for terrestrial RA systems. Initially suggested by US Department of Agriculture (USDA) definition of the soil health as "*capacity of the soil to function as a vital living ecosystem that supports plants, animals, and humans*" (USDA, 2019, 1) has been further developed by other authors by adding in the term the concept of fertility, quality and security of soil, as well as methods for properly measure healthy soil (Fierer et al., 2021; Lehmann et al., 2020). This is not surprising as microorganisms can have a positive effect on soil regeneration even if the soil has been heavily altered by human agricultural activity (Coban et al., 2022). However, according to the recent study, due to the lack of RS for soil microbial diversity, authors gave the lowest impact priority for microbial diversity as an indicator to evaluate agricultural management (Simona et al., 2024). On the other hand, although the USDA had previously confirmed that microbial diversity could be used as a representative indicator for soil health assessment but did not provide some proper metrics. However, the USDA also emphasized a big potential of such indicator. (USDA, 2019.) Later was proposed more advanced list of what microbial metrics can be used for evaluating soil health, which includes microbial biomass, abundance, ratio, community and gene composition as well as specific extents of the microbial contribution to biochemical cycles and symbiotic relationships with plants (Fierer et al., 2021). Notably, most of these metrics can be analyzed thanks to eDNA, which were presented in Chapter 2.2 and 4.1.

The guidelines for using eDNA-related methods for assessing soil health have been presented with in-depth review of the characteristics of methods and indicators (Djemiel et al., 2022).This was proven by Djemiel et al. (2022) and confirmed in my literature review that due to microorganisms (predominately bacteria, fungi and plants) being bioindicators, it is possible to assess various soil land use types in different farming systems and even determine the rate of soil contamination. Bioindicator are organisms (usually microorganisms) that are based on specific patterns (e.g. behavior, presence or absences, abundance, etc.) shows discernible information about the environment. As authors emphasized, such indicator should be easy to use in the field (in terms of practicality and financial affordability), accurate (i.e. precise results with low false positive/negatives rates), duplicatable (i.e. sensitive enough with possibility to repeat in another study area) and universal for different microbiological habitats. (Djemiel et al., 2022.)

There is a big perspective to use PNV and health soil (sustained including by microbe diversity) values as RSs for biodiversity LCA in RA which have been taken through eDNA-related methods for several reasons. As emphasized in the unpublished article led by Järviö (submitted), results of eDNA samples taken from (semi-) natural systems can be integrated to RS for biodiversity LCA due to eDNA's advantages such as more actual (current data and not taken from outdated databases) and accurate (inclusion rare species and hence bigger taxa numbers) species richness estimates. In addition, the authors noted another advantage of eDNA methods, through which the BR of anthropogenically modified lands could be assessed. This is possible by assessing species composition and comparing them between natural or semi-natural sites and farms with agroecological principles, which is not yet realized in LCA.

Where it is impossible to determine RS for a particular area, the Delphi method introduced by Alejandre et al. (2023) can be used to fill such gaps and determine RS. Authors conducted a comprehensive anonymous Delphi survey of experts from different countries with various scientific background and knowledge on relative abundance of wild pollinators in different land management types with different intensities and taxa covered in 45 countries. The Delphi method consisted of 3 rounds of assessments, each of them refined the previous results through feedback received from other experts, eliminating any contradictory results by the end of the survey. More details about Delphi method can be found in their article. After evaluating each case with 3 level of pollinator's abundance (lowest, typical, highest), experts determined natural grassland as RS, and ranked shrubland second as possible RS. Authors cautioned that the Delphi method is a good method when RS has not been previously identified, and Delphi is based on expert analytical judgment and may differ from actual local measurements (Alejandre et al., 2023.)

4.3.1. Baseline for biodiversity LCA in RA

Determining a baseline (i.e. inception of the assessment) for biodiversity LCA on RA farms is also difficult, mainly because there are no standardized requirements as to how many years must elapse before RA practices can show a meaningful positive effect on BD. As was discussed in the Chapter 2.1, the larger number of LCA-related methods do not have specification of exact year as a baseline due to their adaptability to context of some particular situation of BD impact assessment (Damiani et al., 2023). However, there are some LCA-related methods which set the baseline as the current year (e.g. Damiani et al., 2021) or suggest to choose a specific year from their dataset (Damiani et al., 2023). For example, in LCA model written by Chaplin-Kramer et al (2017), authors used 2007 baseline, which contradicts the GNF (2019) recommend for BD assessment.

When selecting an LCA method for assessing BD on RA farms, the primary aspect which should be considered is the year of adoption of RA practices on a particular farm. Depending on what recommendations or standards of organizations promoting RA practices or awarding

RA certificates a farmer follows to achieve RA status for their farm, the time it takes to achieve this status can vary, but on average it requires a minimum of 5 years of RA practices. For example, BSAG (2024) and agricultural consortium AgriCaptureCO2 (Nyssens-James et al., 2023), promoting RA principles, have not got any requirements about years of implementation of RA practices on specific area. Other organizations which provide RA certification have different levels before being awarded the 100% RA farm status, but in general it must take at least 5 years to declare that certified RA farm fully realizes the RA principles. At the same time, they require applying crop rotation for 7 years with a minimum of 3 years of herbaceous perennials (except for woody perennial cropping systems such as vineyards or orchards). (ROA, 2023.) The most comprehensive standardized certification of RA farms was done by AGW (2024). They specified in detail that in order for a farm to be granted RA status, different RA practices must be applied for different land use types from their list of standards. For example, if farms have been following RA principles for at least 5 years and every year AGW reviews or monitors how such farms are implementing their RA principles, they can be granted RA status. However, if a farmer has asserted that he/she is applying RA principles, which cannot be confirmed by third parties and/or AGW representatives, RA status can only be granted after 15 years of implementing RA practices. (A Greener World, 2024.)

In conducted literature review the minimum years of utilizing some of RA practices was 2 years (e.g. Herren et al., 2020) and maximum over 20 (e.g. Dyson et al., 2024a), but some of the researchers did not specify how many years farmers implemented RA practices on their agricultural systems (e.g. Colley et al., 2020). It is also complicated to select a valid baseline for biodiversity LCA because some of the authors studied only some of the RA practices (e.g. no tillage), but not an integrated RA practices on farm (Frøslev et al., 2022). However, in most of the articles, as well as according to recommendations from Fenster et al. (2021a) and Dickie et al. (2018) the minimum years where RA practices presented positive effect on BD was 3 years, which can be chosen as a baseline for biodiversity LCA.

4.4. Proposals how to improve CFs for biodiversity LCA on RA thanks to eDNA

As mentioned in Chapter 2.1 and emphasized in the scientific literature, since land use plays a major role as one of the main impacts on terrestrial agricultural BD, researchers are more often studying PDF or SOC as two main indicators for analyzing impacts of land transformation or land occupation on BD and soil quality (Lago-Olveira et al., 2024). Even though it has been proven (see Chapter 2.3) that AEPs can improve soil quality and increase BD (e.g. Fenster et al., 2021b), there are limitations to the ability to estimate BD in various farms with different land use types through existing LCA-related methods. The limitations are related either to incomplete assessment of all land use types, or levels of farm management intensities, or both, and may differ from one LCA-related model to another. (Damiani et al., 2023.)

One of the main limitations of CFs is that they were, and still are, sometimes not well specified in terms of spatial distribution (Curran et al., 2016; Scherer et al., 2023). This could lead to inconsistencies when using the same CF for assessing BD in different ecoregions with similar land use types, but completely divergent BD (Hayashi, 2020; Teillard et al., 2016). As was pointed out earlier by several authors (e.g. Curran et al., 2016; Järviö et al., submitted) that considering spatial distribution (i.e. regionalization) is crucial for BD assessment in farm with different land use management practices.

BD changes can be assessed in different spatial scale levels though LCA using different CFs. An article about the importance of correlation between farm- and field-scale BD indicators with different AEPs (Lüscher et al., 2017), found through a literature review, was previously discussed in Chapter 4.1. An equally important paper elucidating the interrelation of field- and regional-scale BD indicators and outlining the possibility of using LCA-related methods for BD assessment on agricultural systems with different land use management techniques was presented by Hayashi (2020). However, both of these studies chose not to delve into adjustments of their CFs, including land transformation due to other scope of their studies, although the model they used originally had this possibility (Chaudhary and Brooks, 2018). Hayashi (2020), in his article, compared correlations between CFs from different scales of BD assessment (ecoregion and field), using two BD indicator cases. In the first case, he used PDF at ecoregion level without specification of taxa taken from (UNEP/SETAC, 2017) to determine the BD indicator for each ecoregion and six land use types (annual and permanent crops, as well as pastures, urban, extensive and intensive forestry) and taken from Chaudhary and Brooks' model (2018) for defining intensity levels (minimal, light and intense use). For BD assessment in the field-scale level, the author estimated species richness (but without abundance) taken from surveys from farmers and Japanese Ministry of Agriculture, Forestry, and Fisheries. (Hayashi, 2020.)

Hayashi concluded that it is complicated to assess BD in field-scale from ecoregion-scale BD. He indicated two reasons for this. First, is a necessity to take into account the different effects of land use intensity levels on different taxa (light use in his study case showed positive correlation with plants, but negative or sometimes neutral with amphibians). Second, greater species richness on OA farms than on CA farms can lead to different results during comparing. These two reasons resulted in higher absolute values of coefficients in correlations for plants when assessing potential species change. Moreover, the author compared the impact of limitations in the study sites and the number of years of these studies, and the first one caused greater discrepancies. Thus, the utilization of CFs from ecoregions and different land use management types was insufficient for assessing BD consequences, which also should be considered. (Hayashi, 2020.)

More versatile LCA-related method (i.e. GLAM) has recently been proposed to assess regional and global BD in different land use types with three levels of land use intensity (Scherer et al., 2023), but is not yet available in LCA. The GLAM has already been discussed in Chapter 2.1, but it is crucial here to highlight its advantages over other operational LCA models, which has recently been done in an article led by Järviö (submitted). The GLAM model overperforms other LCA methods, because it compiled the best (i.e. more comprehensive and accurate) aspects of previous LCIA methods for assessing BD in different scales and locations, with different intensities levels, land use types and impacts on BD, as well as including impacts on BD from landscape fragmentation. As in the IW+ method (Bulle et al., 2019), GLAM can take into account the possible differences between countries in terms of variety of impacts on species richness of different agricultural practices. Since IW+ does not estimate the absolute loss of species richness within three different land use intensity levels, it was used the same logic in GLAM for variable as it is in the LC-Impact method (Verones et al., 2020). (Järviö et. al., submitted.) Authors of the GLAM honestly stated that they studied impacts on species richness only (considered 5 main taxa such as plants and extant tetrapod) due to more easier data collection methods and did not estimate species abundance and their functional diversity. Moreover, without including belowground microorganisms' assessment and covering less than 9% of known vascular plants, it is might be hard to see the high representativeness of the BD loss rates.

Besides all advantages of GLAM, its authors emphasized importance to use their model in combination with some other methods of BD assessment for more comprehensive species sample (Scherer et al., 2023). However, they did not refer to eDNA which can facilitate species determination and therefore refine the CFs, as well as assess functional diversity and abundance of species. Probably these omissions and the previously described advantages of GLAM prompted Järviö et al. (submitted) to take the GLAM model as a basis and integrate the data obtained from eDNA-related analyses into the CFs equations. Such improvements of current biodiversity LCA-related methods allow to refine not enough updated datasets and add new values from previously uncounted species or even taxa (Järviö et al., submitted).

As shown by Burton et al. (2022) there is a predominance of studies focusing on the effects from various land use types with different intensities on aboveground species rather than on soil microorganisms, despite the big role of soil microbial diversity on the environment and soil health discussed earlier (e.g. Djemiel et al., 2022; Hermans et al., 2023). The ratio of number of studies and total covered taxa was approximately 4 to 1 for above- and below-ground species, correspondingly (Burton et al., 2022). This may explain the lack of CFs for soil microorganisms and below- and aboveground invertebrates. The non-inclusion of CFs for soil microorganisms and invertebrates leads to an incomplete BD assessment through LCA in different land use types. Scherer et al. (2023) called to enhance the GLAM model by including invertebrates. This thesis proposes, besides using suggestions by Järviö et al. (submitted) to incorporate results from eDNA-related methods into CFs, separately proposes to expand the list of invertebrates and microorganisms in GLAM by adding approaches of using CFs for missing taxa for better BD assessment in LCAs methods. Such missing data of arthropods and microorganisms can be collected through eDNA-related methods.

