Biogeosystem Technique

Journal is being issued since 2014.
E-ISSN 2413-7316
2021. 8(1). Issued 2 times a year

Journal is indexed by: **Cross Ref** (USA), **Electronic scientific library** (Russia), **MIAR** (Spain), **Open Academic Journals Index** (USA), **CiteFactor – Directory of International Reseach Journals** (Canada).

All manuscripts are peer reviewed by experts in the respective field. Authors of the manuscripts bear responsibility for their content, credibility and reliability.
Editorial board doesn't expect the manuscripts' authors to always agree with its opinion.

Postal Address: 1367/4, Stara Vajnorska str., Bratislava – Nove Mesto, Slovak Republic, 831 04

Release date 25.06.2021
Format 21 × 29,7/4.

Website: http://ejournal19.com/en/index.html
E-mail: kalinitch@mail.ru

Headset Georgia.

Founder and Editor: Academic Publishing House Researcher s.r.o.

Order № B-19.

Biogeosystem Technique

2021

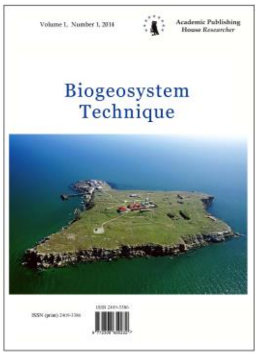
Is. 1

C O N T E N T S

Articles

Published in the Slovak Republic
Biogeosystem Technique
Has been issued since 2014.
E-ISSN: 2413-7316
2021. 8(1): 3-33

DOI: 10.13187/bgt.2021.1.3
www.ejournal19.com



Articles

Is Inorganic Nitrogen the Normal Plant Fertilizer? Or Do Plants Grow Better on Organic Nitrogen? (Critical Review)

Anton O. Nigten [a, *]

[a] Salt of the Earth, Wageningen, Netherlands

Paper Review Summary:
Received: 2021, February 20
Received in revised form: 2021, June 22
Acceptance: 2021, July 2

Abstract

Mineralized nutrients from artificial fertilizers, and from animal dung and plant residuals, are the cornerstone of modern conventional and organic agriculture. But they form a very risky strategy for fertilizing crops. Mineralized nitrogen is not the only way in which plants can get their nitrogen. In addition to the uptake of inorganic nitrogen, there are five other ways in which plants get their nitrogen. Inorganic nitrogen is not a safe way for plants to get their nitrogen. Ammonia, urea, and nitrate disturb the physiological processes in the plants, and, in consequence, the plants are an easy prey for pests and diseases. Ammonia and nitrate reduce the biodiversity in the patsures and the fields within a few years. But on the other side, not all the organic nitrogen is good for plants. When the symbiotic microbes are put aside by putrefactive microbes, the latter produce a lot of rotting compounds and toxins which hinder and even block the growth of the plants. The cations in conventional and biological products are not in balance, and many trace elelements are missing, with the result that not all nitrogen and sulphur are converted into real proteins. In the 19th and the beginning of the 20th century farmers developed systems to transform animal dung and plant residuals in a healthy plant food by mixing it with earth, heather sods or ditch dredge. By doing this, they kept the nutrients in the mixture and prevented the evaporation and washout of them into the air or the water (ground water). Other farmers added sea minerals to the farmyard manure and the compost. In this way, they gave the crops the necessary sodium, magnesium and trace elements. Crops fed with these products grow well. Still others used dung worms to convert animal dung and compost into valuable fertilizers for the soil and the plants. This vermicompost is a much better fertilizer than animal dung or warm compost. In organic agriculture, the yields are lagging behind because the plants can't get enough mineralized nitrogen and sulphur, and at the same time they are not able to get the organic nitrogen and sulphur compounds from the soil, because the symbiotic bacteria and fungi which can bring these organic nutrients directly into the plants, are not present, or blocked. The fertilizing systems in organic and conventional farming are

* Corresponding author
E-mail address: aonigten@hotmail.com (A.O. Nigten)

based on the same system of mineralization before the uptake of the nutrients by the plants. For that reason, there are only minor differences in quality of the products from both systems. Inorganic nitrogen blocks the nitrogen fixation of leguminous and non leguminous plants, if the levels of it in the soil are too high. As they often are. Practical solutions were developed in the past by many farmers and some scientists. Most of them are forgotten, and must therefore be rediscovered. This article is part of a series in which I try to find out why the yields in organic farming are lagging behind in comparison to conventional farming, and why the quality of both product groups is comparable: not good enough. Lack of available (organic) nitrogen and sulphur seems to be the common key.

Keywords: inorganic nitrogen, sulphur, plant, fertilizer.

1. Introduction

Modern agriculture is based on seven pillars. The use of inorganic salts as plant feed is one of them. Ammonia and nitrate are the two salts, which farmers use to give as nitrogen fertilizers. And urea. Urea is broken down in ammonia and carbonic acid before the plants can use it. There are five other ways in which the plants can get nitrogen form the air or from the soil. With or without the help of symbionts. In all these cases, the plants take or get organic nitrogen.

The direct uptake of atmospheric nitrogen by the plants themselves is systematically overlooked by present-day agricultural science, although many scientists in the past have proven that plants can get nitrogen directly from the atmosphere. One of them found out with which organ plants assimilate atmospheric nitrogen directly without the help of symbionts.

Inorganic nitrogen salts disturb the physiological processes in plants, animals and men.

Very often the organically bound nitrogen in animal dung and warm compost in organic agriculture is not or not sufficiently available for the growing plants, although large amounts are given. During the stable time, the storage and the spreading of the dung, and during warm composting many nutrients are already lost. By adding earth, heather sods or ditch dredge to the farmyard dung and the compost these losses can be kept low. Artificial fertilizers also loose big amounts of nutrients into the environment.

Farmyard manure and warm compost contain many growth inhibiting substances which can be converted into good plant nutrients when these materials are given in the autumn. The conversion goes on during winter, but in the Netherlands the organic fertilizers are mostly given during the spring. So during the first weeks the growth of the young plants is inhibited. Farmers in the Netherlands are not allowed to spread organic fertilizers after the September begin.

Nitrogen fixation is disturbed when too large amounts of inorganic nitrogen is given. For nitrogen fixation, phosphor is the most important nutrient.

Inorganic nitrogen also lowers the amount of vitamin C in plants. Vitamin C is important for the natural resistance of plants against pests and diseases.

The differences in quality between organic and conventional agricultural products are small and often inconsistent: less nitrate and more magnesium and zinc in organic crops. In general, you can say that organic crops are, like conventional crops, also out of balance for its macro-elements. Besides, these products contain too much non-protein nitrogen, like ammonia, nitrate, and probably many other non-protein N compounds.

In three articles, I will investigate why artificial fertilizers and the organic fertilizers, as used today, are a risk for plants, animals and men. At the same time, this is a high risk for the environment and the biodiversity. Further, I will demonstate how farmers in the past were able to avoid these problems, and what we can learn from them. I did not study he important question why modern agricultural science failed in solving these problems, although all the solutions were available in the older publications of the last two hunderds years.

Six ways by which plants can get their nitrogen

Inorganic nitrogen

In modern agriculture, the dominant view is that plants always get their nitrogen in an inorganic form – as ammonium or as nitrate. This is one of the cornerstones of the modern paradigm. Questioning this fundamentally means that an important building bloc of this paradigm is torn down. Other beliefs of the dominant paradigm are the overestimated role of potassium, the underestimation of the importance of carbon, sodium, silicon, magnesium, and trace elements. Another cornerstone is insufficient appreciation of the symbiotic relations between plants and microbes. Also the effects of all these on the health of plants, animals and men are systematically underestimated. Problems with pests and diseases, according to this paradigm, can be solved with pesticides and genetic engineering, and the hunger in the world can be solved with more and better artificial fertilizers and smart pesticides.

So inorganic nitrogen is one of seven pillars. In this article, this pillar is put under the magnifying glass.

Nitrogen.

In addition to ammonium and nitrate, there are at least five other ways by which plants can get their nitrogen.

These are the possibilities:

Direct absorption of organic nitrogen

Plants can absorb amino acids and proteins directly from the soil (Näsholm et al., 2000; Näsholm et al, 2009) or through the leaves (Krasil'nikov, 1958). There are plants that secrete proteases themselves. These are enzymes that break down proteins into peptides and amino acids, which are then directly absorbed by the plants through endocytose without first reducing them to ammonium or nitrate. And there are plants that even directly absorb the proteins as a whole (Paungfoo-Lonhienne et al., 2008).

Endosymbionts

Important groups of plants get amino acids from their rhizosphere symbionts, or from their above ground symbionts in leaves and stems:

- the rhizobia in the root nodules of the leguminosae. There is a very comprehensive literature about them. And recently scientists found bacteria which don't belong to the rhizobia but which are also able to form root nodules and assimilate nitrogen: in India it is proven that Caulobacter, a bacterium in the root nodules of Horse gram, also assimilates nitrogen. Just like the rhizobia, the Caulobacter belongs to the alpha proteobacteria (Edulamudi et al., 2011). Shiraishi and his colleagues discovered in 2010 that also Pseudomonas and Burkholderia form root nodules and assimilate nitrogen in the roots of Robinia Pseudoacacia (Shiraishi et al., 2010). They belong to the gamma and the beta proteobacteria respectively.

- Nitrogen assimilating cyanobacteria in special glands on the stems of the Gunneraceae (among which Giant rhubarb) (Santi et al., 2013). And Nostoc cyanobacteria in the leaf cavities of Azolla ferns in the wet rice cultivation;

- Frankia bacteria in the actinorhizia plant families – mostly trees and shrubs (Santi et al., 2013);

- The nitrogen fixing bacteria Azoarcus, Azospirillum en Herbaspirillum in the roots of grasses

and of rice. Although certain azospirillum groups also have positive effects on some wheat varieties, this is not a consequence of nitrogen assimilation but of other growth stimulating compounds from Azospirillum according to some authors (Clemente et al., 2016; Ribeiro et al, 2018). Karimi got higher wheat yields (plus 18 %) with azospirillum under drought stress. But this was, he said, a consequence of better root development through a higher indole acetic acid production by Azospirillum Zea Sp. 2. from wild wheat varieties (Karimi et al., 2018).

Lafferty Doty questions this:

"*In greenhouse studies with wheat, only inoculation with the wild-type strain of K. pneumoniae resulted in increased height and greenness under N-limited conditions compared to inoculation with a nif mutant or killed control strain, or uninoculated. These studies indicate that, while phytohormone modulation, vitamin synthesis, and increased mineral uptake and stress tolerance conferred by diazotrophic endophytes are important, N-fixation is also a key factor in the benefit of inoculation"* (Doty, 2017).

These four groups are so-called endosymbionts, because they offer their services in special organs or in the root surface of the plants with which they are connected. So they live inside the plants.

Free living nitrogen fixers

There are on the other hand free-living nitrogen fixers in the soil: Examples of these are azotobacter; actinomycetes, clostridium, azospirillum, klebsiella, burkholderia, and cyanobacteria. More and more species are being discovered that can bind atmospheric nitrogen. Many nitrogen-fixing bacteria belong to the proteobacteria.

Symbionts, which release nitrogen from the soil humus or the soil particles

Bacteria:

Then there are the symbionts, which do not assimilate nitrogen from the air themselves, but make the nitrogen free from the organic material, from the humic acids, or from the soil particles by consuming it. Humic acids can bind inorganic nitrogen. This group of symbionts includes bacteria, fungi and maybe also yeasts. These symbionts are then absorbed and "emptied by the plant roots", after which they go back into the soil and again collect nutrients over there (White et al., 2018).

White and his colleagues have demonstrated plausibly that plants, in order to obtain nitrogen, digest their soil symbionts by breaking down their cell membranes with aggressive oxygen compounds (H_2O_2). White speaks in this context of a rhizophagy cycle:

"*In the rhizophagy cycle, symbiotic microbes go from plants into the soil, acquire nutrients of various kinds, and carry nutrients back to plants, enter plant root cells where plants oxidatively extract nutrients from microbes, then plants deposit* [the] *microbes back into the soil from tips of root hairs to continue the* cycle "(White, 2019; see also White et al., 2018).

In his 2018 article, White published beautiful photos that give us an idea of these processes. The drivers are partly bacteria that release nitrogen from the organic material such as Micrococcus luteus and Bacillus amyloliquefaciens, and partly free-living nitrogen fixers such as Klebsiella and Burkholderia. It concerns **species-related symbionts**. Micrococcus luteus, for example, does stimulate tomatoes, but inhibits grasses and roots of other species.

