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Biological nitrogen recirculation to food protein – A review

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ABSTRACT

Nitrogen is a part of a complex cycle with transformative reactions being not only an essential element for living organisms, but also facilitating negative environmental impacts as eutrophication and climate change. To reduce the negative environmental impacts, closing the nitrogen loop, reducing inputs of fossil-based synthetic nitrogen fertilizers, and returning nitrogen-rich material and waste streams back into the food system are essential. This review investigates the potential of nitrogen transformation technologies to return nitrogen to food systems from existing material streams, levelling the imbalances of the nitrogen cycle. Review of both conventional and biotechnological pathways for nitrogen recovery, as well as of legal aspects and safety issues uncovers the knowledge gaps, potentials, and barriers for making nitrogen circular in a food system context. Further a few technologies aiming the recirculation of the nitrogen disclosed as a basis for potential industrial scale up and implementation.

1. Introduction

For a sustainable supply of food, agricultural nutrients must be managed properly. Crop production, food processing, distribution, and consumption are just a few of the interwoven aspects and procedures that make up the food system. The soil nutrients exhausted during crop production must be restored in order to guarantee productivity and security of the food chain (Leip et al., 2021). The management of byproducts or additional resources, known as side streams, within the food system, plays a role in minimizing waste and optimizing resource efficiency. (Gliessman, 2016; Meybeck and Gitz, 2017). Composting, anaerobic digestion, and recycling are effective waste management techniques that help reduce the environmental impact of disposing off food waste. By converting agricultural wastes into valuable components or biofuels, the usage of side streams can also aid in the creation of a more sustainable and circular food system. In addition to advantages for the environment, an effective food system also helps public health, livelihood, and other social and economic factors. We can work to build a more resilient, egalitarian, and sustainable food supply chain that advances both human well-being and the health of the planet by making improvements to the food system (Berry, 2019).

The FAO/WHO emphasized the necessity of resource efficiency and the significance of sustainable food production during the 1992 International Conference on Nutrition ("Nutrition and Development: Global Challenge 1", 1992). By 2050, the population of the world is expected to reach above 9.74 billion and significantly rising food demand, sustainable and efficient food production practices are crucial (Davis and White, 2020). As a necessary and limited ingredient for plant development, nitrogen is important for human nutrition and the production of sustainable food (Leip et al., 2021). However, an imbalance in nitrogen

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use can result in climate change, soil degradation, air and water pollution, and a loss of biodiversity (Rockström et al., 2020; Leip et al., 2015).

Nitrous oxide (N_2O), a powerful greenhouse gas with a global warming potential far higher than carbon dioxide, is one example of a molecule that contains nitrogen (Myhre et al., 2013). and the lifetime of the disturbance (the time required for the pulse release of H_2O to reach zero concentration) is 121 years (Timma et al., 2020). The main anthropogenic source of N_2O emissions are agricultural activities related to the use of synthetic fertilizers and the handling of livestock manure. Natural sources of N_2O emissions are soils, oceans, lightning, as well as inland and coastal waters. Atmospheric N_2O contributes not only to climate change, but also to the destruction of the stratospheric ozone layer at a rate increasing by 2% per decade (Tan et al., 2020) and a considerable rise of about 30% in N_2O emissions is responsible for about 10% of global yearly N_2O emissions. Important repercussions for global greenhouse gas emissions and climate change result from this increase in N_2O emissions. Compared to carbon dioxide (CO_2), nitrous oxide (N_2O) has a far higher warming potential. It is mostly released during agricultural processes like burning agricultural waste, using nitrogen-based fertilizers, and managing livestock manure. The observed increase in N_2O emissions of about 30% emphasizes the need for efficient strategies to lessen its impact on climate change. N_2O emissions can be decreased by employing practices like enhanced nitrogen management in agriculture, which includes the use of precision farming methods and optimal fertilizer delivery. Promoting organic matter management and soil health as priorities in agricultural practices can also help reduce N_2O releases from agricultural sources (Velthof and Oenema, 1997; WallisDeVries and Bobbink, 2017). It is estimated that over the period from 1750 to 2019, increase in greenhouse gases (GHG) concentration has contributed to global warming by about 0.15°C with an effective radiation exposure of about 0.3 W m^{-2}.

Inert dinitrogen (N_2 (nitrogen gas)) not only makes up the largest fraction of the atmosphere, but is also a very important element in the biochemistry of all existing life. Being the main component of amino groups and other amino acid constituents, nitrogen (N) is necessary as a building component of proteins. While complex organic compounds containing H and protein, are necessary for the diet of animals, primary producers, such as plants, may require nitrogen in various inorganic forms, such as ammonia (NH_3), ammonium (NH_4^+), nitrate (NO_3^-), and nitrite (NO_2^-) and organic compounds such as urea $CO(NH_2)_2$ (Harper, 2015). A complex network of biological and geochemical processes of oxidation and reduction that affect the transfer of nitrogen between the atmosphere, biosphere, soil and water is called the nitrogen cycle (Fowler et al., 2013).

To enhance plant growth and protein production, people use fertilizers. It has been repeatedly shown that the use of nitrogen alone or in combination with other nutrients can increase both yield and protein content in conventional crops such as wheat. Sometimes, existing nitrogen reserves in biomass are obtained by organic fertilization with manure, compost, or using the ability of some microorganisms to convert P_2 (phosphorus) into other nitrogen compounds. Mineral nitrogen sources or synthetically produced nitrogen fertilizers make a significant contribution to plant nutrients in agriculture (Erisman et al., 2008). However, some of these additional nitrogen compounds may enter water bodies as a result of excessive use or uncontrolled disposal of biogenic waste (Grizzetti et al., 2013), creating environmental problems such as harmful algal blooms, eutrophication and deterioration of water quality in some areas, as well as accelerating climate change and causing air pollution (Sutton et al., 2021). In places with intensive agricultural practices, related to animal husbandry, excess nitrogen can lead to a noticeable deterioration in the quality of groundwater and surface water. This was observed in the Netherlands due to an excess of introduced nitrogen (Enema et al., 2005).

In addition to environmental problems such as climate change and environmental degradation (Shpirts, 2009), the loss of nitrogenic compounds is a food security problem, being an integral part of a reliable protein supply. Nitrogen losses during disposal, seepage or in the form of ammonia into the air are the main sources of these losses. With a growing global population that has a growing demand for protein, the gap between protein production and demand will grow (Boland et al., 2013). This gap is being filled with synthetic fertilizers. They consume energy during their production and contribute to nitrogen losses in the environment (Liu B et al., 2021). Many researchers believe that this demand will not be met in a sustainable way using exclusively traditional methods, since arable land is limited, and many established methods of producing protein-rich foods, especially animal husbandry, are inefficient and cause environmental damage (Hilborn et al., 2018). In this area, increasing the efficiency of nitrogen use in agriculture by improving fertilizer application methods (Sikora J. et al., 2020), an increase in the intake of organic nitrogen into the soil, which strongly stimulates the growth of heterotrophic microbes (Sabir M. S. et al., 2021) or improving nitrogen uptake by plants (Vivia et al., 2021).

Direct human consumption using traditional or new plant-based diets (Sabate and Soret, 2014), as well as new methods of feed and food production (Rau et al., 2020) are of great interest to the scientific community, as well as to the food industry and consumers.

A good option may be to use available protein and other nitrogen compounds in available streams of organic material. Considering the possibilities of using the previously mentioned materials, the purpose of this article is to explore the possibilities of using nitrogen and describe the potential of its conversion technologies for the return of nutrients to food systems and for leveling the imbalances of the nitrogen cycle. As a first step, data on the protein content in various waste streams will be collected. Combined with inventories covering fluxes at the national and international levels, this will help future studies determine where large amounts of nitrogen are available. This will be the first indicator for selecting suitable technologies for more efficient use of these materials. Such technologies are based on biotechnological concepts, on the use of biological cells or organisms, such as algae, fungi and insects, designed to create high-quality biomass from by-streams that would be considered as waste. There may be more traditional technologies that have already been created and are finding industrial applications, such as the production and use of digestate and compost from by-streams. The use of biomass as an energy source or carbon and nitrogen for growing edible, protein-rich biomass is already possible without involving more limited resources in cycles (Fig. 1). This article will use a holistic approach to system analysis. This means that it will account for direct and indirect changes upstream and downstream from the main nitrogen reuse process. Nitrogen bound in various compounds and various material flows will be considered (Fowler et al., 2013). This paper discusses a participatory and integrated approach to managing nirogen flows through the food system to stay within the local and global nitrogen boundaries, as well as its synergistic contribution towards sustainable and healthy protein-rich diets for humans and animals, together with the need for formulating an effective nitogen policy and holistic legal framework to address environmental and circularity facts that have never been addressed in the literature.

2. The nitrogen cycle

In this section, the concept of the nitrogen cycle will be explained in more detail to illustrate its complexity and relevance to the food system and global ecosystems. Nitrogen is a key biogenic element in the world of plants and animals (Yang et al., 2017). Nitrogen on Earth exists in three forms: diazote (N_2), organic nitrogen bound to carbon (for example, in proteins), and nitrogen nutrients in the form of nitrogen ions or nitrogen oxides (Socolow, 1999). The Earth's atmosphere contains 78% nitrogen in the molecular gaseous form N_2, which is directly inaccessible to plants (Fields, 2004).