There are few examples of articles that have examined CFs for different land use type and intensity levels affecting invertebrates (Alejandre et al., 2023; Elshout et al., 2014; Jaroenkietkajorn et al., 2021) (marked as (1), (2), and (3), respectively, for convenience). They are all unified in that they considered how land occupation affected arthropods using similar logic as in the GLAM model, adding their personal updates for CFs in specific cases. Each has advantages and limitations that should be taken into account before integrating these methods into LCA methods. For example, (1) and (2) assessed impacts of different land use categories globally, while (3) used CFs in Thailand. Moreover, (1) examined forests, natural grasslands, man-made pastures, shrublands, annual and permanent crops with in-depth analysis of latter 2 land use categories; (2) studied 5 out of 14 worldwide biomes with focusing on different annual, permanent crops, where natural ecosystem was as RS. Among these 3 studies, (3) might be interesting to consider for purposes of this thesis only because it is only one, in which authors studied local and regional CFs for insect density (without specifying exact taxa) using the same logic as in GLAM method. However, since researchers focused only on oil palm plantations and did not compare different land use practices (but only the effect from the plantation age on BD), this article can be only useful to consider in terms of integrating logic from different spatial scales when development CFs for insects BD assessment.

Notable features of articles led by Alejandre et al. (2023) and Elshout et al. (2014) are that in (1) the authors assessed relative abundance of wild pollinators, while in (2) the relative species richness of arthropods was studied. In (1), one of the CF variables determining RS was generated using the Delphi method based on a comprehensive independent analytical evaluation of experts from different countries but not actual local measurements (see Chapter 4.3.1), whereas RS in (2) were obtained by averaging data for each land use type taken from 155 publications. In both papers, researchers stated that such RS can be used in cases where RS has not been previously determined. In addition, since both of these studies used same LCIA methods ((Huijbregts et al., 2017; Verones et al., 2020) in (1) and (De Baan et al., 2013) in (2)) as in GLAM, these methods can be integrated to LCIA for more comprehensive BD assessment due to different land use impacts. However, none of these 3 studies focused on impact on BD from land transformation, which also required further elaboration.

As far as I know, there are no articles describing methods for using CFs to microorganismal diversity assessment. The only exception is Wardani's (2018) paper, where author developed CF to estimate contamination of livestock from the common aquatic pathogen (*Cryptosporidium*). It is important to emphasize that Wardani only used microbe concentration and population number per grid cell in person as one of the variables of CF but did not develop such CF to directly assess microbial diversity. Hence, this method cannot be used within current LCA methods for BD assessments, which requires further development.

There are other benefits of improving BD assessment by combining eDNA-related methods with LCA. Since it can be feasible to obtain as accurate and up-to-date as possible BD results in a particular farm with specific AEPs through eDNA methods and then integrate them into

CFs for BD assessment, it might be assumed that it would be conceivable to reduce extinction debt and show more recent and realistic results, thus enhancing RS. The logic can be applied here that the more relevant eDNA-derived BD data can be put into the CFs, the less extinct debt there will be. However, this assumption requires further elaboration. Moreover, thanks to integration of eDNA-related methods to LCA models it might be also possible to consider other factors of BD depletion (and not only popular such as PDF), caused from air and water pollution (e.g. acidification or eutrophication) (Järviö et al., submitted; Lago-Olveira et al., 2024). In overall, integration eDNA results to CFs for biodiversity LCA can show more accurate and distinguishable results on BD where RA principles are applied, and hence, this allows to popularize RA practices over CA.

5. Conclusion

This thesis explored possible ways how through eDNA metabarcoding it could be possible to improve BD assessment in LCA for RA in general and with some specific guidance for Finland, considering local features of climate and geographical location.

The pattern initially pointed out by Järviö et al. (submitted) of the lack of a combined approaches of using "LCA", "eDNA", "RA" and "BD"-related studies to accurately assess BD was firstly, as far as I know, proven within this thesis through a literature review. Throughout in-depth literature review of all possible correlations between these 4 research terms, it was found that none of them exhaustively included each of the above terms together (more detailed analysis see Chapter 4.1 and Figure 1). For example, LCA methods were used to assess BD on farms with AEPs in 18 studies, where only 7 of them used all or some of the RA practices (rather than, for example, OA practices). Among all eDNA-related studies, only 0.6% (309) focused on the use eDNA methods on farms with AEPs, where only 32 of them were used for assessing BD, and only 8 articles used some or all actual RA practices (rather than OA practices). Most of the papers (91%) assessed microorganismal BD through eDNA-related methods on farms with AEPs, which can be explained by the high impact of microorganisms on the soil quality.

In order to integrate taxa/species results from eDNA metabarcoding into LCA models for assessing BD in RA systems, it is necessary to conduct the proper eDNA sampling. Based on the literature review, an updated eDNA sampling guideline for specifically RA systems has been developed, with additional recommendations for conducting it in Finland. This eDNA sampling strategy plan takes into account previous protocols and guidelines for eDNA sampling in agricultural systems and has been updated specifically for RA. This sampling plan incorporates the sampling universe with all included and excluded areas and clearly defined boarders, sample plot size and number of samples per plot, volume and depth of samples, considering also pilots, subsamples, identified species, as well as following storage and transportation of the taken samples. See Chapter 4.2 and Figure 2 for a more detailed description of all eDNA sampling guidelines.

Also, the literature review allowed to summarize and extract the necessary information in terms of suggestions for improvement of CFs, RS, baselines for BD assessment in RA through LCA. Nowadays, the main issue with the baselines for BD assessment through LCA is that there is no unified method for implementing this estimation in RA systems. Possible reasons for this lack is a no clear standardization of what RA system is, because different organizations which granted RA status to the farm have different requirements for farmers. Thus, baselines for LCA which assess BD might be different too. However, after analyzing many articles, it is proposed that a valid baseline for LCA when researchers are going to compare BD change due to RA and CA practices should be at least 3 years according to reviewed scientific articles (or 5 years according to certification programs) of implementing RA practices. Researchers also faced issues in defining proper RSs for their studies in LCA, because more often scholars use an indicator of PNV for BD assessment, but since BD in some ecosystem is heterogeneous, several indicators are needed for more accurate and profound analysis. Soil health values were suggested as another indicator to help determine RS for LCA, since a bioindicator such as microorganisms can correctly indicate soil health and show any changes in soil quality. It has also been proposed to integrate updated in terms of accuracy the SALCA model and the BioImpact method into LCA for BD assessment in RA systems. The SALCA model can help to evaluate different agricultural practices based on different land use types and their correlation with BD of some specific field (Lüscher et al., 2017; Prechsl et al., 2017), whereas the BioImpact method can rank the most relevant BD impact on species and hence allow a preliminary assessment and selection of the most appropriate impact for BD assessment (Turner et al., 2019).

The current issue with some CFs for BD assessment through LCA is that they are not enough developed in terms of spatial distribution, especially in farms with different land use practices. Otherwise, this might lead to inconsistencies when researchers can use the same CF for BD assessment in different ecoregions with the same or similar land use types and intensity levels, but fully different taxa. Within this thesis, the best insights on using CF for assessment of BD through LCA, extracted and compiled from different scattered sources. It was also proposed to expand the coverage of taxa in the CFs by adding invertebrate and microorganismal data. The baselines, RSs and CFs need to be further elaboration and validation of their applicability in real study cases.

Notably, some authors emphasized further refinements and improvements of LCA methods for BD assessment in terms of more accurate and broad detection methods but did not refer to use eDNA-related methods (e.g. Scherer et al., 2023). Being one of the integrated methods for BD assessment, GLAM only considered the 5 main aboveground taxa (plants, amphibians, reptiles, mammals and birds), without covering belowground species and not assessing species abundance and their functional diversity, causing BD assessment not to be fully representative. Integrating eDNA metabarcoding results with LCA methods to estimate BD in RA systems may fill gaps in the incomplete species-related data currently used for LCIA. It has also been assumed that this combination of eDNA metabarcoding and LCA allows to reduce extinction debt and therefore shows more recent and realistic results than in some relatively outdated datasets. Furthermore, by using these two methods together it would be possible to consider other factors on BD loss, for example, to study more in-depth effects on BD from water and air pollution. In addition, such integration could also promote the proliferation of RA practices over CA due to more precise and discernible results on BD on agricultural systems where RA practices have been used. Overall, the integration of eDNA metabarcoding to improve BD assessment in LCA for RA may also align with the first and fourth best practice recommendations for improving LCA from Curran et al. (2016), which related to applying a multidimensional approach to LCA indicators and determining the values of different land use practices, demonstration environmental and economic benefits for RA, encouraging farmers to adopt RA practices. All of these have a wide perspective for future implementation and can contribute to improving on-farm BD globally and in Finland particular. This thesis complements the “Vision and Action Plan for 2022-2025” in Finland, which is part of the “European Biodiversity Strategy for 2030” and can be useful for its further optimization.

References

A Greener World, 2024. Certified Regenerative by AGW Standards [WWW Document]. URL https://agreenerworld.org/certifications/certified-regenerative/certified-regenerative-standards/ (accessed 4.15.24).

Ahlgren, S., Morell, K., Hallström, E., 2022. Mapping of biodiversity impacts and hotspot products in Nordic food consumption. Bioeconomy and Health. Agriculture and Food.

Alejandre, E.M., Scherer, L., Guinée, J.B., Aizen, M.A., Albrecht, M., Balzan, M. V., Bartomeus, I., Bevk, D., Burkle, L.A., Clough, Y., Cole, L.J., Delphia, C.M., Dicks, L. V., Garratt, M.P.D., Kleijn, D., Kovács-Hostyánszki, A., Mandelik, Y., Paxton, R.J., Petanidou, T., Potts, S., Sárospataki, M., Schulp, C.J.E., Stavrinides, M., Stein, K., Stout, J.C., Szentgyörgyi, H., Varnava, A.I., Woodcock, B.A., van Bodegom, P.M., 2023. Characterization Factors to Assess Land Use Impacts on Pollinator Abundance in Life Cycle Assessment. Environ Sci Technol 57, 3445–3454. https://doi.org/10.1021/ACS.EST.2C05311/SUPPL_FILE/ES2C05311_SI_003.XLSX

Andermann, T., Antonelli, A., Barrett, R.L., Silvestro, D., 2022. Estimating Alpha, Beta, and Gamma Diversity Through Deep Learning. Front Plant Sci 13, 839407. https://doi.org/10.3389/FPLS.2022.839407/FULL

Ariza, M., Fouks, B., Mauvisseau, Q., Halvorsen, R., Alsos, I.G., de Boer, H.J., 2023. Plant biodiversity assessment through soil eDNA reflects temporal and local diversity. Methods Ecol Evol 14, 415–430. https://doi.org/10.1111/2041-210X.13865

Armstrong, G., Cantrell, K., Huang, S., McDonald, D., Haiminen, N., Carrieri, A.P., Zhu, Q., Gonzalez, A., McGrath, I., Beck, K.L., Hakim, D., Havulinna, A.S., Méric, G., Niiranen, T., Lahti, L., Salomaa, V., Jain, M., Inouye, M., Swafford, A.D., Kim, H.C., Parida, L., Vázquez-Baeza, Y., Knight, R., 2021. Efficient computation of Faith's phylogenetic diversity with applications in characterizing microbiomes. Genome Res 31. https://doi.org/10.1101/gr.275777.121

Asselin, A., Rabaud, S., Catalan, C., Leveque, B., L'haridon, J., Martz, P., Neveux, G., 2019. Product Biodiversity Footprint e A novel approach to compare the impact of products on biodiversity combining Life Cycle Assessment and Ecology. https://doi.org/10.1016/j.jclepro.2019.119262

Banchi, E., Ametrano, C.G., Stanković, D., Verardo, P., Moretti, O., Gabrielli, F., Lazzarin, S., Borney, M.F., Tassan, F., Tretiach, M., Pallavicini, A., Muggia, L., 2018. DNA metabarcoding uncovers fungal diversity of mixed airborne samples in Italy. PLoS One 13. https://doi.org/10.1371/JOURNAL.PONE.0194489

Banerjee, P., Stewart, K.A., Dey, G., Antognazza, C.M., Sharma, R.K., Maity, J.P., Saha, S., Doi, H., De Vere, N., Chan, M.W.Y., Lin, P.Y., Chao, H.C., Chen, C.Y., 2022. Environmental DNA analysis as an emerging non-destructive method for plant biodiversity monitoring: a review. AoB Plants. https://doi.org/10.1093/aobpla/plac031

Bass, D., Cavalier-Smith, T., 2004. Phylum-specific environmental DNA analysis reveals remarkably high global biodiversity of Cercozoa (Protozoa). Int J Syst Evol Microbiol 54. https://doi.org/10.1099/ijs.0.63229-0

Beacham, J.D., Jackson, P., Jaworski, C.C., Krzywoszynska, A., Dicks, L. V., 2023. Contextualising farmer perspectives on regenerative agriculture: A post-productivist future? J Rural Stud 102, 103100. https://doi.org/10.1016/J.JRURSTUD.2023.103100

Beckmann, M., Gerstner, K., Akin-Fajiye, M., Ceaușu, S., Kambach, S., Kinlock, N.L., Phillips, H.R.P., Verhagen, W., Gurevitch, J., Klotz, S., Newbold, T., Verburg, P.H., Winter, M., Seppelt, R., 2019. Conventional land-use intensification reduces species richness and increases production: A global meta-analysis. Glob Chang Biol 25, 1941–1956. https://doi.org/10.1111/GCB.14606

Beng, K.C., Corlett, R.T., 2020. Applications of environmental DNA (eDNA) in ecology and conservation: opportunities, challenges and prospects. Biodivers Conserv 29, 2089–2121. https://doi.org/10.1007/S10531-020-01980-0/METRICS

Bhattacharyya, P., Roy, K.S., Das, M., Ray, S., Balachandar, D., Karthikeyan, S., Nayak, A.K., Mohapatra, T., 2016. Elucidation of rice rhizosphere metagenome in relation to methane and nitrogen metabolism under elevated carbon dioxide and temperature using whole genome metagenomic approach. Science of the Total Environment 542. https://doi.org/10.1016/j.scitotenv.2015.10.154

Biggs, J., Ewald, N., Valentini, A., Gaboriaud, C., Dejean, T., Griffiths, R.A., Foster, J., Wilkinson, J.W., Arnell, A., Brotherton, P., Williams, P., Dunn, F., 2015. Using eDNA to develop a national citizen science-based monitoring programme for the great crested newt (Triturus cristatus). Biol Conserv 183. https://doi.org/10.1016/j.biocon.2014.11.029

Biodiversa + [WWW Document], 2024. URL https://www.biodiversa.eu/ (accessed 4.11.24).