This seems to be the most interesting group for our organic fertilizer discussion, just like the mycorrhizae, because they make the organically or minerally bound nitrogen also available for the plants. Without mineralization*.

Mycorrhizae:

The mycorrhizal fungi. These fungi also release nitrogen and phosphorus from the soil and humus and transport it to the plant roots in the form of amino acids and organic phosphor. Nitrogen-binding bacteria are sometimes also found in special cavities (tubercles or nodules) of these fungi. Paul and his colleagues have demonstrated this for a pine species (*Pinus contorta*) that grows on poor soils (Paul et al., 2007).

Yeasts:

Yeasts can also bind nitrogen from the air and thus contribute to the better growth of crops. Sherry yeast is an absolute topper in this respect (Schanderl, 1947). In cereals (oats, wheat), sherry yeast strongly stimulated atmospheric nitrogen assimilation. It is not yet clear how it works. Are the yeasts doing the nitrogen fixation themselves and then 'eaten' by the plants (see White), or do they excrete their nitrogen in the rhizosphere zone after nitrogen fixation? Or do they produce stimulating compounds for the cereals which make the nitrogen fixation by the plants themselves possible? We do not know yet.

Schanderl demonstrated that specially selected wheat could extract up to 50 % of its nitrogen from the air by itself (Schanderl, 1947).

"*Mit Weizen habe ich folgendermassen experimentiert: 1939 habe ich auf einer der beiden hiesigen grossen Kaolinsandhalden 10.000 Weizenkörner ausgesät, von den Gedanken ausgehend, dass wohl 99 % der Pflanzen zugrunde gehen würden, dass aber diejenigen welche es sich unter den denkbar schwierigsten Bedingungen der N-Ernährung zu einer Körneransatz brächten, zur Luftstickstoffassimilation fähig sein müssten. In der tat sind von den*

---

* Modern agriculture uses the word mineralization. By this they mean the freeing of inorganic elements and compounds from organic material and minerals: potassium from feldspath or biotite; phosphor from apatite; nitrate and ammonium, hydrogen sulphide, hydrogen cyanide and phosphine from organic materials or from the clayparticles. Minerals are however something completely different. Minerals are stones, inert materials which contain often silicates and aluminium plus metallic and non metallic elements. Nitrogen is not a part of these stoney materials. Nitrogen is in the air and in the organic compounds in the soil. Ammonium can be loosely bound to clay particles. Nitrate in the soil can bind with for example calcium. Together with calcium or as a salt ion, nitrate is lost very quickly to the groundwater or the drainwater. Nitrate can also bind to humic acids, but these are most of the time insufficiently present.
I have used the word mineralization in this text only because it has become the normal word for salts and salt ions. In fact we should call it salts and the proces salinization. Minerals normally are inert and stable. Easily soluble salts are agressive and lost easily.

10.000 Pflanzen 9997 kläglich verhungert, nür 3 brachten es zu Ähren, und nur eine davon zu normaler Samenbildung. Mit Samen von dieser auf N-Anspruchslosigkeit selektionierten Pflanze wurde 1940 ein Gefässversuch mit Sand von 15 mg % N-Gehalt angesetzt und eine N-Bilanz aufgestellt. Das Wachstum dieser Selektions-Nachkommenschaft war für Weizen erstaunlich gut. ***Die N Bilanz ergab dass der Weizen 50 % seines N-Bedarfes aus der Luft gedeckt haben musste*** *(..).*

1942 würde je einem Gefäss eine zusätzliche Dünging von 25 mg N in Form von Hefe, und 140 mg N in Form von KNO_3 gegeben. Der N-Gewinn betrug im ersten Fall 170 mg, im zweiten Falle nur 53 mg" (Schanderl, 1947: 173-174).

In his later trials in 1943, Schanderl found that some wheat grains were able – again – to collect 53 % of its nitrogen from the air (Schanderl, 1943). But, according to Schanderl, all wheat (and other crops with small seeds) need a sufficient nitrogen fertilization in the soil to start with, in order to get good N fixation rates from the air. And first you have to select those wheat grains which still have the faculty to fix nitrogen from the air. All hybrid wheat varieties can be excluded because Graem Sait[*] (Sait, 2018) has shown that these varieties are no longer able to take up kobalt. Kobalt is, like molybdenum, necessary for nitrogen fixation.

Special nitrogen fixing hairs on the leaves

The plants can also assimilate themselves nitrogen through the special hairs on their leaves (Jamieson, 1910). Jamieson stated that his research has shown that all plants have such hairs. In his book of 1910, he first described why the observations and conclusions of Boussingault and Lawes that plants cannot absorb nitrogen (N_2) from the air were scientifically spoken untenable and that Ville with his trials was right. In 1853, Ville had proven that plants are able to absorb nitrogen from the air – nitrogen (N_2), not ammonia (Ville, 1853). Chevreul et al., appointed by the French government in a special committee, verified and confirmed it (Chevreul et al., 1855). But Ville didn't know which organ absorbed the nitrogen from the air. And the leading agricultural scientists of his time – Boussingault and Lawes – started their own trials, which 'proved that plants did not absorb nitrogen from the air'. So they rejected both the results of Ville and the confirmation by the Chevreul commission. But later on Atwater, Franck and Jamieson criticized strongly the trials of Boussingault and lawes as completely untenable (Jamieson, 1910).

Jamieson describes how he observed the plant Spergula[†] (*Spergula arvensis*) which is very rich in nitrogen, and noticed the numerous hairs on the leaves of this plant:

"Rigorous microscopic examination, and careful and varied chemical tests, brought out that the hairs actually are absorbers of nitrogen from air, transformers of it into albumen, and conveyers of the albumen into the plant. What held good in Spergula would, it seemed likely, hold good in other plants; and actual examination showed that this was the case, i.e. that absorbers of nitrogen were found to occur, in one form or another, on all plants". (Jamieson, 1910: 101).

See for more detailed information on the research of Jamieson Appendix 1.

The interesting question then is why specialized symbionts are also needed as described above, if all plants have these nitrogen fixing hairs. Schanderl has proven that a double mechanism exists in leguminosae. He has demonstrated that leguminosae are perfectly able to fix nitrogen from the air without their root nodules (Schanderl, 1947). It looks like as if the root nodules produce extra nitrogen for the next generation, because Schanderl observed that these nodules break open and give their assimilated nitrogen to the soil, and not to the plants. Schanderl was also of the opinion that the root nodules offer the plants which with they are connected the special nitrogen fixation stimulating compounds and not nitrogen. "The nitrogen in the nodules comes from the leaves, not from the nodules". So just the other way round, according to Schanderl. Krasil'nikov agreed with this:

[*] Graeme Sait: "The hybridized, green revolution grains, upon which most of our modern bread is based, attracted a Nobel prize for Norman Bourlag. (..)He did not use traditional hybridization techniques to create this more squat variety, which was much less prone to lodging. Instead, he irradiated the original wheat varieties and selected a mutant that became our main food. (..)There is one mineral that this compromised cereal can no longer uptake at all. This is the rarely-considered trace mineral, cobalt. (..) Cobalt is the building block for an incredibly important nutrient called vitamin B12. A key reason that many of us are now lacking this vitamin relates to the loss of cobalt in our most popular food" (Sait, 2018).

[†] In the past spargel was an important fodder crop in the Netherlands. Mostly as a stubble crop.

"There are reasons to believe that root-nodule bacteria, as well as the bacteria from the nodules on the leaves of the above-mentioned plants, act favorably through their metabolites. According to our data, leguminous plants, in symbiosis with the nodule hacteria, fix molecular nitrogen for themselves from the air. The bacteria, due to their metabolic products, act as biocatalysts, activating the nitrogen-fixing ability" (Krasil'nikov, Korenyako, 1946).

This is at least an interesting question: is the nitrogen from the leaves going down to the nodules, or from the nodules going up into the leaves? Let us look at it with an open mind. See the recent debate on the role of Azospirillum in wheat on page 4: Doty versus Karimi, Clemente, and Ribeiro. Maybe plant promoting compounds go – also – from the nodules to the leaves…

In my article about the work of Schanderl I have listed up all the pro's and contra's, and my conclusion is that Krasil'nikov and Schanderl were wrong on this point, but many arguments of

Schanderl regarding the root nodules and rhizobia are not yet answered. It is still urgent to answer them (Nigten, 2019b).

For cereals Jamieson observed that these 'grasses' have only small and few hairs for the collection and fixing of nitrogen from the air. Maybe for this reason you could think, grasses need special symbionts for nitrogen fixation: azoarcus; azospirillum etc (Paungfoo-Lonhienne et al., 2008). But the results of Schanderl (see above), Lipman and Taylor (1922) and of Rigg (1838) contradict this.

Probably the special hairs that Jamieson describes as nitrogen fixers are the filaments of the cyanobacterium in the plant cells. In free-living cyanobacteria, these filaments or hairs are called heterocysts. Air nitrogen is assimilated through these heterocysts. Heterocysts are extra protected against oxygen, because the splitting of the air nitrogen in an oxygen-rich environment is almost impossible (Figure 1). The cyanobacterium is one of the four primordial symbionts that together form the plant cells.

This would then be the same ancient symbiont that also takes care of photosynthesis in the leaves, (in) the chlorofyll granules, descendants of a cyanobacterium (Nigten, 2020). The other main symbionts of the plant cell are adapted forms of proteobacteria (the mitochondrium), of spirochetes (for the transport and cell division system (mitosis)), and the mother cell in which all symbionts are included (an archaea, probably Thermoplasma acidophilum[*]) (Margulis, Schwartz, 1988; Margulis et al., 2009). In addition, more and more "specialised" symbionts are being discovered.

This concerns both fungi, bacteria and beneficial viruses (Béchamp, 1883; Pradeu et al., 2016).The research into viruslike structures in bacteria and their roles points out that these semi-organelles fullfill important roles as symbionts in the bacteria:

"*The number of bacteria that are found to depend on phages for crucial functions increases almost by the day*" (..) *"It seems that some phages[†] have become almost permanent components of bacteria"* (Hunter, 2008).

All the above described routes provide organically bound nitrogen.

In a similar way, organically bound other elements such as phosphorus, potassium, calcium and magnesium are also supplied by soil life (Krasil'nikov, 1958). Iron, for example, is bound by siderophores[‡] and transported into the cells. Zinc is said to use quercetin as the ionophore[§]. Many ionophores are produced by microbes, some by the plants themselves. Phosphorus is also released from the soil particles by the mycorrhizal fungi and delivered (organically bound) to the plant roots.

[*] Yutin et al abnegate the theory of Margulis regarding Thermoplasma acidophylum as the primal cell of the eukaryotes.
They think , based on phylogenetic analysis, that an extinct archaea is the one (Yutin et al., 2008).

[†] Phages are bacteriophages, literally bacteria eating viruses.

[‡] *"Siderophores (Greek: "iron carrier") are small, high-affinity iron-chelating compounds that are secreted by microorganisms such as bacteria and fungi and serve primarily to transport iron across cell membranes, although a widening range of siderophore functions is now being appreciated. Siderophores are among the strongest soluble Fe^{3+} binding agents known"*. Source Wikipedia english: siderophore.

[§] For more information on ionophores – ion carriers (see Wikipedia english: ionophore).

Fig. 1. Heterocysts. With the cyanobacterium (left under);
only the heterocyst in the hairs (right, see the arrow)

The crux of the problem.

• Nitrate and ammonia … disturbing nutrients.

As early as 1912, Schreiner plausibly argued that nitrate and ammonium are not the preferable nitrogen sources. For example, he clarified for nitrate that the plants must first extract the oxygen from the nitrate – NO_3. That takes a lot of energy. And then, the entire formation of amino acids still has to take place before the built up of proteins can start (Schreiner, Skinner, 1912a). This is all very inefficient. Visser goes a step further in his thesis from 2010 and basing on the extensive literature research concludes that nitrate and ammonium are actually the most harmful nitrogen forms for the plants (Visser, 2010). The plants are overfed with it, and the carbon is missing in both ammonium and nitrate. According to Jones, this leads to an exhaustion of soil carbon, and through this, the soil life is declining (Jones, 2015).

Due to these phenomena, in combination with a lack of magnesium and trace elements, the plants cannot convert all the inorganic nitrogen absorbed into complete proteins, which weakens its resistance (Anjana and Umar, 2018). The plant becomes an easy prey for diseases and pests (Hornick, 1994; Visser, 2010). Microbes, nematodes and insects love nitrate, ammonium, ureum, nitric oxide, nitrosamines and even whole amino acids in the plant, but not proteins. They can't eat the proteins in the plants (Chaboussou, 2005).