The presence of nitrogen in soil and water limits the production of primary biomass and the conversion of inorganic carbon into organic carbon (Duce et al., 2008). Atmospheric nitrogen is constantly

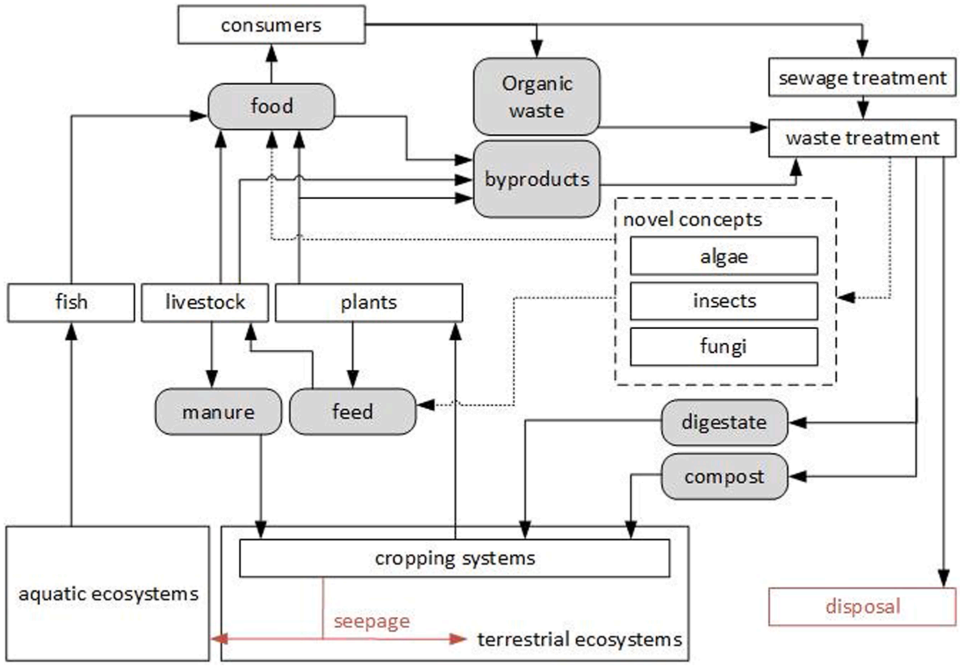

Fig. 1. Scheme of the relevant nitrogen flows influenced by humans and perspective for novel methods of return to the food nitrogen cycle (own figure).

supplemented by nitrogen compounds released from soils and waters in the forms NH_3 (volatilization of NH_4^+), NO_2^- (not contained in organic material) and N_2O and N_2 (denitrification), as well as lightning (Galloway et al., 1995).

In terrestrial ecosystems, the availability of nitrogen for photosynthetic organisms is limited by biological processes of ammonification and nitrification of organic matter into available forms of NH_4^+ and NO_3^-, as well as biological fixation of N_2. If nitrogen compounds in the soil are not absorbed by the soil sorption complex (NH_4^+), are incorporated into soil organic matter (NO_2^-, NH_4^+) or are absorbed by plants and microorganisms (NH_4^+, NO_3^-), then ionic nitrogen oxides (NO_2 and NO_3^-) are reduced by denitrification to gaseous oxides (NO and N_2O) and next to N_2 (Firestone, 1982), some effects of N_2 accumulation in the environment are presented (Galloway et al., 2003). The main Nr transformations of nitrogen from organic matter are associated with nitrogen alkalinization during mineralization (consumption of H^+), but this effect is compensated by nitrification (production of $2H^+$), and the combined process leads to acidification if NO_3^- (consumption of H^+) is not consumed (Table 1) (Welthoff et al., 2011).

N_2-related soil quality involves changes in soil organic content, soil acidification, loss of soil diversity by changes in the structure of soil organisms, and negative influence on food and biomass production and biodiversity. In the conditions of nitrogen over-fertilization, the microorganisms can change soil processes towards emissions of harmful N_r to water and atmosphere. In aquatic ecosystems the similar biological processes of nitrogen fixation involve biological conversion of nitrogen compounds in oxic and anoxic environments to the forms of dissolved inorganic nitrogen (DIN: NH_4^+ NO_3^-, NO_2^-), dissolved organic nitrogen (DON) and particulate organic nitrogen (PON) (Oljam D., 2018).

Only a few kinds of micro-organism can fix molecular nitrogen. The most important are species of Rhizobium in legume root nodules, Azotobacter, blue-green algae and Clostridium, although new species of free-living micro-organisms able to reduce nitrogen are being discovered almost yearly. The nitrogen-reducing enzyme system – nitrogenase –comprises two Fe-proteins, one also containing Mo. It equally well reduces acetylene (an isostere of nitrogen) to ethylene and this reaction is a convenient and widely used measure of nitrogenase activity. World shortage of food proteins is stimulating basic and applied research on nitrogen fixation, especially by forage and grain legumes, and on the many factors – genetic, physiological and environmental – that influence it; some of this work is supported by the International Biological Programme. Industrialization and modern agricultural technology have important impacts on the nitrogen cycle. The over-reliance on fertilizer nitrogen in highly intensive systems of crop production can be wasteful of nitrogen resources and lead to net energy losses (fuel-subsidized agriculture). Similar trends are also seen in the agricultures of some developing countries where legumes could most quickly and massively augment production of food proteins (Nutman, 1971).

Humans can both positively and negatively affect the supply of nitrogen to ecosystems. Sowing crops that fix N_2 and pastures can have a positive effect. The negative impact is caused by industrial nitrogen fixation, mainly in nitrogen fertilizers, and the use of internal combustion engines (McNeill and Unkovich, 2007). Most of the reactive nitrogen enters the environment, polluting waterways and the coastal zone, accumulating in terrestrial systems and adding a number of gases to the atmosphere (Rockström et al., 2009). Today, nitrogen emissions by humans exceed the global thresholds of safe planetary boundaries within which humanity can continue to develop (Steffen et al., 2015). Globally, agriculture, the burning of fossil fuels, the production of synthetic nitrogen fertilizers, the restriction of legume crops and a number

Table 1
Generation (acidification) and consumption (alkalinisation) of protons (H^+) in nitrogen transformation processes (Velthof et al., 2011).

Process	Reaction (R in the reactions means organic carbon compounds)	H^+, mol/mol N
Biological N-fixation	$4ROH + 2N_2 + 3CH_2O \rightarrow 4RNH_2 + 3CO_2 + H_2O$	0
Mineralization of organic N	$4RNH_2 + H_2O + H^+ \rightarrow 4ROH + NH_4^+$	-1
Urea hydrolysis	$(NH_2)_2CO + 3H_2O \rightarrow 2NH_4 + 2OH^- + CO_2$	-1
Ammonium assimilation	$ROH + NH_4^+ \rightarrow RNH_2 + H_2O + H^+$	+2
Nitrate assimilation	$ROH + NO_3^- + H^+ + 2CH_2O \rightarrow RNH_2 + 2CO_2 + 2H_2O$	-1
Ammonia volatilisation	$NH_4^+ \rightarrow NH_3 + H^+$	+1
Denitrification	$5CH_2O + 4NO_3^- + 4H^+ \rightarrow 2N_2 + 5CO_2 + 7H_2O$	-1

of other human activities have significantly increased and accelerated nitrogen circulation. This increases the availability and mobility of nitrogen in large areas around the globe, which leads to serious and long-term environmental, economic and social consequences (Vitousek et al., 1997). Current strategies and technologies are shown in the following sections.

2.1. (Bio)chemistry of nitrogen

The following section illustrates conversion processes of different nitrogen compounds with relevance in nature as well as industry. Transformation can help understand the nitrogen cycle and conceptualize intervention measures.

2.1.1. Haber-Bosch process

The development of a process for the reduction of nitrogen with hydrogen to form NH_3 (Eq. 1) was a milestone to produce fertilizer. The so-called Haber-Bosch requires a temperature of 400-500°C and 100-300 bar and an iron catalyst (Rouwenhorst et al., 2021). The current production exceeds 150 million tons globally (Kyriakou et al., 2020).

$$3\ H_2\ (g) + N_2\ (g) \leftrightarrow 2\ NH_3\ (g) \tag{1}$$

It should be noted that the Haber-Bosch process consumes 50% of the hydrogen produced in the world, which usually comes from fossil resources (Wang et al., 2018). To overcome dependence on fossil resources, the goal is to develop a process that is fully driven by renewable energy in an environment with water and air acting as reagents (Martin et al., 2019). New approaches to the formation of NH_3 are based on the extraction of hydrogen after the oxidation of methane to carbon dioxide and the subsequent reduction of nitrogen. It is reported that such an electrochemical process "can synthesize NH_3 with only 50% of carbon dioxide emissions and 25% of energy" compared to the usual Haber-Bosch process. This application took place in a single proton-ceramic membrane reactor based on $BaZrO_3$ and is at the pilot stage and has not yet been introduced into industry (Kiriakou et al., 2020 The conversion of methane into NH_3, the generation of environmentally friendly hydrogen from biomass (biomass to NH_3 conversion), and the utilization of renewable power (energy conversion into NH_3) were the three methods used to compare the processes for producing environmentally friendly NH_3. The first method includes turning methane, a powerful greenhouse gas, into NH_3, which lowers methane emissions while also making use of it as a resource. The second method focuses on producing ecologically friendly hydrogen from biomass and using it to create NH_3 later. This strategy encourages the use of sustainable and renewable feedstocks for the manufacture of NH_3. The third method, which reduces dependency on fossil fuel-based electricity generation and reduces carbon emissions, uses renewable electricity to power the creation of NH_3 (Rouwenhorst et al., 2021). We may assess various manufacturing methods' individual efficacy, effects on the environment, and economic feasibility by contrasting them (Ghavam et al., 2021). A more eco-friendly and energy-efficient NH_3 industry can be developed with the help of this analysis, which offers insightful information about the most efficient and sustainable methods for NH_3 manufacturing (Zhang et al., 2020). Their calculations showed that "the conversion of energy to NH_3 provides the highest efficiency of the system - more than 74%, which is much higher than the conversion of biomass to NH_3 (44%) and methane to NH_3 (61%)."