Blanchet, F.G., Cazelles, K., Gravel, D., 2020. Co-occurrence is not evidence of ecological interactions. Ecol Lett 23. https://doi.org/10.1111/ele.13525

Bohmann, K., Evans, A., Gilbert, M.T.P., Carvalho, G.R., Creer, S., Knapp, M., Yu, D.W., de Bruyn, M., 2014. Environmental DNA for wildlife biology and biodiversity monitoring. Trends Ecol Evol 29, 358–367. https://doi.org/10.1016/J.TREE.2014.04.003

Brannock, P.M., Halanych, K.M., 2015. Meiofaunal community analysis by high-throughput sequencing: comparison of extraction, quality filtering, and clustering methods. Mar Genomics 23, 67–75. https://doi.org/10.1016/J.MARGEN.2015.05.007

Brunbjerg, A.K., Bruun, H.H., Brøndum, L., Classen, A.T., Dalby, L., Fog, K., Frøslev, T.G., Goldberg, I., Hansen, A.J., Hansen, M.D.D., Høye, T.T., Illum, A.A., Læssøe, T., Newman, G.S., Skipper, L., Søchting, U., Ejrnæs, R., 2019. A systematic survey of regional multi-taxon biodiversity: Evaluating strategies and coverage. BMC Ecol 19, 1–15. https://doi.org/10.1186/S12898-019-0260-X/FIGURES/7

Brunner, J.L., 2020. Pooled samples and eDNA-based detection can facilitate the “clean trade” of aquatic animals. Scientific Reports 2020 10:1 10, 1–11. https://doi.org/10.1038/s41598-020-66280-7

Brys, R., Halfmaerten, D., Neyrinck, S., Mauvisseau, Q., Auwerx, J., Sweet, M., Mergeay, J., 2021. Reliable eDNA detection and quantification of the European weather loach (Misgurnus fossilis). J Fish Biol 98, 399–414. https://doi.org/10.1111/JFB.14315

BSAG, 2024. Regenerative Agriculture [WWW Document]. URL https://www.bsag.fi/en/regenerative-agriculture/ (accessed 4.16.24).

Bulle, Margni, M., Patouillard, L., Boulay, A.M., Bourgault, G., De Bruille, V., Cao, V., Hauschild, M., Henderson, A., Humbert, S., Kashef-Haghighi, S., Kounina, A., Laurent, A., Levasseur, A., Liard, G., Rosenbaum, R.K., Roy, P.O., Shaked, S., Fantke, P., Jolliet, O., 2019. IMPACT World+: a globally regionalized life cycle impact assessment method. International Journal of Life Cycle Assessment 24, 1653–1674. https://doi.org/10.1007/S11367-019-01583-0/FIGURES/6

Burian, A., Mauvisseau, Q., Bulling, M., Domisch, S., Qian, S., Sweet, M., 2021. Improving the reliability of eDNA data interpretation. Mol Ecol Resour 21, 1422–1433. https://doi.org/10.1111/1755-0998.13367

Burton, V.J., Contu, S., De Palma, A., Hill, S.L.L., Albrecht, H., Bone, J.S., Carpenter, D., Corstanje, R., De Smedt, P., Farrell, M., Ford, H. V., Hudson, L.N., Inward, K., Jones, D.T., Kosewska, A., Lo-Man-Hung, N.F., Magura, T., Mulder, C., Murvanidze, M., Newbold, T., Smith, J., Suarez, A. V., Suryometaram, S., Tóthmérész, B., Uehara-Prado, M., Vanbergen, A.J., Verheyen, K., Wuyts, K., Scharlemann, J.P.W., Eggleton, P., Purvis, A., 2022. Land use and soil characteristics affect soil organisms differently from above-ground assemblages. BMC Ecol Evol 22, 1–9. https://doi.org/10.1186/S12862-022-02089-4/TABLES/3

Buxton, A., Matechou, E., Griffin, J., Diana, A., Griffiths, R.A., 2021. Optimising sampling and analysis protocols in environmental DNA studies. Sci Rep 11. https://doi.org/10.1038/s41598-021-91166-7

Carof, M., Godinot, O., Le Cadre, E., 2022. Biodiversity-based cropping systems: A long-term perspective is necessary. Sci Total Environ 838. https://doi.org/10.1016/J.SCITOTENV.2022.156022

Chaplin-Kramer, R., Sim, S., Hamel, P., Bryant, B., Noe, R., Mueller, C., Rigarlsford, G., Kulak, M., Kowal, V., Sharp, R., Clavreul, J., Price, E., Polasky, S., Ruckelshaus, M., Daily, G., 2017. Life cycle assessment needs predictive spatial modelling for biodiversity and ecosystem services. Nat Commun 8. https://doi.org/10.1038/ncomms15065

Chaudhary, A., Brooks, T.M., 2018. Land Use Intensity-Specific Global Characterization Factors to Assess Product Biodiversity Footprints. Environ Sci Technol 52, 5094–5104. https://doi.org/10.1021/ACS.EST.7B05570/SUPPL_FILE/ES7B05570_SI_001.XLSX

Chaudhary, A., Verones, F., De Baan, L., Hellweg, S., 2015. Quantifying Land Use Impacts on Biodiversity: Combining Species-Area Models and Vulnerability Indicators. Environ Sci Technol 49, 9987–9995. https://doi.org/10.1021/ACS.EST.5B02507

Clare, E.L., Economou, C.K., Faulkes, C.G., Gilbert, J.D., Bennett, F., Drinkwater, R., Littlefair, J.E., 2021. eDNAir: Proof of concept that animal DNA can be collected from air sampling. PeerJ 9, e11030. https://doi.org/10.7717/PEERJ.11030/TABLE-1

Coban, O., de Deyn, G.B., van der Ploeg, M., 2022. Soil microbiota as game-changers in restoration of degraded lands. Science (1979) 375. https://doi.org/10.1126/SCIENCE.ABE0725/ASSET/87962C22-9471-4ED5-A872-9CB734500694/ASSETS/IMAGES/LARGE/SCIENCE.ABE0725-F4.JPG

Coghlan, S.A., Shafer, A.B.A., Freeland, J.R., 2021. Development of an environmental DNA metabarcoding assay for aquatic vascular plant communities. Environmental DNA 3. https://doi.org/10.1002/edn3.120

Colley, T.A., Olsen, S.I., Birkved, M., Hauschild, M.Z., 2020. Delta Life Cycle Assessment of Regenerative Agriculture in a Sheep Farming System. Integr Environ Assess Manag 16. https://doi.org/10.1002/ieam.4238

Creer, S., Deiner, K., Frey, S., Porazinska, D., Taberlet, P., Thomas, W.K., Potter, C., Bik, H.M., 2016. The ecologist's field guide to sequence-based identification of biodiversity. Methods Ecol Evol 7, 1008–1018. https://doi.org/10.1111/2041-210X.12574

Crenna, E., Marques, A., La Notte, A., Sala, S., 2020. Biodiversity Assessment of Value Chains: State of the Art and Emerging Challenges. Environ Sci Technol. https://doi.org/10.1021/acs.est.9b05153

Crenna, E., Sozzo, S., Sala, S., 2018. Natural biotic resources in LCA: Towards an impact assessment model for sustainable supply chain management. J Clean Prod 172, 3669–3684. https://doi.org/10.1016/J.JCLEPRO.2017.07.208

Cristescu, M.E., Hebert, P.D.N., 2018. Uses and Misuses of Environmental DNA in Biodiversity Science and Conservation. https://doi.org/10.1146/annurev-ecolsys-110617-062306 49, 209–230. https://doi.org/10.1146/ANNUREV-ECOLSYS-110617-062306

Curran, M., De Souza, D.M., Antón, A., Teixeira, R.F.M., Michelsen, O., Vidal-Legaz, B., Sala, S., Milà I Canals, L., 2016. How Well Does LCA Model Land Use Impacts on Biodiversity? - A Comparison with Approaches from Ecology and Conservation. Environ Sci Technol 50, 2782–2795. https://doi.org/10.1021/ACS.EST.5B04681/ASSET/IMAGES/LARGE/ES-2015-04681B_0002.JPEG

da Costa, T.P., da Costa, D.M.B., Murphy, F., 2024. A systematic review of real-time data monitoring and its potential application to support dynamic life cycle inventories. Environ Impact Assess Rev. https://doi.org/10.1016/j.eiar.2024.107416

Damiani, M., Roux, P., Loiseau, E., Lamouroux, N., Pella, H., Morel, M., Rosenbaum, R.K., 2021. A high-resolution life cycle impact assessment model for continental freshwater habitat change due to water consumption. Science of The Total Environment 782, 146664. https://doi.org/10.1016/J.SCITOTENV.2021.146664

Damiani, M., Sinkko, T., Caldeira, C., Tosches, D., Robuchon, M., Sala, S., 2023. Critical review of methods and models for biodiversity impact assessment and their applicability in the LCA context. Environ Impact Assess Rev 101. https://doi.org/10.1016/j.eiar.2023.107134

Darling, J.A., Jerde, C.L., Sepulveda, A.J., 2021. What do you mean by false positive? Environmental DNA 3. https://doi.org/10.1002/edn3.194

De Baan, L., Alkemade, R., Koellner, T., 2013. Land use impacts on biodiversity in LCA: A global approach. International Journal of Life Cycle Assessment 18. https://doi.org/10.1007/s11367-012-0412-0

De Schryver, A.M., Brakkee, K.W., Goedkoop, M.J., Huijbregts, M.A.J., 2009. Characterization factors for global warming in life cycle assessment based on damages to humans and ecosystems. Environ Sci Technol 43, 1689–1695. https://doi.org/10.1021/ES800456M/SUPPL_FILE/ES800456M_SI_001.PDF

De Stefano, A., Jacobson, M.G., 2018. Soil carbon sequestration in agroforestry systems: a meta-analysis. Agroforestry Systems 92. https://doi.org/10.1007/s10457-017-0147-9

Deiner, K., Bik, H.M., Mächler, E., Seymour, M., Lacoursière-Roussel, A., Altermatt, F., Creer, S., Bista, I., Lodge, D.M., de Vere, N., Pfrender, M.E., Bernatchez, L., 2017.