Not only nitrate can disturb physiological processes in plants but also ammonium. Britto and Kronzucker have given an overview in 2002 (Britto, Kronzucker, 2002).

The most interesting of their findings are these:

- Charles Darwin already in 1882 saw growth inhibition in euphorbia peplus through ammonium. (In the same period, Julius Hensel condemned the use of farmyard manure, because he thought the ammonia in it was harmful for the crops. Instead of that, he stimulated the use of rock flour as a fertilizer).

- In humans ammonium can cause neurological and insulin disorders. And: *"Sensitivity to NH_4^+ may be a universal biological phenomenon, as it has also been observed in many animal systems".*

- In the majority of ecological systems, the values of NH_4^+ in the soil solution vary between 0.4 mmol/l and 4 mmol/l. In agricultural soils, it often ranges from 2 to 20 mmol/l.

- The large amounts of human-made ammonium in ecosystems affect not only individual species but also e.g. the decline of forests;

- The threshold at which ammonium becomes harmful differs per plant species. Britto gives some examples of sensitive plants, among which tomatoes and potatoes. Other plants like rice, onions and leeks are highly adapted to ammonium. But these plants can also suffer from ammonium toxicity if the amount given is too high. When also nitrate and potassium were given this ammonium toxicity became less;

- Symptoms of ammonium toxicity in the sensitive species appear mostly at NH_4^+ in the soil solution above 0.1 to 0.5 mmol/l. Some symptoms are: lower seed germination, and less seedling establishment; chlorosis in the leaves; diminished growth; a possible (still much discussed) intracellular disturbed pH; lower yields and even plant death;

- *"Symptoms not so readily visible, but equally important, can include a decline in mycorrhizal associations".*

- Less potassium, magnesium and calcium, and more phosphate, sulphate and chloride in the plant tissues through ammonium. Some acids like malate acid go also down, while amino acid concentration increase; rhizosphere acidifies after ammonium uptake, while alkalizing happens after uptake of nitrate. So most plants which tolerate ammonium are also acid tolerant.

- The way in which ammonium enters the root cells is still highly debated. One of the ideas is that there is a lack of regulation for the uptake of ammonium. Maybe because the potassium channels for the uptake of potassium are also used for the uptake of ammonium;

- The content of sugar and starch in plants decreases with ammonium fertilization. This can be induced through damage of the photosynthetic centers. A possible side effect is less ascorbate because of less carbon. Ascorbate is the anion of vitamin C.

- Ammonium inhibits the uptake of nitrate to a certain degree. But extra nitrate can alleviate ammonium toxicity in ammonium sensitive plants;

In later research, Balkos and Britto found out that for rice it helps to give extra potassium to mitigate the harmful effects of ammonium. Rice is a non-sensitive species (Balkos, Britto, 2010).

More interesting is what Britto and his colleagues did not investigate. Eight questions:

- Are there no alternatives for ammonium and nitrate like organic nitrogen compounds?

- Which other elements are necessary for mitigating the harmful effects of ammonium (and nitrate)? Potassium plays only a minor role in the conversion of ammonia and nitrate into real proteins.

- Why is the general level for harmless ammonium – under 0.1 to 0.5 mmol/l for sensitive species – so low? What is the level of ammonium in the top layer of the soil if you spread for instance 100 kg NH_{4+} N/ha as ammonium fertilizer? And what if you do this ten years or more in a row?

- And what is the general level for the amount of harmless ammonium for non-sensitive species? And for soil life? The results of the Bernburg trials (1910-1962) gives detailed information for differences in bacterial numbers depending on different treatments*. See Appendix 2.

- In which way is ammonium in natural ecosystems bound in the soils (clay particles; humic acids; fulvic acids; other organic matter)? And is there a maximum level for binding ammonium in different soils? Or, in other words, at what level is ammonium lost from soils? Or is it always lost to some degree?

- What are the consequences for human and animal health if crops contain ammonium in their tissues? And is there a safe level?

- Why do you have to spread say 100 kg/ha of ammonium fertilizers to get 40 % in the crops? Is here a physiological mechanism at work? And which? Osmosis?

- Does ammonium (and nitrate) also lower the content of other vitamins than vitamin C?

In a recent study, a negative impact of inorganic fertilizers on the rhizosphere microbiome of wheat plants is proven (Reid et al., 2021):

"The profound negative effect of inorganic chemical fertilizer application on rhizobacterial diversity has been well documented using 16S rRNA gene amplicon sequencing and predictive

* From the trials at the Bernburg experimental station (1910-1962) in Germany we know that the highest bacterial count was found in the fields which were treated with animal dung. 33.86 million bacteria in one gram soil. In the treatment with only nitrogen the count was 11.2 million bacteria. The animal dung parcell had almost the highest nitrogen percentage. The highest was the parcel treated with animal dung plus phosphor and potassium, but no extra nitrogen. In the parcel treated with NPK (N= nitrate) the soil nitrogen was low. Even less than the control: 0,038 against 0,066. And the bacterial count was also low (Poschenrieder and Lesch, 1942). See Appendix 2 for the exact data of all treatments and an interesting ascertainment: the nitrogen of farmyard manure is in the soil, but nevertheless insufficiently available for the plants.

metagenomics. (..).In general, fertilizer addition decreased the proportion of nutrient-solubilizing bacteria (nitrate, phosphate, potassium, iron, and zinc) isolated from rhizocompartments in wheat whereas salt tolerant bacteria were not affected (..). We hypothesized that the addition of chemical fertilizer would reduce putative Plant Growth-Promoting Rhizobacteria populations in wheat. We found that the abundance of rhizobacteria with acquisitional traits for key plant nutrients (endogenous nitrogen, phosphate, potassium, iron, and zinc mobilization) were significantly reduced in wheat grown in soils treated with NPK fertilizer.

This study contributes to our understanding of the impact of fertilizer on wheat rhizobacteria and supports previous studies showing the deleterious effect of chemical fertilizer on plant rhizobacteria, particularly through highlighting the greater abundance of putative Plant Growth-Promoting Rhizobacteria in unfertilized plants. It is assumed that wild relatives have co-evolved with the microbial community of native soils, selecting microbes beneficial to growth and health. Here, we show the probability that wheat plants can select growth-promoting bacteria to their roots to establish mutually beneficial associations and that chemical fertilizer reduces this selection" (Reid et al., 2021).

But 'there is still uncertainty about the exact mechanisms how or why this is caused'. The authors developed a hypothesis about competition between rhizosphere bacteria and non symbiotic bacteria:

"However, it is unclear why fertilizer addition would inhibit root colonization by these bacteria. It is possible that rhizobacteria are less able to metabolize primary nutrients in the form presented in agricultural fertilizers than other members of the soil microbiome. If this is the case, it would follow that they are also less competitive in this environment and this would be reflected in their lower abundance" (Reid et al., 2021).

Future research.

In future research, the methods developed by Reid et al. can be used to check if the rhizosphere bacteria in current organic agriculture are repressed in a comparable way by farmyard manure, slurry or heated composts as in inorganic fertilizing. And at what levels inorganic fertilizers repress the rhizosphere biome? And what is the influence of toxic organic nitrogen compounds on the rhizosphere biome? Are the effects of the toxic organic nitrogen and sulphur compounds in rotting farmyard manure, slurry or in warm compost on the rhizosphere biome comparable to the effect of inorganic fertilizers of which Reid et al. talk?

Organic nitrogen in farmyard manure: present but not available … a complicated problem.

The crux of the problem of farmyard manure seems to be that on the one hand, there is mostly sufficient organic nitrogen in the soil, but on the other hand, at the same time it is not available or at least not sufficiently accessible for the crops.

Lawes and Gilbert (1858) confirmed this in their report about the results of a three years trial on meadowland with 17 treatments, among which a treatment with pure minerals (K + Na + P + Ca + S + Mg; and no ammonium, nor nitrate); a treatment with ammoniacal salts; a treatment with farmyard manure; one with farmyard manure plus ammoniacal salts; one with minerals salts, ammoniacal salts and cut wheat straw and one with farmyard manure plus 200 lbs ammoniacal salts.

Lawes noticed that the pure mineral treatment had very positive effects on the leguminous herbage, and that the pure ammoniacal salts had only positive effects on the gramineous plants. And that farmyard manure in this respect had an in between position:

"That the mineral constituents of the dung had their share of effect, would appear from the fact, that the Leguminous herbage was moderately luxuriant on the dung plot, and that those of the Grasses were the most developed which were increased in their proportion to the rest by the artificial mineral manures. And again, that the nitrogen also of the dung was effective, may be judged, not only from the general development of the Graminaceous

plants under its use, but from the fact of a like fullness in the proportion of the Grasses in flowering and seeding stem, as where ammoniacal salts were employed in conjunction with the mixed mineral manure.

It would appear, however, that a much less proportion of the whole nitrogen supplied to the land was active, when it was provided in the form of farmyard manure, than when in that of ammoniacal salts. There would, in fact, be considerably more of nitrogen applied per acre in the 14 tons of farmyard manure, than in the 400 lbs. of the mixed ammoniacal salts. Nevertheless, the encouragement of the Leguminous plants was much greater, and that of the Graminaceous *ones much less, where the farmyard manure was employed, than where the 400 lbs of ammoniacal salts, together with the mixed mineral manure, were used.*

That the less produce by the farmyard manure, than by the mixed mineral manure and 400 lbs. of ammoniacal salts, was due to a deficiency of available nitrogen, notwithstanding the large actual amount of it in the dung, would appear from the fact, that on the employment of 200 lbs. of ammoniacal salts in addition to the farmyard manure (Plot 17), there was a further average annual increase of 8 3/8 cwts, of hay per acre. Still, even with this addition, there was about ½ a ton less of hay annually than where the " mixed mineral manure" and the "400 lbs. of ammoniacal salts" were applied.

The evidence regarding the action of the farmyard manure goes to show, that, though it is doubtless a very complete and important restorer of both the mineral constituents and the

nitrogen required to repair the exhaustion of this most greedy crop, yet, the amount of these constituents supplied by its means is proportionally much less active within a given time than that provided in the artificial combinations" (Lawes, Gilbert, 1858: 568).

Results of the three years trial on meadowland of Lawes and Gilbert are presented in the Table 1.

Table 1. Treatments with different fertilizers (from Lawes, Gilbert, 1858: 558)

Manures per acre per annum	Average yield of three years, t	Average annual increase by manure	Striking effect
T 1. Unmanured	1.203 ton	-	Grasses and legumes
T 4: 200 lbs ammonia sulphate and 200 Lbs ammonia murate	1.762 ton	Plus 0.559 ton.	Grasses, almost no legumes
T 8 Mixed mineral manure: K + Na + P + Ca + S + Mg	1.66 ton 13 cwt	Plus 0.457 ton	Legumes, almost no grasses
T 10 Mixed mineral manure and 200 lbs ammonia sulphate and 200 Lbs ammonia murate	2.965 ton	1.762 ton	
T 12. Mixed mineral manure; 200 lbs ammonia sulphate and 200 Lbs ammonia murate, and 2000 lbs cut wheat straw	2.711 ton	1.508 ton	Five cwt less than T 10 through the wheat straw
T 16. 14 ton Farmyard manure	2 ton	0.813 ton	Grasses, and some legumes.
T 17. 14 ton Farmyard manure plus 100 lbs ammonia sulphate and 100 Lbs ammonia murate	2.406 ton	1.203 ton	

Here we see that 14 ton farmyard manure – T16 – has a lower yield than T10 – artificial ammonia fertilizers plus mixed mineral manure K + Na + P + Ca + S + Mg, although according to Lawes the 14 ton farmyard manure contains much more nitrogen than the artificial ammonia fertilizers in T10*.

But couldn't plants directly absorb amino acids or proteins as stated above? Yes, but then the organic nitrogen compounds must be amino acids or proteins for most of these plants, and they must be accessible to the plant roots. It seems that the absorption of organic nitrogen for roughly 85-90 % of the plants requires the mediation of the microorganisms in the soil – the symbionts: mycorrhizae or bacteria. But then there must be symbionts available. And also the right symbionts.

* In the Netherlands farmyard manure contains roughly 5% N_{tot} in fresh product (Blgg AgroXpertus, 2011). So in 14 ton FYM there is roughly 700 kg N_{tot}. Indeed, much more than in 400 pounds of the ammonia fertilizers.