Once NH_3 has been obtained via greener routes, the same reactions can be performed, and the nitrogen species obtained as from NH_3 from conventional Haber-Bosch process. The reaction of NH_3 with CO_2 results in the formation of ammonium carbamate (CH_6N2O_2) which subsequently decomposes to urea and water. NH_3 can further react with nitric acid to form ammonium nitrate (NH_4NO_3).

2.1.2. Biological nitrogen conversion

N_2 is the most common compound found in the atmosphere, but its use in biological processes is limited, thus creating N scarcity in many ecosystems. N_2 conversion is carried out through nitrification (van Kessel et al., 2015), commamox (Stein, 2019), denitrification, ammomax (Tan et al., 2020), ammonification (Xia et al., 2018), mineralization (Nakayama et al., 2021), and assimilation (Mus et al., 2016) which are important steps in the nitrogen cycle.

2.1.3. Nitrification

Nitrification is a two-step biological process where microorganisms (from the *Nitrosomonas, Nitrosococcus* and *Nitrosospira* genera (Aakra et al., 2001), convert NH_3 to NO_2^-using NH_3 monooxygenase (Eq. 2.1) and hydroxylamine oxidoreductase (Eq. 2.2) enzymes.

$$NH_3 + O_2 \rightarrow NO_2^- + 3\ H^+ \tag{2.1}$$

$$NH_3 + O_2 + 2\ H^+ \rightarrow NH_2OH + H_2O \tag{2.2}$$

$$NH_2OH + H_2O \rightarrow NO_2^- + 5\ H^+ \tag{2.3}$$

In the second step, after $NO_2^-$is produced in nitrification process, it is converted through NO_2^-oxidizing microorganisms to NO_3^- using NO_2^- oxidoreductase enzyme (Eq. 3). Nitrification process is performed by ***Nitrobacter*** (Poly et al., 2008)***, Nitrospina*** (Sun et al., 2019)***, Nitrococcus*** (Füssel et al., 2017)***, Nitrospira*** (Daims and Wagner, 2018) and other genera.

$$NO_2^- + H_2O \rightarrow NO_3^- + 2H^+ \tag{3}$$

2.1.4. Comammox

Comammox (complete NH_3 oxidation) is a microbial process of complete conversion of NH_3 into NO_3^- by single nitrification process (van Kessel et al., 2015). Comammox based microorganisms can be used for NH_4^+ removal, agriculture biofiltration units, drinking and wastewater treatment (Fowler et al., 2018). The most common comammox organism is *Nitrospira*, whose genome encodes the biochemical pathways both for NH_3 and NO_2^-oxidation (Daims et al., 2015).

2.1.5. Denitrification

Contrary to nitrification, *denitrification* is a microbial specific respiration process and occurs in conditions when soil pore space is filled with more than 60% water, NO_3^- is found in terrestrial or aquatic environment and no oxygen is available. The microbial community reduces NO_3^- to various gases: NO_3^- reductase (Eq.4.1) creates nitrite (NO_2^-), nitrite reductases (Eq. 4.2) create nitric oxide (NO), nitric oxide reductase (Eq. 4.3) creates nitrous oxide (N2O) and nitrous oxide reductase (Eq. 4.4) - dinitrogen (N_2).

$$NO_3^- + 2H^+ \rightarrow NO_2^- + H_2O \tag{4.1}$$

$$NO_2^- + 2H^+ \rightarrow NO + H_2O \tag{4.2}$$

$$2NO + 2H^+ \rightarrow N2O + H_2O \tag{4.3}$$

$$N2O + 2H^+ \rightarrow N_2 + H_2O \tag{4.4}$$

In the denitrification process, microorganisms form 1 mole of N_2 and 6 moles of water from 2 mols of NO_3^-. Microbial community counts more than 125 species and represents 10 – 15% of the bacterial populations found in soil, water, and sediment. The most common ones are *Pseudomonas* (Carlson and Ingraham, 1983), *Alkaligenes* and *Bacillus* (Liu et al., 2003). Denitrification based microorganisms are used to remove the NO_3 from different industrial wastewaters (Law et al., 2012) and more.

2.1.6. Annamox

Anammox is a specific case of anaerobic NH_4^+ oxidation and is found in many microorganisms, which allows the reduction of NH_4^+ and NO_2^- directly into N_2 (Eq. 5):

$$NH_4 + NO_2^- \rightarrow N_2 + 2H_2O \quad (5)$$

This is possible because microorganisms have an additional membrane bound compartment inside the cytoplasm (Boumann et al., 2009). This is considered industrially relevant in wastewater nitrogen removal processes (You et al., 2020). Several other known anammox bacteria genera exist, such as *Brocadia, Jettenia, Kuenenia* and, *Anammoxoglobus* (Shen et al., 2013).

2.1.7. *Ammonification*

Often ammonification is considered a biological process where atmospheric nitrogen is converted into inorganic NH_4^+ and is often mislabelled as a mineralization process. Generally, two different ammonification processes exist: nitrogen fixation and assimilatory and dissimilatory NO_3^- reduction.

Nitrogen fixation is a chemical process where N_2 is converted into NH_3. Generally, microorganisms create two moles of NH_3 from 1 mole of N_2, at the expense of 16 moles of ATP (Eq. 6).

$$N_2 + 8H + 16ATP \rightarrow 2NH_3 + H_2 + 16ADP + 16Pi \quad (6)$$

Many existing aerobic microorganisms like *Azotobacter, Beijerinckia, Klebsiella* and Cyanobacteria, as well as anaerobic microorganisms like *Desulfovibrio* or *Clostridium* independently fix nitrogen. *Rhizobium, Frankia, Azospirillum* and others have evolved to fix nitrogen in symbiosis with plants.

Anaerobic assimilatory and dissimilatory NO_3^- reduction to NH_4^+, also known as nitrate/nitrite ammonification, is a chemical process which occurs in chemoorganoheterotrophic microorganisms using NO_3^- for respiration and reduces it to NH_4 in a two-step process ($(NO_3^- \rightarrow NO_2^- \rightarrow NH_4^+)$). The first step provides periplasmic NO_3^- reductase (Eq. 7.1) (Sparacino-Watkins et al., 2014), which reduces NO_3^- to respiratory NO_2^-. But in the second step, NO_2^- is conversed into NH_4^+ by cytochrome c NO_2^-reductase (Lam and Kuypers, 2011) (Eq. 7.2).

$$NO_3^- + 2H^+ \rightarrow NO_2^- + H_2O \quad (7.1)$$

$$NO_2^- + 6\ Reduced\_cytochrome\_c + 8H^+ \rightarrow NH_4^+ + 6\ Oxidized\_cytochrome\_c + 2H_2O \quad (7.2)$$

2.1.8. *Mineralization*

Mineralization is a biological process, where microorganisms and fungi (decomposers) convert amine or amide groups (of general formula $R\text{-}NH_2$) from organic matter into inorganic nitrogen forms like NH_3 and/or NH_4^+. All large organic parts before decomposition to macromolecules should be depolymerized. This process occurs in soil, sediments or water and depends on the microbial and fungal community composition found in the ecosystem (Fig. 2). For example, key proteases are developed by bacteria *Bacillus, Pseudomonas* and fungus *Pythium* sp., *Cladosporium* sp. (Vranova et al., 2013) and more.

2.1.9. *Nitrogen assimilation*

Nitrogen assimilation is a biological process, where plants, fungi and some bacteria are unable to use N_2 for their metabolism and depend on NO_3^-, NH_3 or NH_4^+ availability in the soil. Microorganisms and plants are capable of consuming a variety of nitrogen-based compounds like, NO_2^-, NO_3^-, NH_3, NH_4^+, CH_4N2O and also amine or amide groups, such as amino acids and many others, which will not be discussed in this review.

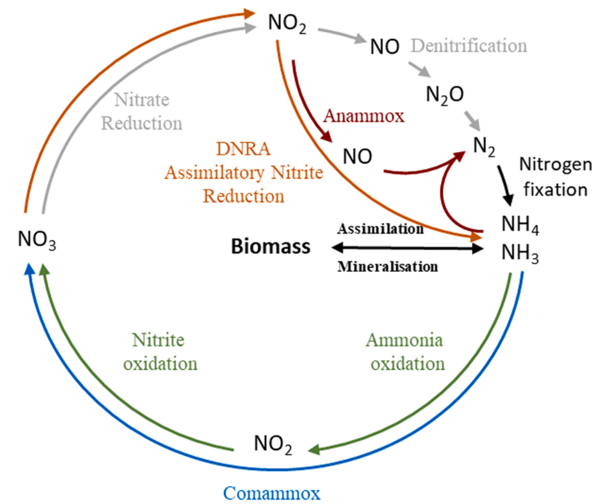

Fig. 2. Basic nitrogen cycle processes. Adapted from (Stein and Klotz, 2016).