Environmental DNA metabarcoding: Transforming how we survey animal and plant communities. Mol Ecol 26, 5872–5895. https://doi.org/10.1111/MEC.14350

Deiner, K., Fronhofer, E.A., Mächler, E., Walser, J.C., Altermatt, F., 2016. Environmental DNA reveals that rivers are conveyer belts of biodiversity information. Nature Communications 2016 7:1 7, 1–9. https://doi.org/10.1038/ncomms12544

DeSalle, R., Goldstein, P., 2019. Review and Interpretation of Trends in DNA Barcoding. Front Ecol Evol 7, 460426. https://doi.org/10.3389/FEVO.2019.00302/BIBTEX

Dias, A., Van Houdt, S., Meschin, K., Von Stackelberg, K., Bago, M.L., Baldarelli, L., Gonzalez Downs, K., Luuk, M., Delubac, T., Bottagisio, E., Kasak, K., Kebabci, A., Levers, O., Miilvee, I., Paju-Hamburg, J., Poncet, R., Sanfilippo, M., Sildam, J., Stepanov, D., Karnauskaite, D., 2023. Using essential biodiversity variables to assess forest ecosystem integrity. Frontiers in Forests and Global Change 6, 1098901. https://doi.org/10.3389/FFGC.2023.1098901/BIBTEX

Dickie, I.A., Boyer, S., Buckley, H.L., Duncan, R.P., Gardner, P.P., Hogg, I.D., Holdaway, R.J., Lear, G., Makiola, A., Morales, S.E., Powell, J.R., Weaver, L., 2018. Towards robust and repeatable sampling methods in eDNA-based studies. Mol Ecol Resour 18, 940–952. https://doi.org/10.1111/1755-0998.12907

Dijk, M., Morley, T., Rau, M.L., Saghai, Y., 2021. A meta-analysis of projected global food demand and population at risk of hunger for the period 2010–2050. Nature Food 2021 2:7 2, 494–501. https://doi.org/10.1038/s43016-021-00322-9

Djemiel, C., Dequiedt, S., Karimi, B., Cottin, A., Horrigue, W., Bailly, A., Boutaleb, A., Sadet-Bourgeteau, S., Maron, P.A., Chemidlin Prévost-Bouré, N., Ranjard, L., Terrat, S., 2022. Potential of Meta-Omics to Provide Modern Microbial Indicators for Monitoring Soil Quality and Securing Food Production. Front Microbiol 13, 889788. https://doi.org/10.3389/FMICB.2022.889788/BIBTEX

Drummond, A.J., Newcomb, R.D., Buckley, T.R., Xie, D., Dopheide, A., Potter, B.C.M., Heled, J., Ross, H.A., Tooman, L., Grosser, S., Park, D., Demetras, N.J., Stevens, M.I., Russell, J.C., Anderson, S.H., Carter, A., Nelson, N., 2015. Evaluating a multigene environmental DNA approach for biodiversity assessment. Gigascience 4, 46. https://doi.org/10.1186/S13742-015-0086-1/2707775

Dyson, K., Nicolau, A.P., Tenneson, K., Francesconi, W., Daniels, A., Andrich, G., Caldas, B., Castaño, S., de Campos, N., Dilger, J., Guidotti, V., Jaques, I., McCullough, I.M., McDevitt, A.D., Molina, L., Nekorchuk, D.M., Newberry, T., Pereira, C.L., Perez, J., Richards-Dimitrie, T., Rivera, O., Rodriguez, B., Sales, N., Tello, J., Wespestad, C., Zutta, B., Saah, D., 2024a. Coupling remote sensing and eDNA to monitor environmental impact: A pilot to quantify the environmental benefits of sustainable agriculture in the Brazilian Amazon. PLoS One 19. https://doi.org/10.1371/JOURNAL.PONE.0289437

Dyson, K., Nicolau, A.P., Tenneson, K., Francesconi, W., Daniels, A., Andrich, G., Caldas, B., Castaño, S., de Campos, N., Dilger, J., Guidotti, V., Jaques, I., McCullough, I.M., McDevitt, A.D., Molina, L., Nekorchuk, D.M., Newberry, T., Pereira, C.L., Perez, J., Richards-Dimitrie, T., Rivera, O., Rodriguez, B., Sales, N., Tello, J., Wespestad, C., Zutta, B., Saah, D., 2024b. eDNA soil sampling protocol. https://doi.org/10.1371/JOURNAL.PONE.0289437.S005

EASAC, 2022. Regenerative agriculture in Europe. A critical analysis of contributions to European Union Farm to Fork and Biodiversity Strategies.

Elbrecht, V., Vamos, E.E., Meissner, K., Aroviita, J., Leese, F., 2017. Assessing strengths and weaknesses of DNA metabarcoding-based macroinvertebrate identification for routine stream monitoring. Methods Ecol Evol 8. https://doi.org/10.1111/2041-210X.12789

Elshout, P.M.F., Van Zelm, R., Karuppiah, R., Laurenzi, I.J., Huijbregts, M.A.J., 2014. A spatially explicit data-driven approach to assess the effect of agricultural land occupation on species groups. International Journal of Life Cycle Assessment 19. https://doi.org/10.1007/s11367-014-0701-x

Erickson, R.A., Merkes, C.M., Mize, E.L., 2019. Sampling Designs for Landscape-level eDNA Monitoring Programs. Integr Environ Assess Manag 15, 760–771. https://doi.org/10.1002/IEAM.4155

Evans, D.M., Kitson, J.J., 2020. Molecular ecology as a tool for understanding pollination and other plant–insect interactions. Curr Opin Insect Sci. https://doi.org/10.1016/j.cois.2020.01.005

Evans, N.T., Li, Y., Renshaw, M.A., Olds, B.P., Deiner, K., Turner, C.R., Jerde, C.L., Lodge, D.M., Lamberti, G.A., Pfrender, M.E., 2017. Fish community assessment with eDNA metabarcoding: Effects of sampling design and bioinformatic filtering. Canadian Journal of Fisheries and Aquatic Sciences 74, 1362–1374. https://doi.org/10.1139/CJFAS-2016-0306/SUPPL_FILE/CJFAS-2016-0306SUPPL.ZIP

Fan, J., Liu, C., Xie, J., Han, L., Zhang, C., Guo, D., Niu, J., Jin, H., McConkey, B.G., 2022. Life Cycle Assessment on Agricultural Production: A Mini Review on Methodology, Application, and Challenges. Int J Environ Res Public Health 19. https://doi.org/10.3390/IJERPH19169817

FAO, 2023. World Food and Agriculture – Statistical Yearbook 2023, World Food and Agriculture – Statistical Yearbook 2023. FAO. https://doi.org/10.4060/cc8166en

Farrell, J.A., Whitmore, L., Mashkour, N., Rollinson Ramia, D.R., Thomas, R.S., Eastman, C.B., Burkhalter, B., Yetsko, K., Mott, C., Wood, L., Zirkelbach, B., Meers, L., Kleinsasser, P., Stock, S., Libert, E., Herren, R., Eastman, S., Crowder, W., Bovery, C., Anderson, D., Godfrey, D., Condron, N., Duffy, D.J., 2022. Detection and population genomics of sea turtle species via noninvasive environmental DNA analysis of nesting beach sand tracks and oceanic water. Mol Ecol Resour 22. https://doi.org/10.1111/1755-0998.13617

Fediajevaite, J., Priestley, V., Arnold, R., Savolainen, V., 2021. Meta-analysis shows that environmental DNA outperforms traditional surveys, but warrants better reporting standards. Ecol Evol 11, 4803–4815. https://doi.org/10.1002/ECE3.7382

Fenster, T.L.D., LaCanne, C.E., Pecenka, J.R., Schmid, R.B., Bredeson, M.M., Busenitz, K.M., Michels, A.M., Welch, K.D., Lundgren, J.G., 2021a. Defining and validating regenerative farm systems using a composite of ranked agricultural practices. F1000Research 2021 10:115 10, 115. https://doi.org/10.12688/f1000research.28450.1

Fenster, T.L.D., Oikawa, P.Y., Lundgren, J.G., 2021b. Regenerative Almond Production Systems Improve Soil Health, Biodiversity, and Profit. Front Sustain Food Syst 5. https://doi.org/10.3389/FSUFS.2021.664359

Fierer, N., Wood, S.A., Bueno de Mesquita, C.P., 2021. How microbes can, and cannot, be used to assess soil health. Soil Biol Biochem 153, 108111. https://doi.org/10.1016/J.SOILBIO.2020.108111

Fonseca, V.G., 2018. Pitfalls in relative abundance estimation using edna metabarcoding. Mol Ecol Resour 18, 923–926. https://doi.org/10.1111/1755-0998.12902

Forstchen, M., 2020. The Elephant in the Lab (and Field): Contamination in Aquatic Environmental DNA Studies. Front Ecol Evol. https://doi.org/10.3389/fevo.2020.609973

Foucher, A., Evrard, O., Ficetola, G.F., Gielly, L., Poulain, J., Giguet-Covex, C., Laceby, J.P., Salvador-Blanes, S., Cerdan, O., Poulenard, J., 2020. Persistence of environmental DNA in cultivated soils: implication of this memory effect for reconstructing the dynamics of land use and cover changes. Scientific Reports 2020 10:1 10, 1–12. https://doi.org/10.1038/s41598-020-67452-1

Francis, C.A., Harwood, R.R., Parr, J.F., 1986. The potential for regenerative agriculture in the developing world. American Journal of Alternative Agriculture 1, 65–74. https://doi.org/10.1017/S0889189300000904

Frøslev, T.G., Nielsen, I.B., Santos, S.S., Barnes, C.J., Bruun, H.H., Ejrnæs, R., 2022. The biodiversity effect of reduced tillage on soil microbiota. Ambio 51, 1022–1033. https://doi.org/10.1007/S13280-021-01611-0/METRICS

Fu, M., Hemery, L., Sather, N., 2021. Cost Efficiency of Environmental DNA as Compared to Conventional Methods for Biodiversity Monitoring Purposes at Marine Energy Sites.

George, P.B.L., Fidler, D.B., Van Nostrand, J.D., Atkinson, J.A., Mooney, S.J., Creer, S., Griffiths, R.I., McDonald, J.E., Robinson, D.A., Jones, D.L., 2021. Shifts in Soil Structure, Biological, and Functional Diversity Under Long-Term Carbon Deprivation. Front Microbiol 12. https://doi.org/10.3389/fmicb.2021.735022

Giller, K.E., Hijbeek, R., Andersson, J.A., Sumberg, J., 2021. Regenerative Agriculture: An agronomic perspective. Outlook Agric 50, 13–25. https://doi.org/10.1177/0030727021998063/ASSET/IMAGES/LARGE/10.1177_0030727021998063-FIG4.JPEG

GNF, 2019. Baseline Report Biodiversity in Standards of the Banana and Pineapple Sector 36.

Goglio, P., Knudsen, M.T., Van Mierlo, K., Röhrig, N., Fossey, M., Maresca, A., Hashemi, F., Waqas, M.A., Yngvesson, J., Nassy, G., Broekema, R., Moakes, S., Pfeifer, C., Borek, R., Yanez-Ruiz, D., Cascante, M.Q., Syp, A., Zylowsky, T., Romero-Huelva, M., Smith, L.G., 2023. Defining common criteria for harmonizing life cycle assessments of livestock systems. Cleaner Production Letters 4, 100035. https://doi.org/10.1016/J.CLPL.2023.100035

Goldberg, C.S., Strickler, K.M., Fremier, A.K., 2018. Degradation and dispersion limit environmental DNA detection of rare amphibians in wetlands: Increasing efficacy of sampling designs. Science of The Total Environment 633, 695–703. https://doi.org/10.1016/J.SCITOTENV.2018.02.295

Goldberg, C.S., Turner, C.R., Deiner, K., Klymus, K.E., Thomsen, P.F., Murphy, M.A., Spear, S.F., McKee, A., Oyler-McCance, S.J., Cornman, R.S., Laramie, M.B., Mahon, A.R., Lance, R.F., Pilliod, D.S., Strickler, K.M., Waits, L.P., Fremier, A.K., Takahara, T., Herder, J.E., Taberlet, P., 2016. Critical considerations for the application of environmental DNA methods to detect aquatic species. Methods Ecol Evol 7, 1299–1307. https://doi.org/10.1111/2041-210X.12595

Goss-Souza, D., Mendes, L.W., Borges, C.D., Rodrigues, J.L.M., Tsai, S.M., 2019. Amazon forest-to-agriculture conversion alters rhizosphere microbiome composition while functions are kept. FEMS Microbiol Ecol 95. https://doi.org/10.1093/FEMSEC/FIZ009

Grealey, J., Lannelongue, L., Saw, W.Y., Marten, J., McRossed D Sign©ric, G., Ruiz-Carmona, S., Inouye, M., 2022. The Carbon Footprint of Bioinformatics. Mol Biol Evol 39. https://doi.org/10.1093/MOLBEV/MSAC034

Grégoire, D.S., George, N.A., Hug, L.A., 2023. Microbial methane cycling in a landfill on a decadal time scale. Nature Communications 2023 14:1 14, 1–18. https://doi.org/10.1038/s41467-023-43129-x

Guerrieri, A., Bonin, A., Münkemüller, T., Gielly, L., Thuiller, W., Francesco Ficetola, G., 2021. Effects of soil preservation for biodiversity monitoring using environmental DNA. Mol Ecol 30, 3313–3325. https://doi.org/10.1111/MEC.15674

Guillardín, L., MacKay, J.J., 2023. Comparing DNA isolation methods for forest trees: quality, plastic footprint, and time-efficiency. Plant Methods 19. https://doi.org/10.1186/s13007-023-01086-y

Hagner, M., Pohjanlehto, I., Nuutinen, V., Setälä, H., Velmala, S., Vesterinen, E., Pennanen, T., Lemola, R., Peltoniemi, K., 2023. Impacts of long-term organic production on soil fauna in boreal dairy and cereal farming. Applied Soil Ecology 189. https://doi.org/10.1016/j.apsoil.2023.104944

Han, X., Gill, M.J., Hamilton, H., Vergara, S.G., Young, B.E., 2020. Progress on national biodiversity indicator reporting and prospects for filling indicator gaps in Southeast Asia. Environmental and Sustainability Indicators 5, 100017. https://doi.org/10.1016/J.INDIC.2019.100017