The research by Paungfoo-Lonhienne (Paungfoo-Lonhienne et al., 2008) showed that a member of the *brassicaceae* family – the sand rocket – was able to absorb proteins directly, without the intervention of microorganisms. The plants examined secrete proteases themselves that break down proteins into amino acids that they can absorb via endocytosis. The *Brassicaceae* don't have mycorrhizal fungi as symbionts. And they don't need them. They are able to process proteins by themselves.

"Digestion and uptake of protein may be widespread in the plant kingdom and may be crucially important for the 10 % of plant species that do not form mycorrhizal symbioses (Paungfoo-Lonhienne et al., 2008: 4527).

And why do the plants in agriculture, based on farmyard manure or slurry not use their special hairs to fix nitrogen from the air?

There are different reasons why this does not happen, or not as much as necessary or possible:

1. Growth inhibitors and toxins from deep litter manure, slurry*, warm compost and their putrefactive bacteria suppress and hinder the plant growth and the symbiotic microbes around the roots. So the plants can't get enough organic nitrogen from the soil to start their growing. First they have to build their young green leaves before the nitrogen fixation in the hairs of their green leaves can begin (Schanderl, 1947);
2. When there is too much nitrate or ammonia in the soil, nitrogen fixation on the leaves stops. On most organic farms, nitrate and ammonia are the only nitrogen compounds available for the plants in the beginning of the growth season, because the organic nitrogen is not accessible (see point 1);
3. All the trace elements necessary for nitrogen fixation should be available. So at least kobalt, molybdenum, zinc and magnesium must be there in sufficient amounts. And probably much more trace elements. In many soils these trace elements are low, missing or not available (Dimkpa, Bindrapan, 2016). And ammonia plus potassium hinder the uptake of many cations, including trace elements;
4. And growth stimulators like auxins, cytokinins and gibberellins support these processes. So they should be available too. For their production symbiotic microbes around the roots are unmissable;
5. Pesticides may disturb the work of the symbionts, and the cooperation between plants and their symbionts. In a recent study in the Netherlands, it became clear that also in organic agriculture there are many different residuals of pesticides (Buijs, Samwel Mantingh, 2019). Partly, they come from outside organic agriculture and partly form the dairy farms themselves. From the past or from actual use. Organic dairies use insecticides and vermicides against flies on, and worms inside the cows. And this is still officially permitted.

What can we learn from the literature about nitrogen availability from farmyard manure, slurry and warm compost in (organic) agriculture?

The farmyard manure, slurry and plant residuals must first be pre-digested by non-symbiotic microbes before the symbionts can start working. Cellulose, hemicellulose and lignin have to be broken down before the cell cytoplasm can be digested. This pre-digestion takes time (Rusch, 1968; Krasil'nikov, 1958). But because the manures in the Netherlands are often applied in the spring, that necessary time is missing. The digestion, which follows then, often also produces substances that temporarily inhibit the growth of the plants. This is partly because the quality of the manure or the compost itself leaves much to be desired. It contains, as you can smell, too much non-protein nitrogen, and non-protein sulphur, NPN and NPS. And the very toxic phosphine, PH_3 and Hydrocyanic acid, HCN. Such manures and compost often stimulate the wrong bacteria – decay bacteria. These putrefactive bacteria produce toxins, such as mercaptans, putrescins, cadaverins, indoles, and skatoles (Hennig, 1996) and eventually these substances are converted into ammonia, hydrogen sulfide, hydrogen cyanide, nitrous oxide (Laughing gas), nitrate, phosphine and gaseous nitrogen (N_2) in the manure, which emit easily. Especially phosphine emits very quickly from the manure heaps and the slurry pits, because it is poorly soluble in water. In the modern emission literature it is never mentioned, but in the 19th century it was already memorized (Bowditch, 1856).

* Not all slurry is giving rotting. We have shown that a slurry with a C/N ratio of twelve and up gives little rotting compounds like ammonia (Vanhoof, Nigten, 2020).

Losses of inorganic nitrogen – and other anions – in historical perspective:

Bowditch, a reverent of St Andrews at Wakefield, studied the losses of nitrogen, sulphur and phosphor from manure heaps, from stored blood from slaughterhouses and other slaughter residuals, and from heaps with vegetable residuals. He showed how much ammonia, hydrogen sulphide and phosphine was lost from these residuals. His purpose was to demonstrate the farmers and workers the losses from these heaps, not with laboratory results but with the smells which escaped from these residuals and with test paper which coloured blue if there was emitted ammonia, hydrogen sulphide or phosphine.

And also he made clear to the farmers how they could keep these valuable elements in the heaps by mixing earth with the residuals, often in a ratio of one to one. Then the heaps gave no more smells at all. And the end product gave very good yields as he demonstrated. He also argued that much phosphor was lost from the heaps by the evaporation of phosphine. Phosphine he said, is not as good dissolved in water, as ammonia and hydrogen sulphide. In fact, he showed, it escapes from the first moment it arises.

"*The former gas [hydrogen sulphide] is therefore twenty four times more soluble than the latter [phosphine]*" (Bowditch, 1856).

And he stated it is the oxide of iron and of alumina and silica of the earth which binds these precious elements. For this he refers to the experiments of Way and Thompson, and to Liebig. He pointed especially to the role of hydrogen ions which catch nitrogen, sulphur, carbon and phospor in a very agressive way, before emitting. He didn't make clear where the hydrogen comes from. I think he supposes it comes from the degradation of proteins.

The main mistake, he says, which is made is that men wants to see quick results, for instance by heating the compost heaps, but nature never works quickly. Processes in nature are always slow. So we should also work slow. Only then we can keep the valuable elements in the residual heaps. Earth, he argues, helps us to work slowly. And he reminds us that the normal treatment of manure heaps and compost heaps by the farmers is such that the precious elements are lost, and in spring these farmers buy the same lost materials from the fertilizer industry:

"What renders the case more noticeable, is, that the burning is the worst[*] *when the evaporation is the greatest, and no spectacle is more familiar to an observer of the fermentation of manure than a cloud of white vapour which completely conceals the workmen who are removing a heap of "firefanged"* [†] *horse dung.*

But every particle of that exhaling moisture was designed by a beneficent Providence to be condensed into a liquid charged with the precious burden which it is now bearing away on the wings of the wind. Elements of corn and cattle are volatilizing with every grain of the steam, and (in towns) are becoming sources of disease and death[‡] *to those whom, if differently managed, they might feed! And why? Simply because man will defeat nature.*

Nature designed putrefaction (combustion) to be slow, and to that end required all decomposing refuse to be buried, in which case its slow but useful conversion was certain. Man on the other hand places the waste substances so that the combustion may be rapid. He employs the light porous material straw to mix with animal excreta, and places the whole so as to ensure a free passage of oxygen among the putrefying mass" (..). [Through the heating the water in the heaps evaporates…]

"But suppose all the water had been retained by the heap. Suppose the oxygen had been supplied to the decaying mass as it is supplied in the soil, abundantly but yet slowly, would there have been any firefang, or would the ammonia and other valuable products have flown away almost as quickly as they were generated? We are always wrong when we can perceive a law of nature and do not conform to it" (Bowditch, 1856: 328).

But not only from farmyard manure and warm compost a lot of nutrients are lost. The same is true for articial fertilizers.

[*] Here Bowditch means, 'that the burning is the strongest..'

[†] Firefang is, I think, something like a smoldering fire. In the modern dictionaries, I couldn't find a translation of firefang.

[‡] Bowditch gave in his tekst an example of his son who got 'typhus' when he got too much of this smelly odors from a manure heap.

In 1856 lawes and Gilbert, two english scientists, published the results of their research into the partial utilization of nitrogen:

"*As a final average it is seen that we have, including all these cases and extending over so many years, in the case of wheat, only 39.9 per cent., and in that of barley only 43.1 per cent, of the nitrogen of the manure recovered in the increase of crop ! (..) So much, then, for the indications of some hundreds of direct experiments on this subject. But we further unhesitatingly maintain, that the general result here arrived at, agrees very closely indeed with that of common experience in the use of guano and other nitrogenous manures for the increased growth of grain*" (Lawes, Gilbert, 1856: 484-485).

In 2017, Yuan and Peng came to similar conclusions for China (Yuan and Peng, 2017): In 1961, when nitrogen fixation and animal dung were the most important sources of nitrogen in China, 59.4 % of all the nitrogen which was brougth to the land was utilized by the crops. In 2012, when artificial fertilizer was the main nitrogen source only 37.5 % was utilized by the crops. The remaining 62.5 % goes in the environment, resulting in dead zones in lakes and seas, in the disappearance of rare plant and animal species in nature and in the pastures and fields, and in pollution of drinking water.

The biggest part of the 37.5 % nitrogen which is taken up by the crops also ends in the environment after being eaten by men and animals, from the slurry pit, the deep litter stables, the compost heaps, slaughter residuals, and the sewage treatments. All three are ideal places for emissions. So roughly about 90 % of the nitrogen fertilizers is lost in one cycle. Jones also comes up with losses of 60-90 % (Jones, 2015).

And the losses from farmyard manure at Rothamsted are even higher:

"*The measurements and calculations in these tests are all so simple in kind that they can hardly be distrusted or devalued. They are simply matters of sampling and analysis and multiplication by the total weights involved. The nitrogen content of the top nine inches of soil has been considered. Figures given are those for an acre. On one plot the only manuring was with farmyard manure each year. This gave to the soil 201 pounds of nitrogen per year. To this supply must be added 7 pounds to cover the rainfall contribution and the nitrogen in the seed sown. So, each year, the soil totally received 208 pounds of nitrogen. But each wheat crop removed 50 pounds of nitrogen per year. So the soil should have gained 158 pounds annually.*

In 1865 the total nitrogen content in the soil per acre was 4,850 pounds; and, by 1914, it was 5,590 pounds. A gain in forty-nine years of 740 pounds, which works out at only just about 15 pounds per year. The theoretical annual gain of 158 pounds is reduced in fact to a mere 15—so there was, therefore, an average loss of 143 pounds of nitrogen per year". (Hopkins, 1956).

That is a yearly loss of 71 %. Even more than from artificial N fertilizers during cropping.

The positive effect of farmyard manure for carbon sequestration in the soil is another question. Although lots of carbon (and nitrogen) are lost in the stable or during composting and in the field the net result for fertilizing with farmyard manure is that the organic matter in the soil stays on level and the same applies for nitrogen during a fifty years trial:

"*At Woburn, continuous cereal cropping from 1876 to 1926 showed a 33 per cent loss in organic matter content where only fertilizers had been used—but little loss where large applications of farmyard manure had been regularly given. This comparison was worked out from the figures for carbon content and nitrogen content in 1876 and in 1926, it being assumed that the organic matter content was fairly proportional to these figures. These were the figures*" (Hopkins, 1956) (Table 2).

Table 2. Carbon and Nitrogen content in the soil under different fertilizing schemes

Fertilizer	Carbon content percentage		Nitrogen content percentage	
	1876	1926	1876	1926
Manured* plot	1.48	1.5	0.155	0.15
NPK plot	1.48	1.0	0.155	0.09

* Manured with farmyard manure.

As Hopkins remarks, in both ways of fertilizing the C/N ratio stays 10 : 1, but the artificial fertilizers give a reduction of carbon ánd nitrogen. The yields, and the total amounts lost are something completely different. (See also Appendix 2 for the nitrogen content in the soil from farmyard manure).

So in 160 years we can say there is no improvement as far as effective use of nitrogen is concerned. And the losses of carbon, potassium, phosphor and sulphur into the air and soil are not even mentioned in many government proposals. Phosphor into the groundwater is the exception.

At the moment, in the Netherlands the goverment has reduced the whole question of losses from the manure into losses of ammonia as far as agriculture is concerned. The measurements of my colleague showed that also hydrocyanic acid (HCN) evaporates from the slurry. And of course there is the loss of laughing gas, NO_x, and N_2. And are there other volatile nitrogen compounds, which we miss while measuring because our equipment can't measure them? Casey sums up many of these compounds (Casey et al., 2006). And sometimes you get the feeling that science and the goverment don't want to measure them at all, because more lost nitrogen means more political problems.

And the losses from the great compost factories, from the sewage treatments or from the processing of cadavers and slaughterhouse residuals are not even mentioned.

According to Hao (Hao and Benke, 2008), from 13 % up to 70 % of the total nitrogen from the beginning of the composting proces can be lost as ammonia. Other losses are laughing gas, nitrate and inert nitrogen (N_2). Hao proposes many interesting methods to diminish these losses:

- Extra straw or woodchip for increasing the C:N ratio;
- Less turnings and smaller heaps;
- Acidification by adding phosphoric acid, $MgCl_2$ or $Al_2(SO_4)_3$; S;
- Binding ammonia with coir, zeolite or peat;
- And the adding of magnesium and phosphor salts for forming Struvite.