3. Nitrogen-rich material streams

It is evident that residual streams have significant potential as a source of proteins. However, a quantified analysis of nitrogen streams is required to prioritize their use. In order to identify further potential of nitrogen recycling, the aim of this section is to identify material flows that present major sources of nitrogen pollution as well as potential substrates (Tables 2 and 3). In order to gain a more quantitative understanding of nitrogen wastage from solid or liquid material streams, information has been collected on such streams and their properties in terms of constituent nitrogen compounds and their concentrations. These data present a starting point for further analysis of nitrogen streams in the food system.

Table 2
Crops and by-products biomass nitrogen amount.

Crops	Other nitrogen compounds	Unit	References
Wheat root	20 – 30	mg/g plant	(Guo et al., 2019)
Wheat stem	2.5 – 4	mg/g plant	(Guo et al., 2019)
Barley leaf	20 – 75	mg/g FW	(Comadira et al., 2015)
Chickpea stem	5.5 – 8.1	mg/g DW	(Comadira et al., 2015)
Chickpea leaf	18.3 – 20.3	mg/g DW	(Comadira et al., 2015)
Lentil stem	8.5 – 14.3	mg/g DW	(Comadira et al., 2015)
Lentil leaf	14.6 – 17.5	mg/g DW	(Comadira et al., 2015)
Lupin stem	4.3 – 7.9	mg/g DW	(Comadira et al., 2015)
Lupin leaf	7.5 – 16	mg/g DW	(Comadira et al., 2015)
Pea stem	14.2 – 17.3	mg/g DW	(Comadira et al., 2015)
Pea leaf	23.3 – 28.6	mg/g DW	(Comadira et al., 2015)
Iceberg lettuce	2.3	mg g plant^{-1}	(Ziarati, 2012)
Romania lettuce	2.2	mg/g plant	(Ziarati, 2012)
Celery	3.2	mg/g plant	(Ziarati, 2012)
Spinach	3.6	mg/g plant	(Ziarati, 2012)
Cabbage	1.3	mg/g plant	(Ziarati, 2012)
Chinese cabbage	3.9	mg/g plant	(Ziarati, 2012)

Table 3
Major accumulating agricultural and municipal residues to be utilized by algae and insects, their protein content and total or NH_3-N contents as well as their current use.

Source of residues	Residues	Protein content [%, w w^{-1}]	Other nitrogen compounds	Current use	References
Agricultural residues	Fresh chicken manure	34.5	Total nitrogen 5.5% (w w^{-1})	Fertilizer	(Singh et al., 2018)
	Composted chicken manure	27.1	Total nitrogen 4.3% (w w^{-1})	Fertilizer	(Singh et al., 2018)
	Pig manure	-	Total nitrogen 3-6% (w w^{-1})	Fertilizer	(Wu et al., 2021)
	Cattle manure	-	N content of 1.04-1.78% (w w^{-1})	Fertilizer	(Leitner et al., 2021)
	Piggery wastewater	-	NH_3-N 1.4 g N L^{-1}, total nitrogen 1.0 g L^{-1}	Disposal	(Wang et al., 2012)
Municipal residues	Sewage sludge after anaerobic digestion	29.5	-	Incineration	(Pleissner et al., 2021)
	Food waste	10.0	-	Disposal	(Pleissner et al., 2014)
	Municipal solid waste compost	16	-	Nitrogen source in fermentation	(Izaguirre et al., 2020)

3.1. Agricultural and municipal residues

In agriculture harvesting and processing of many crops produces significant amounts of biomass in by-products. These have potential for secondary industrial bioconversion. Different crops and their structural components have different nitrogen contents, which can vary from 2.5 to 28.6 mg g^{-1} (dry basis) (Table 2).

Agricultural and municipal residues are produced in rural and urban areas respectively. The protein content can vary (Table 3) ranging between 10 and 29.5% (w/w), and utilization approaches should be designed in a way to make proteins available. A direct use as protein source is hindered by hygienic issues. However, conversion strategies via insects or microalgae are promising approaches (Nagdalian et al., 2018).

Nitrogen return of new and developing technologies to the food system should be analysed to decrease the negative impact of biomass of by-products, such as eutrophication or emission of NH_3 and NO_2 (Leip et al., 2021, 2015).

3.2. Food industry by-products

The by-products produced during food processing, which contribute to the overall make-up of waste streams in the food industry, will be covered in this section. It is significant to note that additional sources, such as side streams from farms, are not mentioned, even though this section concentrates on the specific by-products produced during food processing. First off, due to the enormous volume produced at processing facilities around the world, the production of by-products during food processing is a serious concern. These byproducts frequently come in a variety of compositions, some of which may contain useful ingredients like organic matter and minerals. We want to emphasize the potential of the by-products of food processing as valuable resources that may be further exploited rather than being viewed as trash by investigating them. These byproducts might include important proteins, fibers, oils, or bioactive substances that could be extracted or converted into components for a variety of uses in the food, feed, or pharmaceutical sectors (Caponio et al., 2022). By optimizing resource efficiency and limiting waste production, exploring the possibilities of these byproducts can help the food sector establish more circular and sustainable processes. In this section, the emphasis is on the by-products produced during food processing, with side streams from farms excluded. Agricultural residues and manure are examples of side streams from farms that are major sources of organic matter and nutrients, but they are normally treated separately due to their unique features and management techniques (Haldar et al., 2022). These side streams are frequently addressed in the context of nutrient recycling or agricultural waste management initiatives. The linkages between the food sector and agricultural production must be understood, though. The efficient use of side streams from farms should be included in the management of by-products in the food business, which should ideally be integrated with sustainable practices throughout the whole food supply chain. To create a more complete and sustainable circular food system, holistic techniques that consider both agricultural side streams and by-products of food processing are required (Galanakis, 2012).

3.2.1. Fruit and vegetable peel

During and after the processing of fruits and vegetables, side streams are generated. These side streams contain peels, seeds, skins and pomace (Sagar et al., 2018). The percentage of total yield ending up as these kinds of by-products depends on the type of fruit or vegetable. This is 35% for bananas, 50% for citrus, 20% for grapes and 15% for potatoes (Joshi et al., 2000). However, these streams still contain interesting molecules, such as polyphenols, dietary fibers, enzymes and proteins (Sagar et al., 2018). Since yearly production of citrus exceeds 120 million metric tons (MMT), the production of bananas 110 MMT, grapes 45 MMT, apples 80 MMT and the production of potatoes even exceeds 3800 MMT on annual basis (FAO, 2017), substantial amounts of side streams are generated. Next to household garbage, the processing of fruit and vegetable produces amps up to the highest amount of waste (Gowe, 2015).

3.2.2. Brewery grains

Brewery grains can be divided into brewer's spent grains and distillers' grains. Brewer's spent grains also called draff is a by-product in the brewing industry. This mainly consists of grain husks and is the solid residue after wort production. These spent grains include approximately 85% of the total by-products in the industry (Mussatto, 2014). Spent grains are usually composed of barley malt grain husks. Results of chemical composition of 15 studies were compared, which showed that the protein content of brewer's spent grains varies between 14.2 and 26.7% based on dry matter (Jackowski et al., 2020). The yearly production of brewer's spent grains in Europe ranges around 3.4 million tons (Steiner et al., 2015).

On the contrary, distillers' grains are a by-product of distillation processes and can further be divided into wet distillers' grains (WDG) and dried distillers' grains with solubles (DDGS). Production of DDGS from WDG is usually done by a drying mill (Lim & Yildirim-Aksoy, 2008). The most used grain is corn, although wheat, sorghum, rice and other grains can also be used. Depending on the types of grains used, the protein content of DDGS ranges between 26.6 and 44.0% based on dry matter, which emphasizes the potential in the feed industry (Lim & Yildirim-Aksoy, 2008).

3.2.3. Animal parts

Ever since the BSE (Bovine spongiform encephalopathy) outbreak in 2001, processed proteins derived from mammalian tissues have been banned within the EU (EG, 2000/766), with the exception as feed in pet feed and aquaculture (EU 142/ 2011, art. 13 & 24). There are a few exceptions however: the use of hydrolyzed proteins, collagen and gelatin or blood products derived from non-ruminants (or parts of non-ruminants) is allowed if it has been produced and placed on the market in accordance with the specific conditions laid down in section C of Chapter IV to annex IV of Regulation (EC) No 999/2001 (IPIFF, 2019). Blood meal used in feed production first has to be heat-treated at 100°C to kill possible pathogens (Mulik, 2014). Blood meal has a protein content of 90-95% (Mulik, 2014). Also, feather has got very significant nutritional value, as the protein content is around 90%. However, this consists mostly of keratin (Moritz and Latshaw, 2001).

3.3. Bygone foods/former foodstuff

Food waste from retail enterprises like supermarkets falls under this category. According to research, the primary causes of food loss in the retail sector are spoilage and expiration dates. It is essential to sort these items every day, even before they pass their "best by" date, to solve this problem. These products are frequently still safe to consume even if they can no longer be sold to customers (Horoś & Ruppenthal, 2021). It is important to recognize, however, that losses and waste in the food industry originate from sources other than retail, and they must not be ignored. For example, food waste can occur during the manufacturing, processing, and distribution phases along with the retail phase. Moreover, the majority of Sweden's 4% food loss or waste is ascribed to fruit and vegetable losses (Eriksson et al., 2012). This highlights the significance of seeking immediate solutions to resolve food waste accumilation at multiple levels of the supply chain. resulting in between 0.3 and 2 kg of waste per individual per year (Stenmarck et al., 2016).