Hänfling, B., Handley, L.L., Read, D.S., Hahn, C., Li, J., Nichols, P., Blackman, R.C., Oliver, A., Winfield, I.J., 2016. Environmental DNA metabarcoding of lake fish communities reflects long-term data from established survey methods. Mol Ecol 25, 3101–3119. https://doi.org/10.1111/MEC.13660

Harrison, J.B., Sunday, J.M., Rogers, S.M., 2019. Predicting the fate of eDNA in the environment and implications for studying biodiversity. Proceedings of the Royal Society B: Biological Sciences. https://doi.org/10.1098/rspb.2019.1409

Hashizume, H., Taga, S., Sakata, M.K., Hussein, M., Siddig, E.E., Minamoto, T., Fahal, A.H., Kaneko, S., 2023. Environmental detection of eumycetoma pathogens using multiplex real-time PCR for soil DNA in Sennar State, Sudan. Trop Med Health 51. https://doi.org/10.1186/s41182-023-00563-3

Hassan, S., Sabreena, Ganiee, S.A., Yaseen, A., Zaman, M., Shah, A.J., Ganai, B.A., 2023. Unraveling the potential of environmental DNA for deciphering recent advances in plant–animal interactions: a systematic review. Planta 258, 1–17. https://doi.org/10.1007/S00425-023-04267-0/METRICS

Hayashi, K., 2020. Inconsistencies between regional- and field-scale biodiversity indicators within life cycle assessment: the case of rice production systems in Japan. International Journal of Life Cycle Assessment 25. https://doi.org/10.1007/s11367-020-01749-1

He, X., Batáry, P., Zou, Y., Zhou, W., Wang, G., Liu, Z., Bai, Y., Gong, S., Zhu, Z., Settele, J., Zhang, Z., Qi, Z., Peng, Z., Ma, M., Lv, J., Cen, H., Wanger, T.C., 2023. Agricultural diversification promotes sustainable and resilient global rice production. Nat Food 4, 788–796. https://doi.org/10.1038/S43016-023-00836-4

He, X., Sutherland, T.F., Abbott, C.L., 2021. Improved efficiency in eDNA metabarcoding of benthic metazoans by sieving sediments prior to DNA extraction. Environmental DNA 3, 716–726. https://doi.org/10.1002/EDN3.172

Hermans, S.M., Lear, G., Case, B.S., Buckley, H.L., 2023. The soil microbiome: An essential, but neglected, component of regenerative agroecosystems. iScience 26, 106028. https://doi.org/10.1016/J.ISCI.2023.106028

Herren, G.L., Habraken, J., Waeyenberge, L., Haegeman, A., Viaene, N., Cougnon, M., Reheul, D., Steel, H., Bert, W., 2020. Effects of synthetic fertilizer and farm compost on soil nematode community in long-term crop rotation plots: A morphological and metabarcoding approach. PLoS One 15, e0230153. https://doi.org/10.1371/JOURNAL.PONE.0230153

Holdaway, R.J., Wood, J.R., Dickie, I.A., Orwin, K.H., Bellingham, P.J., Richardson, S.J., Lyver, P.O., Timoti, P., Buckley, T.R., 2017. Using DNA metabarcoding to assess New Zealand's terrestrial biodiversity. N Z J Ecol 41. https://doi.org/10.20417/NZJECOL.41.28

Horizon Europe - European Commission [WWW Document], 2024. URL https://research-and-innovation.ec.europa.eu/funding/funding-opportunities/funding-programmes-and-open-calls/horizon-europe_en (accessed 4.11.24).

Huijbregts, M.A.J., Steinmann, Z.J.N., Elshout, P.M.F., Stam, G., Verones, F., Vieira, M., Zijp, M., Hollander, A., van Zelm, R., 2017. ReCiPe2016: a harmonised life cycle impact assessment method at midpoint and endpoint level. International Journal of Life Cycle Assessment 22, 138–147. https://doi.org/10.1007/S11367-016-1246-Y/TABLES/2

Hutchins, L., Mc Cartney, A., Graham, N., Gillespie, R., Guzman, A., 2023. Arthropods are kin: Operationalizing Indigenous data sovereignty to respectfully utilize genomic data from Indigenous lands. Mol Ecol Resour. https://doi.org/10.1111/1755-0998.13822 iESTF [WWW Document], 2024. URL https://iestf.global/ (accessed 4.11.24).

Ionescu, D., Bizic, M., Karnatak, R., Musseau, C.L., Onandia, G., Kasada, M., Berger, S.A., Nejstgaard, J.C., Ryo, M., Lischeid, G., Gessner, M.O., Wollrab, S., Grossart, H.P., 2022. From microbes to mammals: Pond biodiversity homogenization across different land-use types in an agricultural landscape. Ecol Monogr 92, e1523. https://doi.org/10.1002/ECM.1523

IPBES, 2019. The global assessment report of the intergovernmental science-policy platform on biodiversity and ecosystem services.

Iso-Touru, T., Tabell, J., Virta, A., Kauhala, K., 2021. A non-invasive, DNA-based method for beaver species identification in Finland. Wildlife Biol 2021. https://doi.org/10.2981/wlb.00808

Jaroenkietkajorn, U., Gheewala, S.H., Scherer, L., 2021. Species loss from land use of oil palm plantations in Thailand. Ecol Indic 133. https://doi.org/10.1016/j.ecolind.2021.108444

Jaureguiberry, P., Titeux, N., Wiemers, M., Bowler, D.E., Coscieme, L., Golden, A.S., Guerra, C.A., Jacob, U., Takahashi, Y., Settele, J., Díaz, S., Molnár, Z., Purvis, A., 2022. The direct drivers of recent global anthropogenic biodiversity loss. Sci Adv 8, 9982. https://doi.org/10.1126/SCIADV.ABM9982/SUPPL_FILE/SCIADV.ABM9982_DATA_S1.ZIP

Järvio, N., Uusitalo, V., Tuomisto, H., Ekroos, J., submitted. Enhancing Biodiversity Life Cycle Impact Assessments Across Diverse Farming Systems through eDNA Analysis.

Ji, F., Yan, L., Yan, S., Qin, T., Shen, J., Zha, J., 2021. Estimating aquatic plant diversity and distribution in rivers from Jingjinji region, China, using environmental DNA metabarcoding and a traditional survey method. Environ Res 199, 111348. https://doi.org/10.1016/J.ENVRES.2021.111348

Johnson, M.D., Fokar, M., Cox, R.D., Barnes, M.A., 2021. Airborne environmental DNA metabarcoding detects more diversity, with less sampling effort, than a traditional plant community survey. BMC Ecol Evol 21, 1–15. https://doi.org/10.1186/S12862-021-01947-X/FIGURES/6

Johnson, M.D., Freeland, J.R., Parducci, L., Evans, D.M., Meyer, R.S., Molano-Flores, B., Davis, M.A., 2023. Environmental DNA as an emerging tool in botanical research. Am J Bot 110, e16120. https://doi.org/10.1002/AJB2.16120

Jusino, M.A., Banik, M.T., Palmer, J.M., Wray, A.K., Xiao, L., Pelton, E., Barber, J.R., Kawahara, A.Y., Gratton, C., Peery, M.Z., Lindner, D.L., 2019. An improved method for utilizing high-throughput amplicon sequencing to determine the diets of insectivorous animals. Mol Ecol Resour 19. https://doi.org/10.1111/1755-0998.12951

Kahlert, M., Bailet, B., Chonova, T., Karjalainen, S.M., Schneider, S.C., Tapolczai, K., 2021. Same same, but different: The response of diatoms to environmental gradients in Fennoscandian streams and lakes – barcodes, traits and microscope data compared. Ecol Indic 130, 108088. https://doi.org/10.1016/J.ECOLIND.2021.108088

Kaunitz, J.D., 2015. The Discovery of PCR: ProCuRement of Divine Power. Dig Dis Sci 60, 2230. https://doi.org/10.1007/S10620-015-3747-0

Kestel, J.H., Field, D.L., Bateman, P.W., White, N.E., Allentoft, M.E., Hopkins, A.J.M., Gibberd, M., Nevill, P., 2022. Applications of environmental DNA (eDNA) in agricultural systems: Current uses, limitations and future prospects. Science of the Total Environment. https://doi.org/10.1016/j.scitotenv.2022.157556

Khangura, R., Ferris, D., Wagg, C., Bowyer, J., 2023. Regenerative Agriculture—A Literature Review on the Practices and Mechanisms Used to Improve Soil Health. Sustainability 2023, Vol. 15, Page 2338 15, 2338. https://doi.org/10.3390/SU15032338

Kirse, A., Bourlat, S.J., Langen, K., Fonseca, V.G., 2021. Metabarcoding Malaise traps and soil eDNA reveals seasonal and local arthropod diversity shifts. Scientific Reports 2021 11:1 11, 1–12. https://doi.org/10.1038/s41598-021-89950-6

Kissling, W.D., Walls, R., Bowser, A., Jones, M.O., Kattge, J., Agosti, D., Amengual, J., Basset, A., van Bodegom, P.M., Cornelissen, J.H.C., Denny, E.G., Deudero, S., Egloff, W., Elmendorf, S.C., Alonso García, E., Jones, K.D., Jones, O.R., Lavorel, S., Lear, D., Navarro, L.M., Pawar, S., Pirzl, R., Rüger, N., Sal, S., Salguero-Gómez, R., Schigel, D., Schulz, K.S., Skidmore, A., Guralnick, R.P., 2018. Towards global data products of Essential Biodiversity Variables on species traits. Nature Ecology & Evolution 2018 2:10 2, 1531–1540. https://doi.org/10.1038/s41559-018-0667-3

Knudsen, M.T., Hermansen, J.E., Cederberg, C., Herzog, F., Vale, J., Jeanneret, P., Sarthou, J.P., Friedel, J.K., Balázs, K., Fjellstad, W., Kainz, M., Wolfrum, S., Dennis, P., 2017. Characterization factors for land use impacts on biodiversity in life cycle assessment based on direct measures of plant species richness in European farmland in the 'Temperate Broadleaf and Mixed Forest' biome. Science of The Total Environment 580, 358–366. https://doi.org/10.1016/J.SCITOTENV.2016.11.172

Krishnamoorthy, A., Gupta, A., Sar, P., Maiti, M.K., 2021. Metagenomics of two gnotobiotically grown aromatic rice cultivars reveals genotype-dependent and tissue-specific colonization of endophytic bacterial communities attributing multiple plant growth promoting traits. World J Microbiol Biotechnol 37, 1–16. https://doi.org/10.1007/S11274-021-03022-5/TABLES/2

Lago-Olveira, S., Ouhemi, H., Idrissi, O., Moreira, M.T., González-García, S., 2024. Promoting more sustainable agriculture in the Moroccan drylands by shifting from conventional wheat monoculture to a rotation with chickpea and lentils. Cleaner Environmental Systems 12. https://doi.org/10.1016/j.cesys.2024.100169

Lago-Olveira, S., Rebolledo-Leiva, R., Garofalo, P., Moreira, M.T., González-García, S., 2023. Environmental and economic benefits of wheat and chickpea crop rotation in the Mediterranean region of Apulia (Italy). Science of the Total Environment 896. https://doi.org/10.1016/j.scitotenv.2023.165124

Lamb, P.D., Fonseca, V.G., Maxwell, D.L., Nnanatu, C.C., 2022. Systematic review and meta-analysis: Water type and temperature affect environmental DNA decay. Mol Ecol Resour 22, 2494. https://doi.org/10.1111/1755-0998.13627

Lammerant, J., Starkey, M., De Horde, A., Bor, A.-M., Driesen, K., Vanderheyden, G., Müller, L., 2021. Assessment of biodiversity measurement approaches for business and financial institutions. Update Report 3. Biodiversity Platform.