Many of these proposals were already made in the 19th century. But then the conclusion was that the simplest, cheapest and best way was adding earth.

And as we know now this old method can be combined with worms to make vermicompost. Pfeiffer saw thousands of worms in these with earth enriched heaps. He didn't even have to add them (Pfeiffer, 1936).

• **Growth inhibiting substances.**

In the period 1905–1915, Schreiner[*] and his colleagues drew up a list of about 20 growth inhibiting substances that are released from the organic conversion or decay processes in the soil. Schreiner suspected that this was due to too little oxygen in the top layer. He identified important growth inhibition through the following substances: dihydroxystearic acid; aldehydes; guanidine; coumarin etc. Dihydroxystearic acid could be detected in all soils with sufficient nutrients yet with poor growth. Laboratory tests confirmed the growth-inhibiting properties of this acid. Guanidine is especially interesting as it is part of urine[†]. The experiments of Schreiner demonstrated that guanidine inhibits the uptake of nitrate, potassium and phosphor:

"*The total phosphate, nitrate, and potash removed by the normal plants was I,608.9 milligrams, against only 1,088.5 milligrams in the guanidin set. The phosphate removed was 427.3 milligrams in the control and 287.0 milligrams in the guanidin set; the potash was 723.7 milligrams for the control and 496.7 milligrams for the guanidin set; the nitrate was 457.9 milligrams for the control cultures and 304.8 milligrams for the guanidin cultures" (..)*

"Guanidin, as carbonate, is shown to be harmful to wheat, corn, cowpeas, and potato plants. It produces an effect similar to a physiological disease. The plant is normal for a few days, then begins to show a spotted appearance on leaf and stem. This effect develops until the plant is

[*] In addition to research into growth-inhibiting substances, Schreiner has also extensively researched which organic nitrogen compounds promote growth. For example, he and his colleagues have extensively tested the following growth-promoting substances: histidine, creatinine and arginine, as well as hypoxanthine, xantine and the nucleic acids. I have written a separate article about the work of Schreiner and his colleagues. In Dutch (Nigten, 2019).

[†] In Wikipedia we find: "*Guanidine is the compound with the formula $HNC(NH_2)_2$ It is a colourless solid that dissolves in polar solvents. It is a strong base that is used in the production of plastics and explosives. It is found in urine as a normal product of protein metabolism"* Source: wikipedia english: keyword: guanidine.

bleached to a considerable extent, with final collapse. This harmful effect of guanidin on plants is augmented by the presence of nitrate and increases with the amount of nitrate present". (Schreiner, Skinner, 1912b).

Especially in combination with nitrate, guanidine was thus very harmful for the wheat plants in this trial. When nitrogen was given as asparagin or creatinin the harmful effects of guanidine did not occur.

Forty years later McCalla and his colleagues continued the work of Schreiner et al. They came to similar conclusions:

"*Soil microorganisms produce a tremendous variety of organic substances during the decomposition of plant and animal residues, and, as numerous studies have shown, some of these substances are phytotoxic. For example, Swaby found that, when soil micro-organisms were present in association with plant residues (lucerne and Phalaris tuberosa), substances inhibitory to plant growth were frequently produced*" (McCalla, Haskins, 1964: 192).

And they validated also the conclusions of Schreiner regarding growth stimulating organic compounds:

"(..)Although green plants can live autotrophically, it is apparent that under suitable conditions they can also live heterotrophically, absorbing organic compounds from their surroundings and metabolizing these compounds. Plants grown in the soil are normally exposed to a tremendous variety of organic compounds which have come directly from plant and animal residues in the soil, or indirectly from these residues through the action of soil micro-organisms. Depending upon their nature and concentration, and on the kind of the plant being grown, these substances may be innocuous, stimulatory, or inhibitory to plant growth" (McCalla, Haskins, 1964: 202).

Kononova found exactly the growth inhibiting substances which Schreiner had found earlier (Kononova, 1961, cited by McCalla, Haskins, 1964).

Krasil'nikov found out that the wintertime is important for a further breakdown of toxic/growth inhibiting compounds and the subsequent growth of bacteria:

"According to our observations, the microbial activity does not always cease in winter. Under a deep snow cover the earth is not always frozen and in such a soil microbiological processes take place. (..) Korenyako has shown that during the winter months of 1952–1954 certain species of actinomycetes (A. globisporus) grew more abundantly, in Moscow Oblast' soils, than during the summer and autumn.

Besides, certain biochemical processes, leading to detoxification of the soil take place in winter" (Krasil'nikov et al., 1955).

"The vigorous growth of microbes in spring is, according to our opinion, not only caused by the warm temperature and by moisture, but also by other factors. First, the toxins are inactivated or decomposed in winter due to low temperature. Second, low temperatures, as was noted above, stimulate the growth and activity of microbes. in addition, many soil nutrients under the action of low temperature, change and become more available to microbes" (Krasil'nikov, 1958: 131, 132).

Bowditch also argues that the manure – enriched with earth and salt[*] – be spread in autumn and winter. Not in the spring. And with practical examples he shows that the yields are better than in the case of spreading the manure in the spring.

And Bowditch points to another advantage: if farmers fertilize in the fall and winter, the grasses start to grow in march. They develop their roots underground, invisible. And the farmers can harvest their grass in June. May and June are the best months because most grasses are in blooming in these months – so it is the time to mow. Mowing in July is too late. The grass quality is then less, more woody and less nutritive, and the chance of rain is much greater.

"*Early growth [of grass] is secured by having all the elements of nutrition thoroughly incorporated with the soil amongst the roots of the grasses, in such a state of decomposition that*

[*] Common salt was a normal fertilizer these days. Bowditch: *"Common salt is included in every manure here recommended, because experience has shown its beneficial action upon grass whenever it has been properly applied, and because the analyses show that grass always contains both its elements, chlorine and sodium.*
The quantity recommended is 1 cwt. per acre [roughly 100kg/ha], which Is more than a sufficient addition to the natural supplies for the largest crop of grass we can imagine as being reaped from an acre" (Bowditch, 1858).

they can be taken up by the roots as soon as the temperature of the earth and the air stimulate the plants into activity. That this occurs very early is certain, for by the following table we perceive that many of the grasses reach maturity in May, and most of them before the middle of June, and therefore we may safely assume that the manure should be well incorporated with the soil (not lying upon its surface), and sufficiently decomposed there to act efficiently in the middle of March" (Bowditch, 1858).

In the same article, Bowditch gives an overview of the grasses, which farmers sowed these days: 21 in total, and of these there is only one flowering in July. Ryegrass is not on the list.

Manuring with stable manure in the summer is not recommended by Bowditch. The reason is interesting. In some districts, Bowditch saw farmers spreading solid manure in the summer. Then the grasses dwarfed, which didn't happen when spreading the same manure in the winter. Liquid manure is another story. He says this manure can be spread in the summer, but only if it is regularly alternated with fertilizing solid manure. Otherwise, liquid manure* results in a few sturdy grasses. The other grasses disappear:

"*My experience* [the experience of farmer Bywater in Leeds, cited by Bowditch]*, however, in the use of tank liquor as a dressing for grassland resulted in the discovery that by its exclusive application in successive years a very strong grass was produced, which appeared to destroy by its rankness the white clover and all the finer and more delicate kind of grasses; whilst a return to the use of farmyard manure, as a change, restored all the various kinds which had usually grown before*" (Bowditch, 1858).

And a last remark of Bowditch in this article concerns the use of sewage. It contains too much water, and the grass will rot quickly if not mown frequently, according to Bowditch. Bowditch advises to let it run into the oceans:

"*It is far better that the sewage of towns should run to waste into the ocean, than that our cultivators should apply it to the land and lose money by the application*".

But he also offered a better solution. Not only for liquid manure from the farms but also for human excreta. For the liquid manure, he advises to mix it with earth and finely sifted ash and 'other materials the farmer can command'. And for human excreta – the best manure for grasses – he advises the sprinkling of clay over it:

"*The present absurd water-carriage of excreta must be abandoned, and sewers employed for their legitimate purpose, viz., to carry away waste water to its natural receptacle the river. Moveable boxes should be attached to every house, and removed weekly in summer, fortnightly in winter. A cistern filled with dry pounded clay would be placed overhead, and a simple mechanical contrivance would throw down a measured quantity of this every time the handle was raised as water is now let down a closet. Nature's deodorizer and disinfectant would prevent the escape of injurious exhalations, and the refuse would be removed by water or other carriage some miles into the country, to await under sheds the farmer's season of use*" (Bowditch, 1858).

Later on in the UK this 'earth toilet' or 'earth closet' was really used. These were evaluated by a.o Voelcker. He published his very interesting results in 1872. Moule, he writes, had constructed, and patented several forms of earth closets. One of his conclusions was that earth toilets were only a good solutions in small towns and villages and for poor people. The earth in these toilets were only used to mix with excreta. Not with the urine. And this excreta rich earth had not a great fertilizing value. But the managers of prisons were very content with the earth toilets. All stench was gone now, and the hygiene in the prisons was much better. The urine in these facilities was collected in great tanks and sold. In his article are many interesting data, among others about the average composition of human excreta and urine in these days (Voelcker, 1872).

Future research.

Bowditch and Lawes have both a chemical view on the processes in the soil. They only talk about mineralization. Krasil'nikov points to the microbes: during the winter cold, the nutrients come available to the microbes (Krasil'nikov, 1958). An interesting question for future research is why the nutrients come available in the winter cold, and how the microbes consume their food in the winter. Do they eat organic compounds directly or do they mainly absorb inorganic nutrients? Or both? Fact is that Krasil'nikov points us to the microbial dimension of all the changes.

* I suppose that liquid manure in these days was muck water or aalt, and not slurry.

The symbiotic phase.

At some point, after decay, the symbiotic phase begins in the soil. Under the influence of the poisonous and growth-inhibiting substances, in most farms in spring it gets going with fits and starts. The manure, spreaded in the spring, must first digest for a long time, the toxic components must be broken down, and the soil must also heat up sufficiently. And the symbionts must gain the upper hand over the putrefactive bacteria.

In turn, in spring, the symbionts must be fed with all kinds of root exudates from the plants in order to put them to work. The plants will probably grow slowly under the influence of the growth-inhibiting substances. The symbionts themselves also suffer from the ammonia, nitrate, hydrogen sulfide, phosphine, hydrogen chloride and all other secretions released by the work of the decay bacteria.

Paul et al. (2007) reported the following for the nitrogen fixers:

"In addition, mineral nitrogen availability is known to reduce nitrogen fixation rates (Sougoufara et al., 1990; Zuberer, 1998; Dianda, Chalifour, 2002) *and therefore TEM [Tuberculate Ectomycorrhizae, author] occurring in stands with high nitrogen availability may display lower nitrogenase activity "*(Paul et al., 2007).

For the negative effects of ammonia and nitrate on nitrogen fixation, see also Lawes and Gilbert (1858), Reich et al. (1987), Poschenrieder and Lesch (1942) and Pfeiffer (1936).

But Jiang et al. (2020) differentiated into the amount of nitrate in relation to nitrogen fixation:

"Our results showed that small amounts of nitrate (2.5 and 5 mM) promoted nodule formation and increased nodule biomass[*], compared with plants in the 0 nitrate control treatment. In contrast, nitrate concentrations over 10 mM inhibited nodulation, resulting in reductions in nodule number and nodule biomass. Nodulation was completely inhibited by 15-mM nitrate in all the genotypes. Regression analyses indicated that 5-mM nitrate is the optimum concentration for promoting nodulation as measured by the total number of nodules formed, the number of effective nodules formed, and the nodule biomass formed". (Jiang et al., 2020).

According to Jones (2015), five kilogram nitrogen per hectare is the maximum you need for the support of nitrogen fixing bacteria, after a gradual reduction of artificial nitrogen fertilizer in say 3 years.

According to her fertilizing with higher amounts of inorganic nitrogen is not good for soil microbial life:

"*When inorganic nitrogen is provided, the supply of carbon* [from the plants, author] *to associative nitrogen fixing microbes is inhibited, resulting in carbon-depleted soils. Reduced carbon flows impact a vast network of microbial communities, restricting the availability of essential minerals, trace elements, vitamins and hormones required for plant tolerance to environmental stresses such as frost and drought and resistance to insects and disease*" (Jones, 2015).