Portion sizes, ignorance, logistical problems, attitudes, and knowledge are blamed for the bulk of food waste in hotels, restaurants, and non-profit catering businesses (Monier et al., 2010; Vinck et al., 2019). Food waste in catering is around 2.5 times more than food waste in retail, or 0.75 to 5 kg per person annually (Stenmarck et al., 2016).

Consumer food waste accounts for more than 50% of all food waste globally, with an average yearly food waste per person of 71 kilograms (Stenmarck et al., 2016). The majority ends up in waste bins, compost bins for home composting or in sewers (mainly liquid foods) (Stenmarck et al., 2016).

4. Nitrogen utilization systems

While some species have the extraordinary power to absorb various nitrogen compounds through the biochemical mechanisms discussed in earlier chapters, others have the potential to transform the protein content present in waste materials into biomass (Martínez–Hernández et al., 2018). The use of diverse species, such as farm animals, insects, algae, and fungus, to digest waste from different phases of the food cycle is explored in this section, showing their potential for achieving sustainable nitrogen management.

Insects and farm animals have long been used in agricultural systems to recycle nutrients. They are effective at turning nitrogen-rich waste, such food scraps and agricultural byproducts, into valuable items like meat, eggs, and insect biomass. These systems offer a sustainable source of protein and nutrients in addition to reducing waste (Meyer-Rochow and Jung, 2020).

The use of fungus and algae as substitute methods for using nitrogen has drawn increasing attention in recent years. Algae have the capacity to produce biomass that is protein-rich sustainably by converting nitrogen molecules into biomass through photosynthesis. On the other hand, fungi may use their enzymatic processes to break down complex organic substances, including nitrogen-containing waste products, and convert them into useable nutrition (Singh, 2021).

4.1. Farm animals

Various waste products of the food industry are used as animal feed, for example, potato peelings, bread and brewing waste. These flows are mainly characterized by a homogeneous composition, constant quality, low price and predictable quantity Caldeira et al. (2019) estimated that there are about 268 million tons (by wet weight) in the EU food residues from primary production and processing and processing industries are used for animal feed.

From a legal point of view, it is necessary to make a clear distinction between food losses and food waste in order to discuss their potential use for nitrogen extraction in animal feed (see Fig. 3). Food loss occurs before it reaches the consumer, as a result of unintended agricultural processes or technical limitations at the stages of production, storage, processing, packaging and distribution. In the EU, about 130 million tons of by-products are generated and 69 million tons of food is lost during the production, processing and distribution stages (Caldeira et al., 2019). Food waste is usually generated at the stages of retail trade and consumption in the form of food that is suitable for human consumption, but is not consumed because they are thrown away (Lipinski et al., 2013). About 60 million tons of food waste are generated in the EU (Caldeira et al., 2019). Since 2002, a ban has been imposed on food waste, which may potentially contain animal by-products for use in animal feed. More specifically, Regulation (EC) No 1069/2009 of the European Commission prohibits the use of catering waste in feed for farm animals, with the exception of fur-bearing animals, while catering waste is defined as "all food waste, including used vegetable oil, originating from restaurants, catering facilities and kitchens, including central kitchens and domestic kitchens". (Commission Regulation (EC) No. 142/2011). This means that all waste coming from the final stage of consumption of the food value chain is legally prohibited for use in animal feed.

On the other hand, reuse of food that is no longer intended for human consumption into/as animal feedstuff is seen as the third most preferable option for food surplus, by-products and food waste management after prevention and reuse in human consumption (JRC, 2020). Also, from an environmental and economic point of view feeding food waste to animals is more beneficial than e.g., energy generation via anaerobic digestion or composting (Shurson, 2020). European Union (EU) guidelines state that food waste should preferentially be used as animal feed though for most food waste this practice is currently illegal, because of disease control concerns (Salemdeeb et al., 2017).

In 2018, the European Commission published Guidelines on the Use of Food Products No Longer Intended for Human Consumption in Feed (EC, 2018), which contains clear guidance on legal actions to convert food waste into feed. The guidelines also include cases in which food products consisting of, containing or contaminated with animal products may be used. Here it is necessary to take into account the differences in the needs of animals in feed (diet) (herbivorous and omnivorous livestock), as well as the type of livestock (ruminants, non-ruminants, aquaculture animals, pets, fur-bearing animals). Some leftovers, such as feed containing milk, eggs, non-ruminant gelatin, are allowed as feed for all animals, while food containing fish can only be used for non-ruminant animals, domestic animals and fur-bearing animals; and food containing ruminant meat is only for domestic animals and fur-bearing animals. While feed producers and government organizations are hesitant to expand the possibilities of using food waste in feed due to disease control issues, several studies have been conducted to explore these possibilities (see, for example, the results of the EU REFRESH project (Luyckx et al., 2019)). (Shurson, 2020) argues that there is a huge potential for processing energy and nutrients from food waste into animal feed, for pigs and poultry, since they require a diet richer in energy and nutrients than for ruminants. A recent systematic review (Rajeh et al., 2021) suggests that modern technologies make it possible to process both food losses (by-products) and food waste into safe animal feed. The crude protein content in food waste is in the range

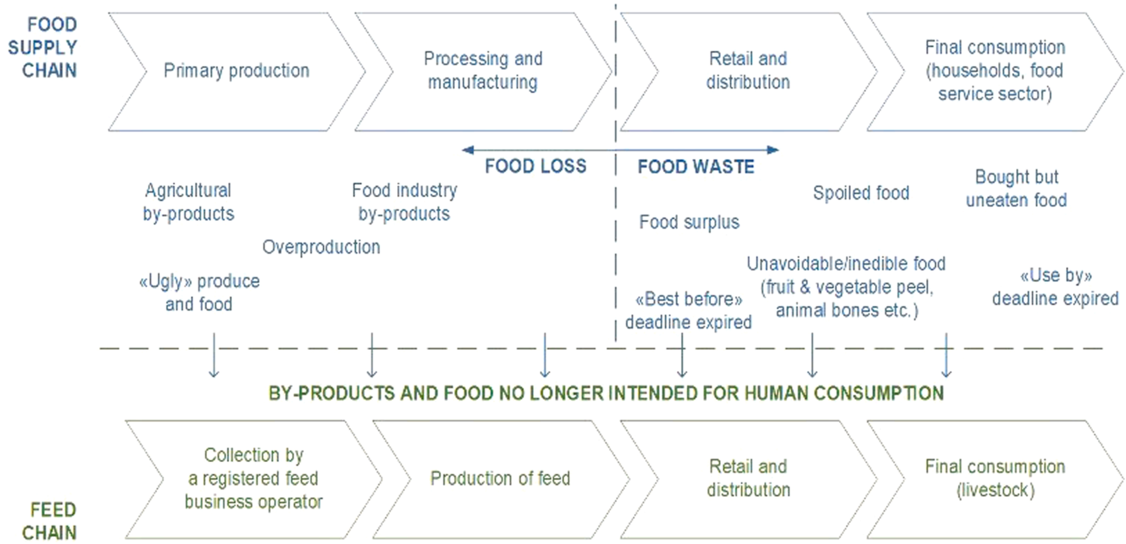

Fig. 3. Sources of food loss and food waste in the food supply chain and food entering the feed chain.

of 13-31%, which is much higher than in corn grain (8-10%). Nevertheless, it is possible to observe a high geographical and seasonal variability in the composition of food waste, therefore, the nutritional value also varies significantly.

Although feeding food waste to farm animals is a common practice in many regions of the world, in particular in the EU, it is currently limited. Nevertheless, since closed-loop bioeconomics occupies an important place on the EU agenda, interest in the potential processing of food waste into animal feed is growing (Liepins et al., 2021). The literature suggests that after overcoming technical barriers (such as proper separation, timely collection and delivery of food waste to the feed producer), systemic barriers (related to the variability of the nutritional profile of food waste) and administrative barriers (such as registration and compliance with almost zero tolerance to pollutants) associated with the current EU legislation has a high potential for cycle closures between food and feed supply chains (Broeze and Luyckx, 2019).

4.2. Insects

The focus of nitrogen utilization processes in this part will be on the yellow mealworm (*Tenebrio molitor*), the house cricket (*Acheta domesticus*) and the black soldier fly (*Hermetia illucens*) (limited with availability of data and research). The main factors in the nitrogen balance are nitrogen uptake and nitrogen excretion. And insects are not an exception. In contrary to vertebrates, nitrogen waste in insects is mainly composed of uric acid instead of CH_4N2O (Weihrauch and O'Donnell, 2021).

For nitrogen uptake, ingested proteins are enzymatically broken down into amino acids and oligopeptides prior to being absorbed by insects (Holtof et al., 2019). After absorption, these can be stored, used as an energy source, used for nucleic acid metabolism or used for protein synthesis (PRICE, 1973). Although chitin synthesis mainly relies on trehalose, nitrogen is incorporated as well, making it a notable factor in the nitrogen balance (Merzendorfer and Zimoch, 2003). The protein efficiency ratio represents the weight gain per gram of protein intake. For *Tenebrio molitor* this ratio is 2.20 (Giannetto et al., 2020), for *Acheta domesticus* it is 2.82 and for *Hermetia illucens* prepupae, this ratio is 3.4 (Poelaert et al., 2018).