Larrey-Lassalle, P., Loiseau, E., Roux, P., Lopez-Ferber, M., Rosenbaum, R.K., 2018. Developing characterisation factors for land fragmentation impacts on biodiversity in LCA: key learnings from a sugarcane case study. International Journal of Life Cycle Assessment 23, 2126–2136. https://doi.org/10.1007/S11367-018-1449-5/METRICS

Leal Filho, W., Wolf, F., Lange Salvia, A., Beynaghi, A., Shulla, K., Kovaleva, M., Vasconcelos, C.R.P., 2020. Heading towards an unsustainable world: some of the implications of not achieving the SDGs. Discover Sustainability. https://doi.org/10.1007/s43621-020-00002-x

Lear, G., Dickie, I., Banks, J., Boyer, S., Buckley, H.L., Buckley, T., Cruickshank, R., Dopheide, A., Handley, K., 2018. Methods for the extraction, storage, amplification and sequencing of DNA from environmental samples. N Z J Ecol 42. https://doi.org/10.20417/nzjecol.42.9ï

Lee, D.J., Kwon, T., Lee, H.J., Oh, Y.H., Kim, J.H., Lee, T.H., 2022. NGSpop: A desktop software that supports population studies by identifying sequence variations from next-generation sequencing data. PLoS One 17, e0260908. https://doi.org/10.1371/JOURNAL.PONE.0260908

Lehmann, J., Bossio, D.A., Kögel-Knabner, I., Rillig, M.C., 2020. The concept and future prospects of soil health. Nat Rev Earth Environ. https://doi.org/10.1038/s43017-020-0080-8

Li, W., Hou, X., Xu, C., Qin, M., Wang, S., Wei, L., Wang, Y., Liu, X., Li, Y., 2021. Validating eDNA measurements of the richness and abundance of anurans at a large scale. Journal of Animal Ecology 90, 1466–1479. https://doi.org/10.1111/1365-2656.13468

Littlefair, J.E., Hleap, J.S., Palace, V., Rennie, M.D., Paterson, M.J., Cristescu, M.E., 2023. Freshwater connectivity transforms spatially integrated signals of biodiversity. Proceedings of the Royal Society B 290. https://doi.org/10.1098/RSPB.2023.0841

Liu, F., Zhang, Y., Liang, H., Gao, D., 2019. Long-term harvesting of reeds affects greenhouse gas emissions and microbial functional genes in alkaline wetlands. Water Res 164. https://doi.org/10.1016/j.watres.2019.114936

Lüscher, G., Nemecek, T., Arndorfer, M., Balázs, K., Dennis, P., Fjellstad, W., Friedel, J.K., Gaillard, G., Herzog, F., Sarthou, J.P., Stoyanova, S., Wolfrum, S., Jeanneret, P., 2017. Biodiversity assessment in LCA: a validation at field and farm scale in eight European regions. International Journal of Life Cycle Assessment 22. https://doi.org/10.1007/s11367-017-1278-y

Mącik, M., Gryta, A., Sas-Paszt, L., Frąc, M., 2023. New insight into the soil bacterial and fungal microbiome after phosphorus biofertilizer application as an important driver of regenerative agriculture including biodiversity loss reversal and soil health restoration. Applied Soil Ecology 189, 104941. https://doi.org/10.1016/J.APSOIL.2023.104941

Maira, L., Byers, O., Lees, C., Nguyend, D., Rodrigueze, J., Smart, J., McGowan, P., 2021. Achieving international species conservation targets: Closing the gap between top-down and bottom-up approaches. Conservation and Society 19, 25–33. https://doi.org/10.4103/CS.CS_19_137

Manfrin, C., Souty-Grosset, C., Anastácio, P.M., Reynolds, J., Giulianini, P.G., 2019. Detection and Control of Invasive Freshwater Crayfish: From Traditional to Innovative Methods. Diversity 2019, Vol. 11, Page 5 11, 5. https://doi.org/10.3390/D11010005

Marques, A., Robuchon, M., Hellweg, S., Newbold, T., Beher, J., Bekker, S., Essl, F., Ehrlich, D., Hill, S., Jung, M., Marquardt, S., Rosa, F., Rugani, B., Suárez-Castro, A.F., Silva, A.P., Williams, D.R., Dubois, G., Sala, S., 2021. A research perspective towards a more complete biodiversity footprint: a report from the World Biodiversity Forum. International Journal of Life Cycle Assessment 26, 238–243. https://doi.org/10.1007/S11367-020-01846-1/METRICS

Mathieu, C., Hermans, S.M., Lear, G., Buckley, T.R., Lee, K.C., Buckley, H.L., 2020. A systematic review of sources of variability and uncertainty in eDNA data for environmental monitoring. Front Ecol Evol 8, 1–14. https://doi.org/10.3389/FEVO.2020.00135

Maxwell, S.L., Cazalis, V., Dudley, N., Hoffmann, M., Rodrigues, A.S.L., Stolton, S., Visconti, P., Woodley, S., Kingston, N., Lewis, E., Maron, M., Strassburg, B.B.N., Wenger, A., Jonas, H.D., Venter, O., Watson, J.E.M., 2020. Area-based conservation in the twenty-first century. Nature 2020 586:7828 586, 217–227. https://doi.org/10.1038/s41586-020-2773-z

McPherson, C., Avanesyan, A., Lamp, W.O., 2022. Diverse Host Plants of the First Instars of the Invasive Lycorma delicatula: Insights from eDNA Metabarcoding. Insects 13. https://doi.org/10.3390/insects13060534

Mehetre, G.T., Leo, V.V., Singh, G., Sorokan, A., Maksimov, I., Yadav, M.K., Upadhyaya, K., Hashem, A., Alsaleh, A.N., Dawoud, T.M., Almaary, K.S., Singh, B.P., 2021. Current Developments and Challenges in Plant Viral Diagnostics: A Systematic Review. Viruses 2021, Vol. 13, Page 412 13, 412. https://doi.org/10.3390/V13030412

Mengist, W., Soromessa, T., Legese, G., 2020. Method for conducting systematic literature review and meta-analysis for environmental science research. MethodsX 7. https://doi.org/10.1016/J.MEX.2019.100777

Morais, D., Pylro, V., Clark, I.M., Hirsch, P.R., Tótola, M.R., 2016. Responses of microbial community from tropical pristine coastal soil to crude oil contamination. PeerJ 2016. https://doi.org/10.7717/peerj.1733

Moutik, B., Summerscales, J., Graham-Jones, J., Pemberton, R., 2023. Life Cycle Assessment Research Trends and Implications: A Bibliometric Analysis. Sustainability 2023, Vol. 15, Page 13408 15, 13408. https://doi.org/10.3390/SU151813408

Nagler, M., Podmirseg, S.M., Ascher-Jenull, J., Sint, D., Traugott, M., 2022. Why eDNA fractions need consideration in biomonitoring. Mol Ecol Resour 22, 2458–2470. https://doi.org/10.1111/1755-0998.13658

Nam, B., Lee, H.J., Choi, Y.J., 2023. Organic Farming Allows Balanced Fungal and Oomycetes Communities. Microorganisms 11. https://doi.org/10.3390/microorganisms11051307

NAS, 2021. The Challenge of Feeding the World Sustainably: Summary of the US-UK Scientific Forum on Sustainable Agriculture. The Challenge of Feeding the World Sustainably 1–36. https://doi.org/10.17226/26007

Navarro-Noya, Y.E., Chávez-Romero, Y., Hereira-Pacheco, S., Lorenzana, A.S. de L., Govaerts, B., Verhulst, N., Dendooven, L., 2022. Bacterial Communities in the Rhizosphere at Different Growth Stages of Maize Cultivated in Soil Under Conventional and Conservation Agricultural Practices. Microbiol Spectr 10. https://doi.org/10.1128/SPECTRUM.01834-21

Nester, G.M., De Brauwer, M., Koziol, A., West, K.M., DiBattista, J.D., White, N.E., Power, M., Heydenrych, M.J., Harvey, E., Bunce, M., 2020. Development and evaluation of fish eDNA metabarcoding assays facilitate the detection of cryptic seahorse taxa (family: Syngnathidae). Environmental DNA 2. https://doi.org/10.1002/edn3.93

Newton, J.P., Bateman, P.W., Heydenrych, M.J., Mousavi-Derazmahalleh, M., Nevill, P., 2022. Home is where the hollow is: Revealing vertebrate tree hollow user biodiversity with eDNA metabarcoding. Environmental DNA 4, 1078–1091. https://doi.org/10.1002/EDN3.313

Newton, P., Civita, N., Frankel-Goldwater, L., Bartel, K., Johns, C., 2020. What Is Regenerative Agriculture? A Review of Scholar and Practitioner Definitions Based on Processes and Outcomes. Front Sustain Food Syst 4, 577723. https://doi.org/10.3389/FSUFS.2020.577723/BIBTEX

Norros, V., Laamanen, T., Meissner, K., Iso-Touru, T., Kahilainen, A., Lehtinen, S., Lohtander-Buckbee, K., Nygård, H., Pennanen, T., Ruohonen-Lehto, M., Sirkiä, P., Suikkanen, S., Tolkkinen, M., Vainio, E., Velmala, S., Vuorio, K., Vihervaara, P., 2022. Roadmap for implementing environmental DNA (eDNA) and other molecular monitoring methods in Finland – Vision and action plan for 2022–2025 71.

Nyssens-James, C., Leake, A., Heinzel, C., Krajcic, J., 2023. Advancing Regenerative. Agriculture in the EU and UK. The importance of enabling policies 19.

O'Donoghue, T., Minasny, B., McBratney, A., 2024. Digital Regenerative Agriculture. npj Sustainable Agriculture 2024 2:1 2, 1–5. https://doi.org/10.1038/s44264-024-00012-6

Ogram, A., Sayler, G.S., Barkay, T., 1987. The extraction and purification of microbial DNA from sediments. J Microbiol Methods 7, 57–66. https://doi.org/10.1016/0167-7012(87)90025-X

Olsen, G.J., Lane, D.J., Giovannoni, S.J., Pace, N.R., Stahl, D.A., 1986. Microbial ecology and evolution: a ribosomal RNA approach. Annu Rev Microbiol 40, 337–365. https://doi.org/10.1146/ANNUREV.MI.40.100186.002005/CITE/REFWORKS

Ordikhani, H., Parashkoohi, M.G., Zamani, D.M., Ghahderijani, M., 2021. Energy-environmental life cycle assessment and cumulative exergy demand analysis for horticultural crops (Case study: Qazvin province). Energy Reports 7, 2899–2915. https://doi.org/10.1016/J.EGYR.2021.05.022

Padhy, S.R., Bhattacharyya, P., Nayak, S.K., Dash, P.K., Mohapatra, T., 2021. A unique bacterial and archaeal diversity make mangrove a green production system compared to rice in wetland ecology: A metagenomic approach. Science of The Total Environment 781, 146713. https://doi.org/10.1016/J.SCITOTENV.2021.146713

Pauvert, C., Buée, M., Laval, V., Edel-Hermann, V., Fauchery, L., Gautier, A., Lesur, I., Vallance, J., Vacher, C., 2019. Bioinformatics matters: The accuracy of plant and soil fungal community data is highly dependent on the metabarcoding pipeline. Fungal Ecol 41. https://doi.org/10.1016/j.funeco.2019.03.005

Pawlowski, J., Apothéloz-Perret-Gentil, L., Altermatt, F., 2020. Environmental DNA: What's behind the term? Clarifying the terminology and recommendations for its future use in biomonitoring. Mol Ecol 29, 4258–4264. https://doi.org/10.1111/MEC.15643

Pedersen Weidema, B., 2008. Using the budget constraint to monetarise impact assessment results. Ecological Economics 68, 1591–1598. https://doi.org/10.1016/j.ecolecon.2008.01.019

Pereira, H.M., Ferrier, S., Walters, M., Geller, G.N., Jongman, R.H.G., Scholes, R.J., Bruford, M.W., Brummitt, N., Butchart, S.H.M., Cardoso, A.C., Coops, N.C., Dulloo, E., Faith, D.P., Freyhof, J., Gregory, R.D., Heip, C., Höft, R., Hurtt, G., Jetz, W., Karp, D.S., McGeoch, M.A., Obura, D., Onoda, Y., Pettorelli, N., Reyers, B., Sayre, R., Scharlemann, J.P.W., Stuart, S.N., Turak, E., Walpole, M., Wegmann, M., 2013. Essential biodiversity variables. Science (1979) 339, 277–278. https://doi.org/10.1126/SCIENCE.1229931/SUPPL_FILE/1229931.PEREIRA.SM.PDF

Pereira, H.M., Ziv, G., Miranda, M., 2014. Countryside Species–Area Relationship as a Valid Alternative to the Matrix-Calibrated Species–Area Model. Conservation Biology 28, 874. https://doi.org/10.1111/COBI.12289

Piggott, M.P., Banks, S.C., Broadhurst, B.T., Fulton, C.J., Lintermans, M., 2021. Comparison of traditional and environmental DNA survey methods for detecting rare and abundant freshwater fish. Aquat Conserv 31. https://doi.org/10.1002/aqc.3474

Pilliod, D.S., Goldberg, C.S., Arkle, R.S., Waits, L.P., 2014. Factors influencing detection of eDNA from a stream-dwelling amphibian. Mol Ecol Resour 14, 109–116. https://doi.org/10.1111/1755-0998.12159

Pochon, X., Zaiko, A., Fletcher, L.M., Laroche, O., Wood, S.A., 2017. Wanted dead or alive? Using metabarcoding of environmental DNA and RNA to distinguish living assemblages for biosecurity applications. PLoS One 12, e0187636. https://doi.org/10.1371/JOURNAL.PONE.0187636

Pranckutė, R., 2021. Web of Science (WoS) and Scopus: The Titans of Bibliographic Information in Today's Academic World. Publications 2021, Vol. 9, Page 12 9, 12. https://doi.org/10.3390/PUBLICATIONS9010012