The symbionts themselves must also have the right 'instruments' to convert the present organic and inorganic nitrogen compounds into compounds that are suitable for their own use. Microorganisms digest the material present. And for that, like plants and animals, they need enzymes. Cofactors are indispensable for enzyme formation. And these cofactors are the trace elements and some macro-elements like magnesium. These are becoming increasingly rare and/or they are no longer released from the soil particles. The latter is an important task of the mycorrhizae. But these have disappeared due to the superphosphate and unbound ammonia, nitrate, phosphine and hydrogen sulfide. Mycorrhiza and rhizobia use the same signaling mechanism.[†]

The work of Poschenrieder and lesch gives information about the influence of the different fertilizer combinations on root nodules:

Poschenrieder gives also an overview of authors, their findings and explanations from the beginning of the 20th century for the negative influence of nitrogen on nodulation.

---

[*] Poschenrieder and lesch demonstrated that the biggest nodules do not have the highest nitrogen fixation. The smaller nodules gave higher yields (Poschenrieder, Lesch, 1942).

[†] "During evolution, the genetic programme for AM has been recruited for other plant root symbioses: functional adaptation of a plant receptor kinase that is essential for AM symbiosis paved the way for nitrogen-fixing bacteria to form intracellular symbioses with plant cells"(Parniske, 2008).

According to Rippel, cited by Pochenrieder and Lesch, the explanation of the inhibitory effect of nitrogen fertilizers on N fixation is the shortage of carbohydrates:

"*Heute wissen wir aus neueren Untersuchungen von Rippel, dass die geschwachte Knöllchenbildung der Leguminosen infolge Zufuhr von gebundenem Stickstoff mit einer Festlegung bzw. erhohten Beanspruchung der Kohlenhydrate durch die Pflanze, die sonst zur Ernahrung der Bakterien dienen, bei der durch die Stickstoffzufuhr gesteigerten Eiweissbildung in Verbindung zu bringen ist*"

And he summarises the results of the field trial in Bernburg as follows:

"Die Stickstoffdüngung übte eine stark hemmende Wirkung auf die Knöllchenbildung aus, die insbesondere bei der einseitig mit Stickstoff gedüngten Versuchsreihe sowie bei der Stallmist plus NPK-Düngung sowohl in einer Abnahme der Knollchenzahl als auch des Knollchen-gewichtes in Erscheinung trat" (Poschenrieder, Lesch, 1942). This is very visible in Table 3.

Table 3. Nutrient intake in the nodules of in total 100 plants*

Manuring	K_2O, mg	P_2O_5, mg	N, mg
Control	459	354	1525
N	109	100	437
P	886	649	2790
K	551	41 7	1751
PK	883	631	2710
NK	174	142	645
NP	371	324	1488
$NPK(NH_4)$	263	197	809
$NPK(No_3)$†	239	176	750
FYM	525	368	1682
FYM+PK	467	303	1272
FYM+NPK	142	102	435

My conclusions based on the amounts in milligrams (whereby the N in the nodules is from N fixation):

1. The highest N fixations is in the treatments with pure P and P + K.

P+K gives somewhat less than pure P.

2. The least N fixation – 435 – is with the most complete fertilizer: FYM + NPK. Followed by pure N (437) and NK (645) respectively;

3. NP gives a N fixation of 1488 mg – 47 % and 45 % less than pure P and PK respectively;

4. K, FYM , FYM + PK, and NP give comparable fixation yields. But in fact these N-yields are almost no yield. Because only K and FYM give somewhat higher N Fixation yields than the Control (1525);

5. Compared to the Control N, NK, NP, $NPK(NH_4)$, $NPK(No_3)$ FYM + PK, and FYM+NPK give a negative N fixation yield.

So, ammonium, nitrate and [FYM + NPK] are counteracting N fixation. Only farmyard manure, and pure potassium give a somewhat higher N fixation than the control.

Phosphor is the real stimulans for nitrogen fixation. Especially N is counteracting it and even K is somewhat counteracting: PK gives less N fixation than pure P.

The yields are very interesting for my research into the causes of the lower yields in organic agriculture. Wabersich has published the yields for potatoes in this long term trial in Bernburg (Wabersich, 1967).

These results are in accordance with those of Lawes and Gilbert (1858). In a three year trial with seventeen different treatments lawes investigated what were the differences in yields and qualities in meadowland. One of their findings was that the legumes grew very well on the

* Here I have left out the columns with the nutrient contents (percentages) of the original table in Poschenrieder and Lesch (1942).

† Sodium nitrate or Chilisalpeter.

treatment with only minerals (K + Na + P + Ca +S + Mg; no ammonium, nor nitrate), and that in all the treatments with nitrogen, the grasses grew well, but the legumes disappeared almost completely, and very quick:

“That the effect of a mixed, but purely mineral manure, upon the complex herbage of permanent meadow land, was chiefly to develop the growth of the Leguminous plants it contained ; and scarcely at all to increase the produce of the Graminaceous plants, or commonly called Natural Grasses.

That the action of purely nitrogenous manures, upon the permanent meadow, was to discourage the growth of the Leguminous herbage, and to increase the produce of the Graminaceous hay.

That by the combination of both nitrogenous and proper mineral manures, the produce of Graminaceous hay was very much increased. In the particular soil and seasons in question, the increase obtained by the combination was far beyond the sum of the increase yielded by the two descriptions of manure, when each of them was used separately.

That farmyard manure gave a considerable increase of chiefly Graminaceous hay. In the soil and seasons in question, however, the artificial combination of nitrogenous and mixed mineral manure yielded a very much larger increase than an annual dressing of 14 tons of farmyard manure. (pp. 571-572).

(..) In fact, where the ammoniacal salts were employed, the increase was exclusively due to the increased growth of Graminaceous plants—the so-called Natural Grasses – there being scarcely a Leguminous plant to be found upon the plot (page 561).

(..) Indeed, notwithstanding the large amount of mineral constituents, and especially of silicious compounds, contained in the cut wheat-straw[*], as compared with the sawdust, there was, whether compared with the produce by the mixed mineral and nitrogenous manure, or with that by the mixed mineral and nitrogenous manure and sawdust, an average annual deficit of 4 to 5 cwts. of first-crop hay, where the cut wheat-straw was employed (page 563).*

(..) It will be shown, on a future occasion, that the percentage of nitrogen in the dry substance of the hay, grown both by ammoniacal salts alone, and by nitrate of soda alone, was comparatively very high—in fact, considerably higher than when the mineral manures were also employed, whereby the Graminaceous produce was much increased. So far then as there was an excessive amount of nitrogen, in the form of elaborated nitrogenous vegetable compounds, where the supplied nitrogen was liberal—the mineral constituents in defect—and the growth restricted thereby—it was that there was a relative deficiency in the formation of the nonnitrogenous vegetable substances (page 566).

(..) But it may be here stated in passing, that the crop grown by the larger amount of ammoniacal salts—supplying as it did the enormous quantity of 200 lbs[†]*. of ammonia per acre per annum—was so over-luxuriant, as to be much laid, matted together, 'and dead at the bottom, some time before the bulk was ready for cutting” (page 564)* (Lawes and Gilbert, 1858).

The farmyard manure in these trials was spread in november and december, the previous year.

Not only nitrogen salts form a risk for plants. The same applies to other plant nutrient salts like potassium chloride (Khan et al., 2013) and superphosphate (Jamieson, 1910). In fact, this is true for all easily soluble salts[‡]. NPK dominates in almost all fertilizer programs. Often combined with chloride, calcium and/or sulphur, and sometimes with magnesium also.

---

[*] In this treatment – plot 12 – lawes and Gilbert had given 2000 lbs cut wheat straw plus 200 lbs ammonia sulphate and 200 lbs ammonia murate. This result is comparable with that of McCalla whos also saw a negative influence of wheat straw on the yields (McCalla, Haskins, 1964). But McCalla noticed also a simpler explanation, namely that the wheat straw took part of soil nitrogen for its breakdown bij microbes. But his own research showed also the toxic compounds in wheat straw (Guenzi, McCalla, 1962).

[†] In fact it was not 200 lbs of ammoniacal salts but 400. There was no treatment with 200 kg, only 400. And even one – T 13 – treatment with 800 kg of the two ammoniacal salts. In fact, the tekst is somewhat confusing. In the overview lawes writes: ‘200 lbs, each, Sulphate and Muriate Ammonia”. From the tekst I concluded that you must read this as: 200 lbs sulphate ammonia, plus 200 lbs muriate ammonia.

[‡] Seasalt can be given as a folium fertilizer provided that less than 2000 ppm is given. In coastal areas – max 25 kilometer from the sea – seasalt, brought by the wind and rain from the seaside, precipitate. I don’t know

Vitamin C

An additional effect of using nitrate or ammonium is that the plants form less vitamin C (Visser, 2010). Vitamin C is an important compound for the natural resistance of plants (Locato et al., 2013), just as in animals and human beings. Visser quotes Wittwer's research here:

"In the 40s a broader recognition wins ground that not all is well with nitrogen fertilizer use, eg Wittwer et al. (1945), giving 'evidence of an inverse relationship between the concentration of vitamin C in plant tissue and nitrogen supplied as fertilizer" (*Wittwer, 1945; Visser, 2010: 193*).*

This sheds new light on the question of why the differences in vitamin C content between conventional and organic agriculture are so small in many studies (Bourn and Prescott, 2002; Wunderlich et al., 2008). If the organic crops do indeed grow at the moment for the biggest part on mineralized nitrogen, as supposed by their scientists, little difference can be expected in this respect from the crops in conventional agriculture. All the more reason to maximize plant growth with the help of organic nitrogen.

Vitamin C was also measured in a TNO/WUR study in 2007 when comparing conventional and organic chicken feed (corn, peas and wheat). The organic food had 10 % less protein and less vitamin B5 and vitamin C.

And these were the results from a study by the same organization in 2006. In this study, the differences for vitamin C and nitrate in 15 organic and conventional vegetables were statistically not significant. Although there were big differences for some vegetables. Only statistically significant differences were found for the dry matter content and dietary fiber. Both were higher in the organic products (Kramer, 2006).

But a more recent international meta-study gives a different result. Especially for flavonoids and carotenoids, organic scores remarkably well here:

"A few years ago, the results of more than 300 studies into differences in the composition of organic and non-organic vegetable foods were listed. Organic products were found to contain more antioxidants, such as 19-69 % more flavonoids, 17 % more carotenoids and 6 % more vitamin C. Remarkably, the content of vitamin E, also an antioxidant, was just 15 % lower. Antioxidants protect body cells against damage and may therefore reduce the risk of diseases " (Rolvink, 2019).

Though, according to Dangour et al. (2009) there are no differences between organic and conventional crops, except for nitrate, magnesium and zinc, phytochemicals and sugars.

"In analysis including all studies (independent of quality), no evidence of a difference in content was detected between organically and conventionally produced crops for the following nutrients and other substances: vitamin C, calcium, phosphorus, potassium, total soluble solids, titratable acidity, copper, iron, nitrates, manganese, ash, specific proteins, sodium, plant non-digestible carbohydrates, β-carotene and sulphur. Significant differences in content between organically and conventionally produced crops were found in some minerals (nitrogen higher in conventional crops; magnesium and zinc higher in organic crops), phytochemicals (phenolic compounds and flavonoids higher in organic crops) and sugars (higher in organic crops).

---

if other salts also can be given as foliar fertilizer. In general you can say that organically bound elements are safer than salts.

* In 1947 Wittwer published a follow-up study on vitamin C and fertilizer for peaches (Wittwer, 1947). Comis (1989) descibes the research findings of Sharon Hornick. And he states: "*Reports of decreasing vitamin C with increased use of nitrogen surfaced in the 1940's with studies on grapefruit. But the availability of cheap nitrogen fertilizer in the 1950's suppressed concerns about quality in favor of yields. Hornick says she has seen renewed interest in crop quality in the 1980's as farmers search for ways to cut chemical use, both to save money and to prevent possible pollution of the groundwater*". The influence of ammonia and nitrate on the other vitamins is as far as I know not investigated. Weston Price compared traditional and modern Western dairy products, and stated that the levels of vitamin A and K2 are much lower in the products in the western countries (Masterjohn, 2008). But Weston Price did not investigate the influence of artificial fertilizers. Vitamin K2 prevents, together with vitamin D and vitamin A the calcification of the weak parts of our body, and they protect at the same time our bones and teeth against discalcification. In the dutch butter the vitamin A content is today indeed low. The milk contains 2,5 times less vitamin A, according to data from Friesland Campina, the dutch Dairy cooperative. The Dutch butter has no longer its typical yellow color in the spring.