For the insect proteins themselves, the true protein digestibility for *Tenebrio molitor, Acheta domesticus* and *Hermetia illucens* is 83.9%, 91.9% and 84.5%, respectively (Traksele et al., 2021). The PDCAAS (Protein digestibility-corrected amino acid score) is used to measure the nutritional quality of proteins, based on amino acid composition and digestibility (Poelaert et al., 2018). A PDCAAS of 1 is the highest, 0 the lowest. *Tenebrio molitor, Acheta domesticus* and *Hermetia illucens* have PDCAAS of 0.86, 0.84 and 0.75, respectively (Poelaert et al., 2018). Digestibility of the dried larvae protein was only 48%. The protein digestibility of the defatted larvae biomass reached 75%. (Traksele et al., 2021). Also, insects themselves are a great protein source, for example *Tenebrio molitor, Acheta domesticus* and *Hermetia illucens* have on average protein contents of 53%, 63% and 42% based on dry matter (Makkar et al., 2014). This makes them a promising protein source for the use in food and feed (Poelaert et al., 2018).

Multiple studies have shown that insect frass of *Tenebrio, Acheta* and *Hermetia* demonstrates a great potential as a fertiliser, which can enhance the circularity in agro- and feed industry (Butnan & Duangpukdee, 2021). Frass produced by the larvae of Hermetia illucens has the potential to recapture N and P from the food chain for reuse as a fertilizer, reducing the need for chemical fertilizers (Schmitt & de Vries, 2020). Because of its fast mineralization and high contents of readily-available nutrients, the effectiveness of, for example, mealworm frass is similar to supplying nitrogen, phosphorus and potassium and to sustain biomass production compared to synthetic NPK fertilizer (Houben et al., 2020).

The studies investigated show that the utilization of insects for novel foods is a good option for the recirculation of proteins of the material streams. The introduction of such applications is hampered by legal hurdles at the present stage.

4.3. Single-cell organisms: fungi and algae

The formation of protein from single-cell organisms has been investigated using a defined medium consisting of single carbon and nitrogen sources. The costs of pure nutrients, however, challenge the implementation of economically feasible production processes. To minimize the cost of nutrients, complex 2nd or 3rd generation biomass to be used as nutrient source have been studied. In Table 4, a couple of examples of single-cell fungi and algae are listed that are able to convert various residues and to accumulate a considerable amount of proteins in biomass.

Fungi are superior organisms as they can secrete enzymes and to degrade a range of even recalcitrant substrates. Zhou et al. investigated the utilization of orange waste rich in pectin and crude fibers (Zhou et al., 2019). The aim was to create protein-rich feed using the three fungal strains *Aspergillus oryzae, Trichoderma koningii* and *Candida tropicalis.* At larger scale 30 L of mixed and sterilized substrate (70%

Table 4
Single-cell fungi and algae species grown on residues for protein formation.

Species	Residues used as culture media	Protein content [%, w w^{-1}]	Reference
Fungi			
Aspergillus oryzae, Trichoderma koningii* and *Candida tropicalis	Orange waste	50	(Zhou et al., 2019)
Candida utilis	Straw	53	(Voutilainen et al., 2021)
Paecilomyces variotii	Grass silage fibers	51	(Pihlajaniemi et al., 2020)
Algae			
Lagerheimia longiseta	Compost	50	(de Medeiros et al., 2020)
Monoraphidium contortum	Compost	50	(de Medeiros et al., 2020)
Scenedesmus quadricauda	Compost	50	(de Medeiros et al., 2020)
Chlorella vulgaris	Agro-waste digestate	35	(Koutra et al., 2021)
Chlorella vulgaris	Municipal organic waste digestate	35	(Koutra et al., 2021)
Chlorella pyrenoidosa	Food waste hydrolysate	30	(Pleissner et al., 2017)
Chlorella vulgaris	Food waste hydrolysate	Ca. 20	(Lau et al., 2014)
Galdieria sulphuraria	Food waste hydrolysate	-	(Sloth et al., 2017)
Galdieria sulphuraria	Digestate and straw	Ca. 40	(Pleissner et al., 2021)

moisture rate, w/w) was inoculated with seed solutions of *A. oryzae, T. koningii* and *C. tropicalis*, and the solid-state-fermentation carried out at 33°C and a pH of 5.52. Within 96 hours a protein content of 17.99% was achieved, while pectin and fibers were continuously degraded with an efficiency of 1.54% and 1.15% between 84 and 96 hours. The produced feed is suitable for chicken and pig. Such an approach is highly relevant as 8×10^9 - 2×10^{10} kg orange waste is produced annually. Voutilainen et al. compared the food protein production with single-cell organisms from agricultural residues (Voutilainen et al., 2021). In their conceptual level techno-economic analysis, four concepts were investigated (three single-cell proteins from *Paecilomyces variotii, Candida utilis*, and *Fusarium venenatum* and one recombinant protein process). The process included steam explosion pretreatment, enzymatic hydrolysis, fermentation, and downstream processing. They show a Sankey (i.e. material flow) diagram that illustrates that 3.434 tons of *Candida utilis* biomass with a protein content 50% (w/w) can be formed from 40,000 tons of straw per year under the use of 150 tons of enzymes, 392 tons of sulfuric acid, 320 tons of sodium hydroxide and 537 tons of NH_3. In accordance with the authors "this study shows considerable potential for food protein production from a lignocellulosic feedstock via cellular agriculture, indicating that the high value of food protein offsets the high costs of lignocellulosic sugar production."

Grass silage is another resource which has been tested as substrate for the formation of protein-rich fungal biomass. Pihlajaniemi et al. used the filamentous fungus *Paecilomyces variotii* to convert hydrolyzed grass silage into a biomass with a protein content of 51% (w/w) (Pihlajaniemi et al., 2020). The grass silage contained around 5% (w/w) proteins. Prior to experiments grass silage was pressed and water extracted to separate proteins and carbohydrates. The carbohydrates were sent to NH_3 pretreatment or steam explosion. The hydrolysate was treated with commercial cellulase Flashenzym Plus to make sugars available as carbon sources. Around 66% of the applied NH_3 could be recovered. The remaining NH_3 served as nitrogen source for *P. variotii* during cultivation.

The marine microalga *Crypthecodinium cohnii* is well known for the accumulation of lipids and for its high content of the polyunsaturated fatty acid docosahexaenoic acid (DHA) (Pleissner and Eriksen, 2012). Cultivations, however, have predominantly been carried out in presence of pure nutrients (de Swaaf et al., 2003). Karnaouri et al. investigated the cultivation of *C. cohnii* in presence of the lignocellulosic biomass beechwood pulp (Karnaouri et al., 2020). After an organosolv pretreatment the authors obtained a cellulose-rich fraction which was sent to enzymatic hydrolysis to obtain free sugars. It is of interest that *C. cohnii* did consume the sugars obtained and accumulated up to 43.5% (w/w) DHA in its biomass. Even though the focus was not on protein formation, the results by Karnaouri et al. revealed the possibility to use lignocellulosic residues as carbon source for the cultivation of *C. cohnii*. This is of interest because *C. cohnii* can accumulate 12-15% (w/w) proteins (Pleissner & Eriksen, 2012). The nitrogen source, which was yeast extract in experiments performed by (Karnaouri et al., 2020) needs to be replaced to allow a further reduction of production costs.

De Medeiros et al. followed the concept and investigated the use of residues (bio-compost of discarded fruits and vegetables) as substrates for the microalgae *Lagerheimia longiseta, Monoraphidium contortum*, and *Scenedesmus quadricauda* under phototrophic conditions (de Medeiros et al., 2020). The compost used in their studies did contain a couple of micronutrients such as calcium, iron, and potassium as well as nitrogen (1.68 g kg^{-1}) and phosphorus (1.25 g kg^{-1}). The culture medium was produced from maturated compost (120 days at 45°C). 1 kg of compost was added to 1 L of distilled water, and the suspension was heated for 30 min, filtered, and finally sterilized. The obtained culture medium contained 63.7 mg L^{-1} NO_3^-, 0.475 mg L^{-1} NO_2^-, 1.3 mg L^{-1} NH_4^+, and 31.6 mg L^{-1} phosphate. *L. longiseta* did show a biomass productivity of 25.35 mg L^{-1} day^{-1}, *M. contortum* 16.53 mg L^{-1} day^{-1}, and *S. quadricauda* 33.73 mg L^{-1} day^{-1}. The biomass contained on an average around 50% (w/w) proteins. The authors concluded "that *S. quadricauda* and *L. longiseta* have significant potential for distinct applications in functional food industries, and the biocompost of discarded fruits and vegetables is a suitable medium for microalgae cultivation." (de Medeiros et al., 2020).