Prechsl, U.E., Wittwer, R., van der Heijden, M.G.A., Lüscher, G., Jeanneret, P., Nemecek, T., 2017. Assessing the environmental impacts of cropping systems and cover crops: Life cycle assessment of FAST, a long-term arable farming field experiment. Agric Syst 157. https://doi.org/10.1016/j.agsy.2017.06.011

Puillandre, N., Brouillet, S., Achaz, G., 2021. ASAP: assemble species by automatic partitioning. Mol Ecol Resour 21, 609–620. https://doi.org/10.1111/1755-0998.13281

Qian, T., Shan, X., Wang, W., Jin, X., 2022. Effects of Temperature on the Timeliness of eDNA/eRNA: A Case Study of Fenneropenaeus chinensis. Water 2022, Vol. 14, Page 1155 14, 1155. https://doi.org/10.3390/W14071155

Quevedo-Cascante, M., Mogensen, L., Kongsted, A.G., Knudsen, M.T., 2023. How does Life Cycle Assessment capture the environmental impacts of agroforestry? A systematic review. Science of The Total Environment 890, 164094. https://doi.org/10.1016/J.SCITOTENV.2023.164094

Ramachandran Nair, P.K., Nair, V.D., Mohan Kumar, B., Showalter, J.M., 2010. Carbon Sequestration in Agroforestry Systems. Advances in Agronomy 108, 237–307. https://doi.org/10.1016/S0065-2113(10)08005-3

Rehberger, E., West, P.C., Spillane, C., McKeown, P.C., 2023. What climate and environmental benefits of regenerative agriculture practices? an evidence review. Environ Res Commun 5, 052001. https://doi.org/10.1088/2515-7620/ACD6DC

Ren, X., Wang, Q., Chen, X., Zhang, Y., Sun, Y., Li, R., Li, J., Zhang, Z., 2021. Elucidating the optimum added dosage of Diatomite during co-composting of pig manure and sawdust: Carbon dynamics and microbial community. Science of the Total Environment 777. https://doi.org/10.1016/j.scitotenv.2021.146058

Riaz, T., Shehzad, W., Viari, A., Pompanon, F., Taberlet, P., Coissac, E., 2011. EcoPrimers: Inference of new DNA barcode markers from whole genome sequence analysis. Nucleic Acids Res 39. https://doi.org/10.1093/nar/gkr732

Richardson, K., Steffen, W., Lucht, W., Bendtsen, J., Cornell, S.E., Donges, J.F., Drüke, M., Fetzer, I., Bala, G., von Bloh, W., Feulner, G., Fiedler, S., Gerten, D., Gleeson, T., Hofmann, M., Huiskamp, W., Kummu, M., Mohan, C., Nogués-Bravo, D., Petri, S., Porkka, M., Rahmstorf, S., Schaphoff, S., Thonicke, K., Tobian, A., Virkki, V., Wang-Erlandsson, L., Weber, L., Rockström, J., 2023. Earth beyond six of nine planetary boundaries. Sci Adv 9. https://doi.org/10.1126/SCIADV.ADH2458/SUPPL_FILE/SCIADV.ADH2458_SM.PDF

Ríos-Castro, R., Romero, A., Aranguren, R., Pallavicini, A., Banchi, E., Novoa, B., Figueras, A., 2021. High-Throughput Sequencing of Environmental DNA as a Tool for Monitoring Eukaryotic Communities and Potential Pathogens in a Coastal Upwelling Ecosystem. Front Vet Sci 8, 765606. https://doi.org/10.3389/FVETS.2021.765606/BIBTEX

Rishan, S.T., Kline, R.J., Rahman, M.S., 2024. New prospects of environmental RNA metabarcoding research in biological diversity, ecotoxicological monitoring, and detection of COVID-19: a critical review. Environmental Science and Pollution Research 2024 31:8 31, 11406–11427. https://doi.org/10.1007/S11356-023-31776-Y

Ritchie, H., Roser, M., 2024. Land Use - Our World in Data [WWW Document]. URL https://ourworldindata.org/land-use#all-charts (accessed 4.16.24).

ROA, 2023. Framework for Regenerative Organic Certified® [WWW Document]. URL https://regenorganic.org/wp-content/uploads/2023/03/Regenerative-Organic-Certified-Framework.pdf (accessed 4.15.24).

Rodríguez-Ezpeleta, N., Zinger, L., Kinziger, A., Bik, H.M., Bonin, A., Coissac, E., Emerson, B.C., Lopes, C.M., Pelletier, T.A., Taberlet, P., Narum, S., 2021. Biodiversity monitoring using environmental DNA. Mol Ecol Resour 21, 1405–1409. https://doi.org/10.1111/1755-0998.13399

Roehe, R., Dewhurst, R.J., Duthie, C.A., Rooke, J.A., McKain, N., Ross, D.W., Hyslop, J.J., Waterhouse, A., Freeman, T.C., Watson, M., Wallace, R.J., 2016. Bovine Host Genetic Variation Influences Rumen Microbial Methane Production with Best Selection Criterion for Low Methane Emitting and Efficiently Feed Converting Hosts Based on Metagenomic Gene Abundance. PLoS Genet 12. https://doi.org/10.1371/journal.pgen.1005846

Rounsevell, M.D.A., Harfoot, M., Harrison, P.A., Newbold, T., Gregory, R.D., Mace, G.M., 2020. A biodiversity target based on species extinctions. Science (1979) 368, 1193–1195. https://doi.org/10.1126/SCIENCE.ABA6592/SUPPL_FILE/ABA6592_ROUNSEVELL_SM.PDF

Ruppert, K.M., Davis, D.R., Rahman, M.S., Kline, R.J., 2022. Development and assessment of an environmental DNA (eDNA) assay for a cryptic Siren (Amphibia: Sirenidae). Environmental Advances 7. https://doi.org/10.1016/j.envadv.2021.100163

Ruppert, K.M., Kline, R.J., Rahman, M.S., 2019. Past, present, and future perspectives of environmental DNA (eDNA) metabarcoding: A systematic review in methods, monitoring, and applications of global eDNA. Glob Ecol Conserv 17. https://doi.org/10.1016/J.GECCO.2019.E00547

Sakata, M.K., Kawata, M.U., Kurabayashi, A., Kurita, T., Nakamura, M., Shirako, T., Kakehashi, R., Nishikawa, K., Hossman, M.Y., Nishijima, T., Kabamoto, J., Miya, M., Minamoto, T., 2022. Development and evaluation of PCR primers for environmental DNA (eDNA) metabarcoding of Amphibia. Metabarcoding Metagenom 6. https://doi.org/10.3897/MBMG.6.76534

Sala, S., Reale, F., Cristóbal-García, J., Marelli, L., Rana, P., 2016. Life cycle assessment for the impact assessment of policies. Life thinking and assessment in the European policies and for evaluating policy options. Joint Research Centre 28380. https://doi.org/10.2788/318544

Sampson, R.N., 1982. Saving agricultural land: Environmental issue of the 1980's. Environmentalist 2, 321–332. https://doi.org/10.1007/BF02603089/METRICS

Sands, B., Machado, M.R., White, A., Zent, E., Gould, R., 2023. Moving towards an anti-colonial definition for regenerative agriculture. Agric Human Values 40, 1697–1716. https://doi.org/10.1007/S10460-023-10429-3/METRICS

Satam, H., Joshi, K., Mangrolia, U., Waghoo, S., Zaidi, G., Rawool, S., Thakare, R.P., Banday, S., Mishra, A.K., Das, G., Malonia, S.K., 2023. Next-Generation Sequencing Technology: Current Trends and Advancements. Biology 2023, Vol. 12, Page 997 12, 997. https://doi.org/10.3390/BIOLOGY12070997

Scherer, L., Rosa, F., Sun, Z., Michelsen, O., De Laurentiis, V., Marques, A., Pfister, S., Verones, F., Kuipers, K.J.J., 2023. Biodiversity Impact Assessment Considering Land Use Intensities and Fragmentation. Environ Sci Technol 57, 19612–19623. https://doi.org/10.1021/ACS.EST.3C04191/SUPPL_FILE/ES3C04191_SI_002.PDF

Schreefel, L., Schulte, R.P.O., de Boer, I.J.M., Schrijver, A.P., van Zanten, H.H.E., 2020. Regenerative agriculture – the soil is the base. Glob Food Sec 26, 100404. https://doi.org/10.1016/J.GFS.2020.100404

Semenchuk, P., Plutzar, C., Kastner, T., Matej, S., Bidoglio, G., Erb, K.H., Essl, F., Haberl, H., Wessely, J., Krausmann, F., Dullinger, S., 2022. Relative effects of land conversion and land-use intensity on terrestrial vertebrate diversity. Nat Commun 13. https://doi.org/10.1038/s41467-022-28245-4

Seppelt, R., Beckmann, M., Ceauşu, S., Cord, A.F., Gerstner, K., Gurevitch, J., Kambach, S., Klotz, S., Mendenhall, C., Phillips, H.R.P., Powell, K., Verburg, P.H., Verhagen, W., Winter, M., Newbold, T., 2016. Harmonizing Biodiversity Conservation and Productivity in the Context of Increasing Demands on Landscapes. Bioscience 66, 890–896. https://doi.org/10.1093/BIOSCI/BIW004

Shendure, J., Ji, H., 2008. Next-generation DNA sequencing. Nature Biotechnology 2008 26:10 26, 1135–1145. https://doi.org/10.1038/nbt1486

Simona, C., Nicola, F., Micol, M., Rodríguez Carmen, M., Raffaella, M., Daniele, P., Andrea, V., Roberto, Z., 2024. A multi-indicator approach to compare the sustainability of organic vs. integrated management of grape production. Ecol Indic 158. https://doi.org/10.1016/j.ecolind.2023.111297

Sirois, S.H., Buckley, D.H., 2019. Factors governing extracellular DNA degradation dynamics in soil. Environ Microbiol Rep 11, 173–184. https://doi.org/10.1111/1758-2229.12725

Smets, W., Leff, J.W., Bradford, M.A., McCulley, R.L., Lebeer, S., Fierer, N., 2016. A method for simultaneous measurement of soil bacterial abundances and community composition via 16S rRNA gene sequencing. Soil Biol Biochem 96, 145–151. https://doi.org/10.1016/J.SOILBIO.2016.02.003

Sniffen, K.D., Price, J.R., Sales, C.M., Olson, M.S., 2017. Influence of Scale on Biomass Growth and Nutrient Removal in an Algal-Bacterial Leachate Treatment System. Environ Sci Technol 51, 13344–13352. https://doi.org/10.1021/ACS.EST.7B03975/SUPPL_FILE/ES7B03975_SI_001.PDF

Stewart, K., Ma, H., Zheng, J., Zhao, J., 2017. Using environmental DNA to assess population-wide spatiotemporal reserve use. Conserv Biol 31, 1173–1182. https://doi.org/10.1111/COBI.12910

Sumberg, J., Giller, K.E., 2022. What is “conventional” agriculture? Glob Food Sec 32, 100617. https://doi.org/10.1016/j.gfs.2022.100617

Taberlet, P., Bonin, A., Zinger, L., Coissac, E., 2018. Environmental DNA: For biodiversity research and monitoring. Environmental DNA: For Biodiversity Research and Monitoring 1–253. https://doi.org/10.1093/oso/9780198767220.001.0001

Taberlet, P., Coissac, E., Hajibabaei, M., Rieseberg, L.H., 2012a. Environmental DNA. Mol Ecol 21, 1789–1793. https://doi.org/10.1111/J.1365-294X.2012.05542.X

Taberlet, P., Prud’Homme, S.M., Campione, E., Roy, J., Miquel, C., Shehzad, W., Gielly, L., Rioux, D., Choler, P., Clément, J.C., Melodelima, C., Pompanon, F., Coissac, E., 2012b. Soil sampling and isolation of extracellular DNA from large amount of starting material suitable for metabarcoding studies. Mol Ecol 21, 1816–1820. https://doi.org/10.1111/J.1365-294X.2011.05317.X

Tamburini, G., Bommarco, R., Wanger, T.C., Kremen, C., van der Heijden, M.G.A., Liebman, M., Hallin, S., 2020. Agricultural diversification promotes multiple ecosystem services without compromising yield. Sci Adv 6. https://doi.org/10.1126/SCIADV.ABA1715

Teillard, F., Maia de Souza, D., Thoma, G., Gerber, P.J., Finn, J.A., 2016. What does Life-Cycle Assessment of agricultural products need for more meaningful inclusion of biodiversity? Journal of Applied Ecology 53, 1422–1429. https://doi.org/10.1111/1365-2664.12683

The Convention on Biological Diversity, 2020. Global Biodiversity Outlook 5: Summary for Policymakers. Secretariat of the Convention on Biological Diversity.