In analysis restricted to satisfactory quality studies, significant differences in content between organically and conventionally produced crops were found only in nitrogen content (higher in conventional crops), phosphorus (higher in organic crops) and titratable acidity (higher in organic crops)" (Dangour et al., 2009).

For livestock products there were found differences for some fats and fatty acids. These were higher in organic products, The same for nitrogen in these products.

De Waart (1998) comes to a somewhat different conclusion for nitrate and vitamin C in organic and conventional products than Dangour:

- Nitrate in organic products is the same or lower than in conventional products;
- Vitamin C in organic products is the same or higher than in conventional products;

And based on some studies she concluded that more nitrogen fertilizer resulted in higher nitrate contents and lower vitamin C contents – an inverse relation.

"As a consequence of a lower nitrogen supply[*] [in organic agriculture, Author] *and the slower availability of it (..) is the ripening phase longer compared to the growth phase. This is reflected in a lower yield, a lower nitrate content, a better taste and a higher vitamin C content"* (de Waart, 1998).

See also Rosen (2010) and Magkos et al. (2006). Rosen made an overview of the health claims by the proponents of organic food. He was not convinced that these claims are right. The debate goes on.

• **Imbalance of the cations**

Furthermore, an excess of potassium impedes to a certain degree the absorption of divalent cations, such as calcium, magnesium, boron (Rinsema, 1981: 81), manganese, zinc and iron[†], but also of the monovalent sodium (for sodium see Arney et al., 1995). On this basis, I suspect that too much potassium also slows down the absorption of the other monovalent and divalent trace elements. Van Baren for instance states that too much potassium inhibits the absorption of silicon (Van Baren, 1934). As a result, the wheat plants at that time got limp stems and there had to be bred short-stemmed varieties. A plant needs enough silicon to protect itself and to keep the stems straight.

Also ammonium hinders the uptake of divalent cations (Britto and Kronzucker, 2002). Because potassium and ammonium are often given together, and because ammonium and potassium use the same entrance in the root cells, it is not clear if the reduced absorption of the other cations is caused by potassium or by ammonium, or by both. Fact is that in many crops the level of potassium is extremely high, and calcium, magnesium and sodium are relatively low (Nigten, 2019c).

German organic dairy farms.

Based on thirteen years of measurements of the grass of organic dairy farms throughout Germany, we know that on average these farms have too much potassium in the grass and too little sodium, calcium and magnesium.

The deviation of the German organic dairy farm silage from ideal ratios is particularly large for potassium. Also the spread is great. The content of potassium in the silage is from 2.5 to 53.8 g potassium/kg DM, with an average of 25.3 g potassium/kg DM. The spread for the other elements was significantly smaller. In 2014, the Dutch average for 1854 dairy farms was 35.2 grams potassium/kg DM. Here too, outliers of 5 to 55 grams potassium/kg DM (DMS, 2015).

The ratios in this German Bio-grass silage are as follows (Table 4).

[*] In many cases we see that the total amount of nitrogen given in organic agriculture is not necessary lower than in conventional agriculture. It is indeed slower to be available, as de Waart states.

[†] The knowledge about the reduced absorption of zinc, manganese and iron due to an excess of potassium is based on practical experience in Dutch greenhouse horticulture. That is why they provide extra zinc and iron in their hydroponics. The Benton practice guide confirms this (Benton, Jones, 2004*). "High concentrations* [of potassium] *interfere with the function Fe, Mn, and Zn. Zinc deficiencies often are the most apparent".* The Mulder map contradicts this. However, this card concerns normal doses (Nigten, 2019c: 22). A high phosphate concentration can also inhibit the absorption of iron and zinc (Royal Brinkman, 2020).

Table 4. Organic dairy farms in Germany. Results for silage (Grünlandsilage) 1997-2010 (data from Leisen, 2011)

The average amounts in the silage (g/kg DM)	Ratio of cations	Ideal ratio's*	Real ratio's in the german silage
	K / Mg	2-5	14.88
K = 25.3	K / Na	2-4	25.3
Na = 1	Ca / Mg	1-2	4.05
Mg = 1.7	Ca / P	1-2 / 1-3†	2.23
Ca = 6.9	Mg / (K+Ca+Na+P)	0.10-0.25	0.046
P = 3.1	N / S	12-15	Unknown. N and S have not been measured

So sodium and magnesium are too low in these grass silages, and potassium is much too high. Sodium and magnesium are necessary for a good conversion of non-protein nitrogen (NPN) and non-protein sulphur (NPS) in real proteins (Chiy, Phillips, 1993). Too much NPN and NPS in fodder and concentrate are a heavy burdening of the liver and the kidneys of the animals. So the imbalance of the cations has a direct effect on the grass quality and the health of the animals.

In a Swedish study was shown that the cattle in organic farming get even much more too little sodium than cattle in conventional farming. The cows on organic farms in Sweden need on average 17 gram per day extra salt against 3,3 to 6,6 gram on conventional diets. 17 gram per day is – for health reasons – too much via the licking of salt (Johansson, 2008). So for these cows salt fertilizing in the pastures is necessary. Salt through the ryegrass has many advantages:

"*Increased herbage digestibility, promoted growth of bacteria that digest fibres in the rumen,*

increased milk yield and decreased somatic cell count are results of using sodium as a fertiliser according to one author" (Johansson, 2008).

Nitrogen and sulfur in the German biogras silage have not been measured, nor their specific compositions. That is an important omission. The levels of non-protein-bound nitrogen and sulfur (NPN and NPS‡) in particular say a lot about the quality. Inorganic N fertilization weakens the plants, comparable to atmospheric N deposition:

"*According to Phoenix et al.* (2012)*, [inorganic] N deposition leads to an increase in the susceptibility of plants to secondary stresses, i.e. increased herbivory, reduced resistance to attack by pathogens or increase in susceptibility to drought or freezing damage"* (cited by Anjana, Umar, 2018: 5).

The iron and manganese levels in the German silage are (very) high, and the copper, selenium and zinc levels are (too) low. Selenium is very low. But the spread is great for all trace elements.

Also for the trace elements, the grass silages are out of balance.

In the next article, I will elaborate on the consequences.

3. Conclusion

Beside N fertilization with ammonia, nitrate or urea plants can collect organic nitrogen with the help of symbiotic microbes, or directly by themselves without this help – in particular the plants who naturally don't have mycorrhizal symbionts. The symbiotic microbes, which collect or produce nitrogen (and other anions), are living inside the plants or outside. Many of them are

* To substantiate these optimal ratios, I refer to an article of my hand from 2018, "Reinventing agriculture" (Nigten, 2018).

† Some authors believe that a Ca/P ratio of up to 3 does not yet lead to problems (Bredon, Dugmore, 1985). Most assume a ratio maximum of 2 (ARC, 1980).

‡ NPN and NPS are abbreviations for Non Protein Nitrogen and Non Protein Sulphur – jargon for nitrogen and sulphur compounds which are not proteins. Maybe also phosphor should be protein-bound.

nitrogen fixers but there are also symbionts which free the nitrogen from the soil particles, from the humic and fulvic acids or other organic matter and bring it into the plants. The source of the nitrogen, which these symbionts consume, can be organic or inorganic, but after the consumption by these microbes, all the nitrogen is organic: microbial protein.

In all these cases, except for the fertilization with nitrogen containing salts, the plants get their nitrogen in an organic form. Directly by the uptake of amino acids, nucleic acids or protein, and indirectly by 'consuming' their symbionts.

Under favorable conditions, plants – all plants – can also fix themselves nitrogen from the air, with special hairs on their leaves. But ammonia and nitrate impede this when the amounts in the soil are too high.

Nitrogen salts are a risk for plants, because the plants have difficulties in getting not too much of it. Too much nitrogen salts can result in non-protein nitrogen in the plants. The same for sulphur and phosphor salts. They weaken the plants and make them vulnerable for pests and diseases. Too much nitrate and ammonia in the crops implies also health risks for animals and men who eat them.

Inorganic fertilizers repress the Plant Growth-Promoting Rhizobacteria.

But at the same time not all organic nitrogen compounds are a good food for plants. Many rotting products from manure, slurry and warm compost also disturb, or delay, or slow down the growth of plants. The putrefactive bacteria, which are producing the rotting, are overruling the symbiotic bacteria. A great deal of the nitrogen in animal dung and warm compost is lost into the environment. Probably, even more than from artificial nitrogen fertilizers. Here is one of the reasons why organic agriculture has lower yields than conventional agriculture.

By adding earth to the animal dung or the vegetal residuals, the rotting and the losses stop.

Sodium, silicon and many trace elements are missing or insufficient[*]. In most crops, also magnesium and calcium are too low[†], because high levels of potassium and ammonium hinder partly the uptake of magnesium, calcium, sodium and trace elements. Extra sodium and magnesium are necessary to counterbalance potassium and for a good NPN and NPS conversion in real proteins. Too much iron and manganese are also a risk for the crops and the animals, which eat them.

References

Anjana, Umar, 2018 – *Anjana, A., Umar, S.* (2018). Nitrogenous Fertilizers – Boon or Bane? SDRP. *Journal of Plant Science*. 2(2).

ARC, 1980 – *ARC* (1980). The nutrient requirements of ruminant livestock. Farnham Royal: Commonwealth Agricultural Bureaux.

Arney et al., 1995 – *Arney, D., Chiy Pc. Phillips C.* (1995). Lactational responses of grazing dairy cows to Na or K fertilization of pastures. *Annales de zootechnie, INRA/EDP Sciences*. 44(Suppl1): 370-370.

Balkos, Britto, 2010 – *Balkos, K., Britto, D.* (2010). Optimization of ammonium acquisition and metabolism by potassium in rice (*Oryza sativa* L. cv. IR-72). *Plant, Cell and Environment*. 33: 23-34.

Baren van, 1934 – *Baren van, F.* (1934). Het voorkomen en de betekenis van kalihoudende mineralen in Nederlandse gronden. Uitgeverij Veenman en zonen, Wageningen.

Béchamp, 1883 – *Béchamp, A.* (1883). Les microzymâs dans leurs rapports avec l'hétérogénie, l'histogénie, la physiologie et la pathologie paris librairie j.-b. Baillière et fils.

Benton, Jones, 2004 – *Benton, J., Jones Jr.* (2004). Hydroponics: A Practical Guide for the Soilless Grower. 2nd ed. Newyork: Taylor & Francis. Pp. 29–70 & 225–229.

BLGGAgroxpertus, 2011 – BLGGAgroxpertus, 2011. [Electronic resource]. URL: https: //www.eurofins-agro.com

[*] The review of Dimkpa et al gives a good overview about the roles and the importance of trace elements for plants, animals and humans (Dimkpa, Bindrapan, 2016).

[†] Fan et al studied the mineral contents of wheat at the Broadbalk wheat experiment: "The concentrations of zinc, iron, copper and magnesium remained stable between 1845 and the mid 1960s, but since then have decreased significantly, which coincided with the introduction of semi-dwarf, high-yielding cultivars" (Fan et al., 2008).

Bourn, Prescott, 2002 – *Bourn, D., Prescott, J. (*2002). A Comparison of the Nutritional Value, Sensory Qualities, and Food Safety of Organically and Conventionally Produced Foods. *Critical Reviews in Food Science and Nutrition*. 42(1): 1-34.

Bowditch, 1856 – *Bowditch, W.* (1856). On the chemical changes in the fermentation of dung. *Journal of the Royal agricultural society of England*. II. Chapter XVI. page 323.

Bowditch, 1858 – *Bowditch, W.* (1858). On the manuring of grassland. *Journal of the Royal agricultural society of England*.

Bredon, Dugmore, 1985 – *Bredon, R.M., Dugmore, T.J.* (1985). Dairying in Kwazulu – Natal. Mineral & Vitamin Nutrition of Dairy Cattle. Department: agriculture and rural development. Province of Kwazulu-Natal.

Britto, Kronzucker, 2002 – *Britto, D., Kronzucker H.* (2002). NH_4 + toxicity in higher plants: a critical review. J. *Plant Physiol*. 159: 567-584.

Buijs, Samwel Mantingh, 2019 – *Buijs, J. and Samwel Mantingh, M.* (2019). Een onderzoek naar mogelijke relaties tussen de afname van weidevogels en de aanwezigheid van bestrijdingsmiddelen op veehouderijbedrijven in Gelderland. Buijs Agro-Services.

Casey, 2006 – *Casey K, Bicudo J, Schmidt D, Singh A, Gay S, Gates S, Jacobson L, Hoff S.* (2006). Air quality and emissions from livestock and poultry production/waste management systems. Animal agriculture and the environment. National center for manure and animal waste maganement. *White papers*. St Joseph Michigan ASABE. Pub Number 913C0306.