The microalga *Chlorella vulgaris* was used by Koutra et al. to valorize liquid digestate from agro-waste, cheese whey and municipal organic waste digestate (Koutra et al., 2021). The carbohydrate concentrations were 0.57 g L^{-1}, 39.0 g L^{-1}, and 0.06 g L^{-1}, respectively, and the total nitrogen concentrations were 4.88 g L^{-1}, 0.81 g L^{-1}, and 0.30 g L^{-1}, respectively. Orthophosphate was present at 0.09 g L^{-1}, 0.24 g L^{-1}, and 0.02 g L^{-1}, respectively. Heterotrophically grown *C. vulgaris* in municipal solid waste digestate reached a protein content of 35% (w/w). The same content was found for *C. vulgaris* grown phototrophically in a mixture of 50% w/w agro-waste digestate and 16% w/w cheese whey. Food waste hydrolysate has been investigated as a nutrient source for the heterotrophic cultivation of *Chlorella pyrenoidosa* (Pleissner et al., 2017), *C. vulgaris* (Lau et al., 2014), and *Galdieria sulphuraria* (Sloth et al., 2017). In all three approaches wasted food consisting of noodles, rice, meat, and vegetables was enzymatically digested and the released sugars and amino acids converted via the three microalgae in algal biomass. In continuous flow cultures of *C. pyrenoidosa,* a protein content of around 30% (w/w) was reached and *C. vulgaris* reached a protein content of around 20% (w/w). *G. sulphuraria* has further been found to grow in a nutrient medium consisting of digestate after proteolytic treatment and straw hydrolysate. The growth rates found were between 0.6 and 0.9 day^{-1} and the protein content around 40% (w/w) (Pleissner et al., 2021).

5. Analysis and discussion

It is well-known that excess of reactive nitrogen presents a range of issues to the environment, and by extension – also to societal well-being and human health. The importance of nitrogen as a building block for protein and for a nutritious diet is obvious. Here, a distinction between atmospheric N_2 and N_r is appropriate. Whilst the former neither has negative environmental impacts nor is useful in nutrition, the latter can be both environmental danger and, in the right chemical forms, limited resource. Of course, the Haber-Bosch process is an established method for producing NH_3 and on that basis – fertilizers which have ensured agricultural productivity for decades. But, even allowing for the reductions of the environmental impact of the process itself, this approach alone presents issues. It has long been known that anthropogenic N accumulates in different sinks (Galloway et al., 1995).

These circular models are to some degree implemented by making organic nitrogen available for assimilation through mineralization processes, e.g., through composting or anaerobic digestion and spreading compost on fields. Whilst presenting an important part of natural and anthropogenic nitrogen cycles and nitrogen recirculation, it cannot be considered the most efficient way to utilize protein. Implementing "short" cycles of N recirculation from underutilized material streams back to food as close to the point of consumption as possible.

It is essential to bring nutrients back onto fields. Whilst some of the N needed can be provided by crops or sustainable use of synthetic fertilizers, some nutrients must remain available for plant production. Furthermore, not all materials are equally usable as protein sources. Selecting the most suitable residue streams should be a priority for implementing sustainable pathways of nitrogen return to food systems. As presented in Section 5, protein content and availability, hygienic issues, production locality with influence on logistics and importantly legal status are to be considered.

The practicality of such protein reuse depends also on the approach used. Some organisms capable of producing nutritious food from proteins are presented in Table 4. Whilst farm animals present a practically proven and favorable option (JRC, 2020), not all residues are suitable diets for all livestock and use of some materials, depending on legal status, is severely restricted in locations such as the EU. Insects may present another option due to good protein efficiency ratios and protein digestibility. Since their frass is also usable as a high-quality fertilizer (Houben et al., 2020), they may help closing the loop on plant nutrient N at least as efficiently as farm animals.

Single cell organisms are another option under research but mostly still at lab scale or under conceptualization. The ability of some fungi to digest high-fiber feeds and enhance their protein content may broaden the choice of usable residue streams. This includes high-fiber materials otherwise not usable in food or feed. This approach brings together utilization of challenging waste streams, protein production for food and feed and reduction of costs for fermentation as well as lignocellulosic sugar production. Algae may be less capable of breaking down organic matter, but some strains provide highly nutritious biomass. Furthermore, some strains have been proven on complex organic substrates, including highly digested substrates with broken down forms of protein and inorganic N-compounds. Utilization of residues such as digestates under controlled conditions may be a novel option stemming from this technology. Livestock have a large impact on nutrient cycles, with repercussions on environmental and public health issues (Gerber et al., 2014). Opportunities to reduce nutrient inputs by improving the efficiency of manure use in agriculture were assessed (McCrackin et al., 2018). Although modern agriculture has separated crop production and animal husbandry, they are linked through efficient nutrient use (Swaney et al., 2018). This connection promotes the renewal of used nutrients, but today not all nutrients are used in recycling. Further closing this gap in the cycle, the methods discussed in this paper make another useful contribution.

To sum up, utilization by animals, including farm animals and insects for novel foods, is a technically readily available option for protein recirculation from some residue streams. However, there are remaining challenges on the legal side. Fungal and algal fermentations may make more materials available for recirculation and in some cases present particularly nutritious feed and food, but still face challenges in upscaling and economics of the processes.

The nutritional value and protein level of various waste streams and by-products, such as agricultural residues, food processing waste, and post-consumer food waste, require more study. The prospective sources of protein that can be effectively used and incorporated into food systems may be found with the aid of this research. To increase biomass output and nutrient recovery, research should concentrate on enhancing the conversion technologies for nitrogen-rich waste materials, such as algae, fungus, and insects. This entails investigating various cultivation methods, procedure variables, and scaling up of these technologies. It is crucial to assess how implementing these nitrogen recirculation techniques would affect the environment and society. To help with decision-making and policy creation, research should evaluate total sustainability, including carbon footprint, water use, and economic viability.

Formulating new laws and regulations that encourage the use of waste streams that are high in nitrogen in food production systems. This may include providing subsidies or incentives to encourage the adoption of nitrogen recirculation technologies and the development of waste collection, processing, and distribution infrastructure is an absolute necessity. Incentivize joint efforts between governmental organizations, academic institutions, and the corporate sector to fund investigation and development in nitrogen recirculation. This can be accomplished through financing initiatives, information exchange platforms, and public-private partnerships in order to expedite the adoption of sustainable protein production systems.

Customers should be upskilled on the benefits of employing nitrogen-rich waste sources and sustainable protein synthesis.

5.1. Consumer acceptance of alternative meat products and strategies to improve the acceptance

In recent years, the significance of entomophagy (Practice of eating insects) has been growing. As insects are typically rich in protein, they are generally regarded as meat alternatives. In Western countries, however, the consumption of meat substitutes is actually quite low, primarily due to food neophobia and inferior sensory qualities in comparison to meat. For instance of insects, food neophobia is unquestionably prevalent (Cardello et al., 1985, Hoek et al., 2013, Pelchat and Pliner, 1995, Tuorila et al., 1998), explored that information on proper use, positive flavor or similarity to familiar food ("tastes like food X"), and exposure over time aid in the acceptance of these unfamiliar foods. The first step in reducing food neophobia is to introduce the substitute within the context of a meal to enhance familiarity with the product (Elzerman et al., 2011). Concerning product-related factors, a lack of sensory appeal is a major barrier to meat substitute adoption among non-vegetarian consumers (Hoek & Hoek, 2011). Lastly, occasional consumers of meat substitutes recognize ethical (in terms of animal welfare or environmental impact) or nutritional aspects of these products, but this recognition is insufficient to compensate for negative attitudes and beliefs toward them (De Boer et al., 2013a, Hoek et al., 2011, Tucker, 2014).

Despite the nutritional benefits, environmental benefits with other benefits such as high fecundity rates, with year round breeding, high conversion rates, low environmental impact due to low rate of green house gas emissions, small breeding space requirements and the ability of certain species to recycle organic agriculture and industrial by-products to feed human or livestocks (Bednárová et al., 2013, Defoliart, 1995, DeFoliart, 1997, Rumpold and Schlüter, 2013a, Rumpold and Schlüter, 2013b, Van Huis, 2013, van Huis et al., 2013, Yen, 2009, Yi et al., 2013) in Western countries, insect food neophobia is well established and may be explained by knowledge of the animals' origins and habitats or by anticipated negative post-ingestional consequences (Caparros Megido et al., 2014; Rozin et al., 1999; Schösler et al., 2012; Verbeke, 2015).

A first possible strategy for reducing insect neophobia is to educate consumers about the cultural, nutritional, and ecological constraints associated with entomophagy; however, multiple studies have shown that this method is ineffective (Lensvelt and Steenbekkers, 2014, Mignon, 2002, Verbeke, 2015). A second option is to increase the frequency of edible insect exposure and testing (Caparros Megido et al., 2014). People who have eaten insects have more favourable attitudes towards entomophagy and are more inclined to eat and cook insects in the future (Caparros Megido et al., 2014; Lensvelt and Steenbekkers, 2014). Nonetheless, the incorporation of insects into a preparation (e.g., pizza with insect protein or biscuit with insect flour) and the association of insects with known flavours (e.g., insects coated with paprika or chocolate) appear to elicit less aversion than the presentation of visible and unflavored insects (Caparros Megido et al., 2014, Lensvelt and Steenbekkers, 2014, Schösler et al., 2012, Tan et al., 2016).

According to the expert interviews, efforts to increase insect consumption should consider the flavour and appearance of insect meals. Some of the experts have found that beautifully presented and served insect food increases the desire to consume them, while others have either consumed insect food prepared by a restaurant professional or prepared tastier insect food meals for others (Halonen et al., 2022). To increase the availability of high-quality insect-based foods in restaurants, restaurant kitchens require chefs who can adequately prepare standard insect-based foods. Insect-based food preparation should therefore be incorporated into restaurant training and education, according to some experts. Moreover, the function of chain restaurants in popularising insect consumption should be considered. In addition to their use as a food source, insects may also be utilised for purposes such as animal feed and pet nourishment. Future insect-industry development should also take into consideration the use of insects as animal feed, as nearly all of the experts concurred. Thus, insects that are suitable for human consumption and easy to farm, such as house crickets, mealworm larvae, and honeybee larvae, could be used for human consumption, while insects that humans cannot consume due to neophobia or regulations, such as black soldier flies, could be used as animal feed (Halonen et al., 2022).