Thomas, A.C., Nguyen, P.L., Howard, J., Goldberg, C.S., 2019. A self-preserving, partially biodegradable eDNA filter. Methods Ecol Evol 10, 1136–1141. https://doi.org/10.1111/2041-210X.13212

Thomsen, P.F., Willerslev, E., 2014. Environmental DNA-An emerging tool in conservation for monitoring past and present biodiversity. https://doi.org/10.1016/j.biocon.2014.11.019

Tillotson, M.D., Kelly, R.P., Duda, J.J., Hoy, M., Kralj, J., Quinn, T.P., 2018. Concentrations of environmental DNA (eDNA) reflect spawning salmon abundance at fine spatial and temporal scales. Biol Conserv 220, 1–11. https://doi.org/10.1016/J.BIOCON.2018.01.030

Trottier, G., Turgeon, K., Verones, F., Boisclair, D., Bulle, C., Margni, M., 2021. Empirical Characterization Factors for Life Cycle Assessment of the Impacts of Reservoir Occupation on Macroinvertebrate Richness across the United States. Sustainability 2021, Vol. 13, Page 2701 13, 2701. https://doi.org/10.3390/SU13052701

Tsuji, S., Iguchi, Y., Shibata, N., Teramura, I., Kitagawa, T., Yamanaka, H., 2018. Real-time multiplex PCR for simultaneous detection of multiple species from environmental DNA: An application on two Japanese medaka species. Sci Rep 8. https://doi.org/10.1038/s41598-018-27434-w

Tuomikoski, E., Selonen, V., Merimaa, K., Laaksonen, T., 2024. Diet of the raccoon dog, an invasive mesopredator, during the breeding season of declining waterbird populations. Glob Ecol Conserv 51, e02917. https://doi.org/10.1016/J.GECCO.2024.E02917

Turner, P.A.M., Ximenes, F.A., Penman, T.D., Law, B.S., Waters, C.M., Grant, T., Mo, M., Brock, P.M., 2019. Accounting for biodiversity in life cycle impact assessments of forestry and agricultural systems—the BioImpact metric. International Journal of Life Cycle Assessment 24. https://doi.org/10.1007/s11367-019-01627-5

Turunen, J., Elbrecht, V., Steinke, D., Aroviita, J., 2021a. Riparian forests can mitigate warming and ecological degradation of agricultural headwater streams. Freshw Biol 66, 785–798. https://doi.org/10.1111/FWB.13678

Turunen, J., Mykrä, H., Elbrecht, V., Steinke, D., Braukmann, T., Aroviita, J., 2021b. The power of metabarcoding: Can we improve bioassessment and biodiversity surveys of stream macroinvertebrate communities? Metabarcoding and Metagenomics 5: e68938 5, e68938-. https://doi.org/10.3897/MBMG.5.68938

Ulibarri, R.M., Bonar, S.A., Rees, C., Amberg, J., Ladell, B., Jackson, C., 2017. Comparing efficiency of American Fisheries Society standard snorkeling techniques to environmental DNA sampling techniques. N Am J Fish Manag 37, 644–651. https://doi.org/10.1080/02755947.2017.1306005

UNCCD, 2022. The Global Land Outlook, second edition, 2nd ed. Bonn.

UNEP/SETAC, 2017. Global Guidance for Life Cycle Impact Assessment Indicators Volume 1, Global Guidance for Life Cycle Impact Assessment Indicators.

USDA, 2019. Recommended Soil Health Indicators and Associated Laboratory Procedures.

Uusitalo, V., Kuokkanen, A., Grönman, K., Ko, N., Mäkinen, H., Koistinen, K., 2019. Environmental sustainability assessment from planetary boundaries perspective – A case study of an organic sheep farm in Finland. Science of the Total Environment 687. https://doi.org/10.1016/j.scitotenv.2019.06.120

Valentin, R.E., Kyle, K.E., Allen, M.C., Welbourne, D.J., Lockwood, J.L., 2021. The state, transport, and fate of aboveground terrestrial arthropod eDNA. Environmental DNA 3, 1081–1092. https://doi.org/10.1002/EDN3.229

van der Heyde, M., Bunce, M., Wardell-Johnson, G., Fernandes, K., White, N.E., Nevill, P., 2020. Testing multiple substrates for terrestrial biodiversity monitoring using environmental DNA metabarcoding. Mol Ecol Resour 20, 732–745. https://doi.org/10.1111/1755-0998.13148

Van Der Meer, M., Kay, S., Lüscher, G., Jeanneret, P., 2020. What evidence exists on the impact of agricultural practices in fruit orchards on biodiversity? A systematic map. Environ Evid 9, 1–27. https://doi.org/10.1186/S13750-020-0185-Z/FIGURES/12

van der Werf, H.M.G., Knudsen, M.T., Cederberg, C., 2020. Towards better representation of organic agriculture in life cycle assessment. Nat Sustain 3, 419–425. https://doi.org/10.1038/S41893-020-0489-6

Velasco-Muñoz, J.F., Mendoza, J.M.F., Aznar-Sánchez, J.A., Gallego-Schmid, A., 2021. Circular economy implementation in the agricultural sector: Definition, strategies and indicators. Resour Conserv Recycl 170. https://doi.org/10.1016/j.resconrec.2021.105618

Vélez-Henao, J.A., Weinland, F., Reintjes, N., 2021. Life cycle assessment of aquaculture bivalve shellfish production — a critical review of methodological trends. International Journal of Life Cycle Assessment 26, 1943–1958. https://doi.org/10.1007/S11367-021-01978-Y/TABLES/1

Verones, F., Hellweg, S., Antón, A., Azevedo, L.B., Chaudhary, A., Cosme, N., Cucurachi, S., de Baan, L., Dong, Y., Fantke, P., Golsteijn, L., Hauschild, M., Heijungs, R., Jolliet, O., Juraske, R., Larsen, H., Laurent, A., Mutel, C.L., Margni, M., Núñez, M., Owsianiak, M., Pfister, S., Ponsioen, T., Preiss, P., Rosenbaum, R.K., Roy, P.O., Sala, S., Steinmann, Z., van Zelm, R., Van Dingenen, R., Vieira, M., Huijbregts, M.A.J., 2020. LC-IMPACT: A regionalized life cycle damage assessment method. J Ind Ecol 24, 1201–1219. https://doi.org/10.1111/JIEC.13018

Verones, F., Kuipers, K., Núñez, M., Rosa, F., Scherer, L., Marques, A., Michelsen, O., Barbarossa, V., Jaffe, B., Pfister, S., Dorber, M., 2022. Global extinction probabilities of terrestrial, freshwater, and marine species groups for use in Life Cycle Assessment. Ecol Indic 142. https://doi.org/10.1016/j.ecolind.2022.109204

Voisin, R., Horwitz, P., Godrich, S., Sambell, R., Cullerton, K., Devine, A., 2024. What goes in and what comes out: a scoping review of regenerative agricultural practices. Agroecology and Sustainable Food Systems 48, 124–158. https://doi.org/10.1080/21683565.2023.2270441

Vrasdonk, E., Palme, U., Lennartsson, T., 2019. Reference situations for biodiversity in life cycle assessments: conceptual bridging between LCA and conservation biology. International Journal of Life Cycle Assessment 24, 1631–1642. https://doi.org/10.1007/S11367-019-01594-X/FIGURES/1

Wang, H., Zheng, H., Browne, F., Roehe, R., Dewhurst, R.J., Engel, F., Hemmje, M., Lu, X., Walsh, P., 2017. Integrated metagenomic analysis of the rumen microbiome of cattle reveals key biological mechanisms associated with methane traits. Methods 124. https://doi.org/10.1016/j.ymeth.2017.05.029

Wardani, I., 2018. Developing a characterization factor framework for microbial contamination, in: E3S Web of Conferences. https://doi.org/10.1051/e3sconf/20187411002

Willerslev, E., Davison, J., Moora, M., Zobel, M., Coissac, E., Edwards, M.E., Lorenzen, E.D., Vestergård, M., Gussarova, G., Haile, J., Craine, J., Gielly, L., Boessenkool, S., Epp, L.S., Pearman, P.B., Cheddadi, R., Murray, D., Bråthen, K.A., Yoccoz, N., Binney, H., Cruaud, C., Wincker, P., Goslar, T., Alsos, I.G., Bellemain, E., Brysting, A.K., Elven, R., Sønstebø, J.H., Murton, J., Sher, A., Rasmussen, M., Rønn, R., Mourier, T., Cooper, A., Austin, J., Möller, P., Froese, D., Zazula, G., Pompanon, F., Rioux, D., Niderkorn, V., Tikhonov, A., Savvinov, G., Roberts, R.G., Macphee, R.D.E., Gilbert, M.T.P., Kjær, K.H., Orlando, L., Brochmann, C., Taberlet, P., 2014. Fifty thousand years of Arctic vegetation and megafaunal diet. Nature 2014 506:7486 506, 47–51. https://doi.org/10.1038/nature12921

Willerslev, E., Hansen, A.J., Binladen, J., Brand, T.B., Gilbert, M.T.P., Shapiro, B., Bunce, M., Wiuf, C., Gilichinsky, D.A., Cooper, A., 2003. Diverse plant and animal genetic records from holocene and pleistocene sediments. Science (1979) 300, 791–795. https://doi.org/10.1126/SCIENCE.1084114/SUPPL_FILE/WILLERSLEV.SOM.PDF

Wineland, S.M., Arrick, R.F., Welch, S.M., Pauley, T.K., Mosher, J.J., Apodaca, J.J., Olszack, M., Holmes, J.N., Waldron, J.L., 2019. Environmental DNA improves Eastern

Hellbender (Cryptobranchus alleganiensis alleganiensis) detection over conventional sampling methods. Environmental DNA 1, 86–96. https://doi.org/10.1002/EDN3.9

Winter, L., Lehmann, A., Finogenova, N., Finkbeiner, M., 2017. Including biodiversity in life cycle assessment – State of the art, gaps and research needs. Environ Impact Assess Rev 67, 88–100. https://doi.org/10.1016/J.EIAR.2017.08.006

Winter, L., Pflugmacher, S., Berger, M., Finkbeiner, M., 2018. Biodiversity impact assessment (BIA+) – methodological framework for screening biodiversity. Integr Environ Assess Manag 14, 282–297. https://doi.org/10.1002/IEAM.2006

Woods, J.S., Rødder, G., Verones, F., 2018. An effect factor approach for quantifying the entanglement impact on marine species of macroplastic debris within life cycle impact assessment. Technology (Singap World Sci) 7. https://doi.org/10.1016/j.ecolind.2018.12.018

Woods, J.S., Veltman, K., Huijbregts, M.A.J., Verones, F., Hertwich, E.G., 2016. Towards a meaningful assessment of marine ecological impacts in life cycle assessment (LCA). Environ Int 89–90, 48–61. https://doi.org/10.1016/J.ENVINT.2015.12.033

WWF, 2022. Living Planet Report 2022 - Building a nature-positive society., WWF. ed. Gland, Switherland.

Xavier, C.V., Moitinho, M.R., De Bortoli Teixeira, D., André de Araújo Santos, G., de Andrade Barbosa, M., Bastos Pereira Milori, D.M., Rigobelo, E., Corá, J.E., La Scala Júnior, N., 2019. Crop rotation and succession in a no-tillage system: Implications for CO2 emission and soil attributes. J Environ Manage 245, 8–15. https://doi.org/10.1016/J.JENVMAN.2019.05.053

Yadav, S., Goyal, V.C., 2022. Current Status of Ponds in India: A Framework for Restoration, Policies and Circular Economy. Wetlands 42, 1–16. https://doi.org/10.1007/S13157-022-01624-9/TABLES/2

Yang, C., Lv, D., Jiang, S., Lin, H., Sun, Junqi, Li, K., Sun, Juan, 2021. Soil salinity regulation of soil microbial carbon metabolic function in the Yellow River Delta, China. Science of the Total Environment 790. https://doi.org/10.1016/j.scitotenv.2021.148258

Yang, G., Zheng, Z., Abbott, B.W., Olefeldt, D., Knoblauch, C., Song, Y., Kang, L., Qin, S., Peng, Y., Yang, Y., 2023. Characteristics of methane emissions from alpine thermokarst lakes on the Tibetan Plateau. Nature Communications 2023 14:1 14, 1–11. https://doi.org/10.1038/s41467-023-38907-6

Zhang, H., Mächler, E., Morsdorf, F., Niklaus, P.A., Schaepman, M.E., Altermatt, F., 2023. A spatial fingerprint of land-water linkage of biodiversity uncovered by remote sensing and environmental DNA. Science of The Total Environment 867, 161365. https://doi.org/10.1016/J.SCITOTENV.2022.161365

Zhang, S., Gao, Y., Cai, J., Yang, H., Zhao, Q., Pan, F., 2023. A Novel Bird Sound Recognition Method Based on Multifeature Fusion and a Transformer Encoder. Sensors 2023, Vol. 23, Page 8099 23, 8099. https://doi.org/10.3390/S23198099

Zumsteg, J.M., Cooper, J.S., Noon, M.S., 2012. Systematic Review Checklist: A Standardized Technique for Assessing and Reporting Reviews of Life Cycle Assessment Data. J Ind Ecol 16. https://doi.org/10.1111/J.1530-9290.2012.00476.X