Consumer acceptability of innovative protein sources produced from waste streams can be enhanced through the educational campaigns and new communication platforms. Encourage knowledge sharing and skill building among farmers, food producers, and other stakeholders to increase their proficiency with nitrogen recirculation technology and the benefits it may offer. This can be strengthened through training programs, seminars, and via knowledge sharing website.

5.2. Regulations on insects as food

The development of the insect industry is somewhat hampered by obsolete food and feed regulations governing insect use. In addition, the regulations prohibit the use of all potential insects as food and feed. Legal issues may arise at different phases of production, processing and marketing chain. Insects can thrive on various substrates, including manure and bio-waste, but introducing them into the food chain poses risks. Novel insects as food and feed may be riskier than familiar insects. How product safety regulations can be used to mitigate the risks posed by insects is the primary legal issue associated with insects. If specific government standards are lacking, businesses may decide to rely on existing industry practises or private standards as a guide, or they may decide to postpone further business investments until clear rules are being established (Lähteenmäki-Uutela et al., 2021).

Since 2018, provisions setting insects within the scope of Regulation (EU) 2015/2283 on novel foods have been applicable in terms of insects as food for human consumption. Under this new regulation, insect-based food products may only be marketed after the European Food Safety Authority (EFSA) has authorised their safety. At this time (October 2020), applications for food products from the following insect species have been submitted: house cricket (Acheta domesticus), banded/tropical house cricket (Gryllodes sigillatus), lesser mealworm (Alphitobius diaperinus), black soldier fly (Hermetia illucens), honey bee (Apis mellifera), migratory locust/grasshopper (Locusta migratoria), and yellow mealworm (Tenebrio molitor) (EC, n.d.). A transitional period is in effect for whole insects and their preparation pending EFSA assessments and subsequent Commission decisions on novel food applications. This means that insect foods that were lawfully marketed on January 1, 2018, and for which an application or notification was submitted by January 2019, may continue to be marketed until the Commission reaches a decision regarding the respective application. This implies that a number of insect species may continue to be sold as food in Europe without the approval as a novel food. On October 1, 2020, the European Court of Justice ruled in case C-526/19 that whole insects were not covered by the previous Novel Food Regulation. This necessitated the extension of the transitional measures for whole insects to all EU countries.

Currently, specific hygiene regulations for insects intended as a human a food source are under consideration: a draught Regulation amending Regulation (EC) No 853/2004 was published for public comment in 2018 (Ares(2019)382900). Currently, specific hygiene regulations for insects intended for human a food source are under consideration: a draught Regulation amending Regulation (EC) No 853/2004 was published for public comment in 2018 (Ares(2019)382900). The feedback period ended in February 2019, and adoption is slated for the first quarter of 2019. The proposed amendment would add a new section to Annex III of Regulation No. 853/2004. The substance of the proposed Regulation does not introduce any new provisions for insects; rather, it reaffirms rules from numerous other statutes that were already in effect. Specifically, insects must be authorized as a novel food under Article 3 of the proposed Regulation (EU) No 2015/2283. In addition, insects may only be raised on substrates of vegetable origin or specifically permitted materials of animal origin, such as fishmeal and hydrolyzed proteins from non-ruminants (Article 4 of the proposed Regulation), but this was already the case under Regulation (EC) No 1069/2009 and No 142/2011. Lastly, according to the proposed Regulation (Article 5), the "substrate for feeding insects must not contain manure, catering waste, or other waste"; however, this was already in the case under Regulation (EC) No. 1069/2009 (.Lähteenmäki-Uutela et al., 2021).

6. Conclusions and future perspectives

The presence of nitrogen in soil and water limits the production of primary biomass and the conversion of inorganic carbon into organic carbon. Future trends clearly predict an increase in meat production, and potential technologies used for nitrogen processing in food chains need to be reviewed. However, farming methods should be optimized to make more efficient use of the nitrogen contained in these materials and reduce waste and environmental pollution. Particular attention should be paid to residual streams that are currently being disposed of, such as sewage, sediment and food waste. An additional source of protein can be by-products, such as underutilized parts of plants, which can serve as raw materials for bioconversion processes.

The beneficial effects of nitrogen recovery have resulted in the development of an assortment of novel technologies. Electrochemical and bio-electrochemical methods, traditional stripping procedures (ammonia air stripping), struvite precipitation techniques, and membrane separation processes constitute some of these technologies (Beckinghausen et al., 2020; Righetto et al.,2022; Ye et al., 2022). Combinations of these technologies with varying degrees of advancement have also been acknowledged in the literature. Membrane separation techniques stand out among these technologies due to their maturity, practicability, and comparatively low energy need. Consequently, the application of various membrane technologies to recover nitrogen from various effluent streams would be a future direction of research with the objective of recirculating nitrogen into the system. Nitrogen can typically be recovered from waste streams as enriched streams, high-purity ammonium compounds, or biomass (e.g., algae). Pressure- and osmotically-driven membranes, in addition to electro- and biologically-enhanced membranes, generate enriched nitrogen streams. High-purity ammonium solutions have been generated by thermally-driven membranes and gas permeable membranes (GPM). Through photobioreactor membranes (PBRM) processes, nitrogen- and carbon-rich biomass can be obtained. Large-scale implementation of membrane technologies as standalone or hybrid systems that produce marketable nitrogen products along with energy and high-quality water is likely to be considered. The most promising of all membrane recovery technologies is GPM. Hybrid systems based on GPM that have been suggested in the literature and proposed in this study are likely to overcome the scalability barrier and achieve industrial implementation before other technologies. More consideration should be given to the feasibility of recovery technologies, and the requirements and priorities of the wastewater industry as well as the potential future consumers of recovered nitrogen products ought to be constantly kept in mind (Al-Juboori et al., 2023).

It has been determined that achieving greater synchrony between N supply and demand across a wide variety of cropping systems and environments in which crops are cultivated is the best way to simultaneously meet crop N requirements and protect environmental quality. However, it was challenging to locate reliable and relevant field data on N fertilizer efficiency (NFE, also known as recovery efficiency of fertilizer N) of major food crops, as measured by the proportion of applied N that was absorbed by the crop. Such data are essential for monitoring and mapping N losses in order to recognize crops and regions with the greatest potential or regions where progress has been made.

Despite the magnitude of the challenge, we are optimistic that damage to the environment and human health issues caused by N losses from modern, high-yield agriculture can be eradicated within 30 years. But our confidence is contingent on policymakers having the foresight to make adequate R&D investments with a ruthless focus on accelerating yield growth of major food crops on existing farmland while simultaneously increasing NFE, as well as transparent environmental performance standards and robust, low-cost metrics that enable farmers to monitor progress towards these standards. To meet this grand challenge, policies must include adequate public investment in education and human resource development, as well as free public access to high-quality data on soil properties, historical and real-time weather data, and water resources at a spatial resolution sufficient to drive innovation toward ever-more-precise crop and soil management in farmers' fields to maximize productivity and avoid harmful impacts on the environment (Cassaman & Dobberman, 2021).

Traditional nitrogen processing technologies in the food system are focused on the return of nitrogen to the soil or to animal feed. Composting, anaerobic digestion, scattering of manure and residues in the fields are aimed at providing crops with the necessary nitrogen.

Biotechnical solutions, including insect cultivation, can be applied to develop "small cycles" when nitrogen is returned to the food system near the place of consumption (consumer). The cultivation of microalgae, unicellular proteins and insects on the remains of agricultural products creates stable prerequisites for the effective use of nitrogen. Organic residues can potentially serve as a source of proteins. Residues low in nitrogen and protein, but rich in carbon, may also contribute to protein consumption in the future. With the help of bioconversion strategies using unicellular organisms or insects, it is possible to obtain a biomass rich in nitrogen (proteins). At the same time, such technologies are currently only available on a laboratory scale, and industrial development is limited due to obstacles associated with consumer adoption, economic costs and legal barriers. It is necessary to provide a broader research base confirming the scale of potential applications of conversion technologies when nitrogen can be processed in safe and economically feasible conditions.

CRediT authorship contribution statement

Shahida Anusha Siddiqui: Conceptualization, Methodology, Validation, Data curation, Writing – original draft, Writing – review & editing, Visualization, Project administration, Investigation, Resources. **Daniel Pleissner:** Methodology, Writing – original draft, Validation, Writing – review & editing. **Agris Pentjuss:** Methodology, Writing – original draft, Writing – review & editing. **Janusz Gołaszewski:** Methodology, Writing – original draft, Writing – review & editing. **Anna Karwowska:** Writing – original draft, Writing – review & editing. **Elina Dace:** Methodology, Writing – original draft, Writing – review & editing. **Maximillian Pahmeyer:** Writing – original draft, Writing – review & editing. **Sabine Van Miert:** Methodology, Writing – original draft, Writing – review & editing. **Lotte Frooninckx:** Methodology, Writing – original draft, Writing – review & editing. **Laurens Broeckx:** Writing – original draft, Writing – review & editing. **Volker Heinz:** Validation, Writing – review & editing. **Sergiy Smetana:** Conceptualization, Writing – review & editing, Validation, Supervision, Resources, Funding acquisition.

Declaration of Competing Interest

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

Data availability

No data was used for the research described in the article.

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