Chaboussou, 2005 – *Chaboussou, F.* (2005). Healthy Crops: A New Agricultural Revolution. Jon Carpenter Publishing.

Chevreul et al., 1855 – *Chevreul, D., Regnault, P., Decaisne, P.* (1855). Recherches expérimentales sur la végétation par M.Georges Ville. Absorption de l'azote de l'air par les plantes. Imprimerie de L. Martinet.

Chiy, Phillips, 1993 – *Chiy, Pc. and Phillips C.* (1993). Sodium fertilizer application to pasture. 1. Direct and residual effects on pasture production and composition. *Grass and Forage Science*. 48(2): 189-202.

Clemente et al., 2016 – *Clemente. J., Condé, A., Andrade, A., Cardoso, C., da Mata Flor, I., Dias Martins,.F, de Lima, W., Burnier de Oliveira, C.* (2016). Azospirillum brasilense and nitrogen fertilization affecting wheat productivity. *African journal of agricultural research*. 11(24): 2179-2184.

Comis, 1989 – *Comis, D.* (1989): Nitrogen overload may shrivel vitamin content. *Agric. Res*. 37(10).

Dangour et al., 2009 – *Dangour, A. Dodhia, S. Hayter, A. Aikenhead, A. Allen, E. Lock, K. Uauy, R.* (2009). Nutritional quality of organic foods: a systematic review. *Am J Clin Nutr*. 90(3): 680-85.

Dianda, 2002 – *Dianda, M., Chalifour, F.P.* (2002). Effect of mineral nitrogen and plant genotype on the growth and nodulation of Faidherbia albida. *Canadian Journal of Botany*. 80: 241-254.

Dimkpa, Bindrapan, 2016 – *Dimkpa, C. and* Bindraban, P. (2016). Fortification of micronutrients for efficient agronomic production: a review. *Agron. Sustain. Dev*. 36: 7.

DMS, 2015 – *DMS* (2015). Mr Dirksen of Dirksen Management Support (DMS) has given me the data about the mineral composition of roughages of hundreds of dairy farms in the Netherland in 2014. (in Print).

Doty, 2017 – *Doty, S.* (2017). Endophytic N-Fixation: Controversy and a Path Forward. In: Doty S. (eds) Func-tional Importance of the Plant Microbiome. Springer, Cham. DOI: https://doi.org/10.1007/978-3-319-65897-1_2

Edulamudi et al., 2011 – *Edulamudi, P., Masilamani, A., Divi, V. and Konada, V.* (2011). Novel root nodule bacteria belonging to the genus Caulobacter. *Letters in Applied Microbiology*. 53: 587-591.

Fan et al., 2008 – *Fan, M., Zhao, F., Fairweather-Tait, S., Poulton, P.R., Dunham, S.J., McGrath, S.P.* (2008). Evidence of decreasing mineral density in wheat grain over the last 160 years. *Journal of Trace Elements in Medicine and Biology*. 22: 315-324.

Guenzi and McCalla, 1962 – *Guenzi, W. and McCalla T.* (1962). Inhibition of germination and seedling development by crop residues. *Soil Sci. Soc.Am. Proc*. 26: 456-458.

Hao, 2008 – *Hao, X. and Benke, M.* (2008). Nitrogen transformation and losses during composting and mitigation strategies. *Dynamic soil, dynamic plant 2*. (Special issue 1): 10-18. Global science books.

Hennig, 1996 – *Hennig, E.* (1996). De geheimen van een vruchtbare bodem. Agriton Noordwolde Zuid.

Hopkins, 1956 – *Hopkins, D.* (1956). Chemicals, Humus and the soil. A simple presentation of contemporary knowledge and opinions about fertilizers, manures, and soil fertility. Ed. Faber & Faber LTD, London.

Hornick, 1994 – *Hornick, S.* (1994). Nutritional quality of crops as affected by management practices. Agricultural Research Service, U.S. Department of Agriculture, Beltsville, Maryland, USA. [Electronic resource]. URL: https://www.infrc.or.jp/knf/PDF KNF Conf. Data/C3-3-070.pdf

Hunter, 2008 – *Hunter, P.* (2008). Not so simple after all. A renaissance of research into prokaryotic evolution and cell structure. *EMBO rep*. 9(3): 224-226.

Jamieson, 1910 – *Jamieson, Th.* (1910). History of the progress of agricultural science in Great Britain. Printed and published by Messrs C. & R. Anderson. "North British agriculturist", Edinburgh.

Jiang et al., 2020 – *Jiang, Y., MacLean, D., Perry, G., Marsolais, F., Hill, B., Pauls, K.* (2020). Evaluation of beneficial and inhibitory effects of nitrate on nodulation and nitrogen fixation in common bean (*Phaseolus vulgaris*). *Legume Science*. 2: e45.

Johansson, 2008 – *Johansson, K.* (2008). Salt to ruminants and horses. Examensarbete 269
Swedish University of Agricultural Sciences Uppsala 2008. Department of Animal Nutrition and Management.

Jones, 2015 – *Jones, C.* (2015). Nitrogen: the double-edged sword. [Electronic resource]. URL: https://www.amazingcarbon.com

Karimi et al., 2018 – *Karimi, N., Zarea, M., and Mehnaz, S.* (2018). Endophytic Azospirillum for enhancement of growth and yield of wheat. *Environmental Sustainability*. 1: 149-158.

Khan et al., 2013 – *Khan, S.A., Mulvaney, R.L. and Ellsworth, T.R.* (2013). The potassium paradox: Implications for soil fertility, crop production and human health. *Renewable Agriculture and Food Systems*. 29(1): 3-27. DOI: 10.1017/S1742170513000318.

Kramer, 2006 – *Kramer, G.* (2006). Groenten. Vergelijking van biologische en reguliere groenten. Afdeling onderzoek Consumentenbond. [Electronic resource]. URL: https://www.consu mentenbond.nl.

Krasil'nikov, 1958 – *Krasil'nikov, N.* (1958). Soil microorganisms and higher plants. Academy of Sciences of the USSR Publishing, Moscow.

Krasil'nikov, Korenyako, 1946 – *Krasil'nikov, N.A., Korenyako, A.I.* (1946). The Effect of Root-nodule Bacteria on Nitrogen Fixation of Clover under Conditions of Sterile Cultures. *Microbiology*. 15(4): 279-283.

Krasil'nikov et al., 1955 – *Krasil'nikov, N.A., Korenyako, A.I., Mirchink, T.G.* (1955). On the Toxicosis of Podsol Soils. *Proceedings of the Academy of Sciences of the USSR. Biological Series*. 3: 34.

Lawes, Gilbert, 1856 – *Lawes, J., and Gilbert, J.* (1856. Vol XVI). On some Points connected with Agricultural Chemistry. *The Journal of the Royal Agricultural society of England*. 484-485.

Lawes and Gilbert, 1858 – *Lawes, J. and Gilbert, J.* (1858). XXV. Report of Experiments with different Manures on Permanent Meadow Land. Part one. *Journal of the Royal agricultural society of England*.

Leisen, 2011 – *Leisen, E.* (2011). (in print).

Lipman, Taylor, 1922 – Lipman, C.B. and Taylor, J.K. (1922). Proof of the power of the wheat plant to fix atmospheric nitrogen. *Science*.

Locato et al., 2013 – *Locato, V., Cimini, S., De Gara, L.* (2013). Strategies to increase vitamin C in plants. From plant defense perspective to food biofortification. *Front. Plant Sci*. 22(4): 152.

Magkos et al., 2006 – *Magkos, F.* Arvaniti, F. and Zampelas, A. (2006). Organic Food: Buying More Safety or Just Peace of Mind? A Critical Review of the Literature. *Critical Reviews in Food Science and Nutrition*. 46: 23-56.

Margulis, Schwartz, 1988 – *Margulis, L., Schwartz, K.* (1988). Five kingdoms. Freeman & Company.

Margulis et al., 2009 – *Margulis, L., Maniotis, A., MacAllister, J., Scythes, J., Brorson, O., Hall, J., Krumbein, W., Chapman, M.* (2009). Spirochete round bodies: syphilis, lyme disease and AIDS: resurgence of the great 'Imitator'? *Symbiosis*. 47: 51-58.

Masterjohn, 2008 – *Masterjohn, C.* (2008). On the Trail of the Elusive X-Factor: A Sixty-Two-Year-Old Mystery Finally Solved. [Electronic resource]. URL: https://westonaprice.org/health-topics/abcs-of-nutrition/

McCalla et al., 1964 – *McCalla, T. and Haskins, F.* (1964). Phytotoxic substances from soil microorganisms and crop residues. *Bacteriological Reviews*. 28(2): 181-207.

Näsholm et al., 2000 – Näsholm, T., Huss-Danell K. and Högberg, P. (2000). Uptake of organic nitrogen in the field by four agriculturally important plant species. *Ecology*. 81(4): 1155-1161.

Näsholm et al., 2009 – Näsholm, T., Kielland, K., and Ganeteg, U. Uptake of organic nitrogen by plants. *New Phytologist*.

Nigten, 2018 – *Nigten, A.* (2018). Re-inventing Agriculture! *Biogeosystem Technique*. 5(2): 213-228.

Nigten, 2019 – *Nigten, A.* (2019). Is de bemesting van de cultuurgewassen met aminozuren een alternatief voor het bemesten met nitraat, ammonium of ureum? Uitgegeven in eigen beheer. [Is fertilization of crops with amino acids an alternative to fertilization with nitrate, ammonium or urea? (in Print).

Nigten, 2019b – *Nigten, A.* (2019). Het werk van Hugo Schanderl. Uitgegeven in eigen beheer. [The work of Hugo Schanderl. (in Print).

Nigten, 2019c – *Nigten, A.* (2019). How Healthy are Our Vegetables? Contours of a New Fertilizing Paradigm. Minerals and non Protein Nitrogen in Vegetables, Grown Organically and Respectively Conventionally. A Quality Assessment (Review). *Biogeosystem Technique*. 6(1): 3-24.

Nigten, 2020 – *Nigten, A.* (2020). Bezit de oersymbiont van de plantencel, de voormalige cyanobacterie, het vermogen om luchtstikstof te assimileren voor alle hogere planten? Nog in concept. [Is one of the primordial symbionts of the plant cells, a former cyanobacteria, able to fix nitrogen from the air, for all higher plants? [in Dutch].

Parniske, 2008 – *Parniske, M.* (2008). Arbuscular mycorrhiza: the mother of plant root endosymbioses. *Nature Reviews Microbiology*. 6 (10): 763-775.

Paul et al., 2007 – *Paul, L., Chapman, B. and Chanway, C.* (2007). "Nitrogen Fixation Associated with Suillus tomentosus Tuberculate Ectomycorrhizae on Pinus contorta var. latifolia". *Annals of Botany*. 99 (6): 1101-1109.

Paungfoo-Lonhienne et al., 2008 – *Paungfoo-Lonhienne, C., Lonhienne, T., Rentsch, D., Robinson, N., Christie, M., Webb, R., Gamage, H., Carroll, B., Peer, M., Schenk, P., and Schmidt, S.* (2008). Plants can use protein as a nitrogen source without assistance from other organisms. *PNAS*. 105(11).

Pfeiffer, 1936 – *Pfeiffer, E.* (1936). De vruchtbaarheid der Aarde. Haar behoud en haar vernieuwing. Het biologisch dynamische principe in de natuur. Publisher: Kluwer Deventer.

Phoenix et al., 2012 – *Phoenix, G.K., Emmett, B.A., Britton, A.J., Caporn S.J.M., Dise, N.B., Helliwell, R., Jones, L., Leith, I.D., Sheppard, L,J., Sowerby, A., Pilkington, M.G., Rowe, E.C., Ashmore, M,R., Power, S.A.* (2012). Impacts of atmospheric nitrogen deposition: responses of multiple plant and soil parameters across contrasting ecosystems in long-term field experiments.*Global Change Biology*. 18: 1197-1215.

Poschenrieder and Lesch, 1942 – *Poschenrieder, H. and Lesch, W.* (1942). Untersuchungen über den Einfluss langjähriger einseitigen Düngungsmassnahmen auf die Ausbildung und Nährstoffaufnahme der Würzelknollchen von Sojabohne. Bodenkunde und Pflanzenernährung 32. Band. Heft I/2.

Pradeu et al., 2016 – *Pradeu, T., Kostyrka, G., Dupre, J.* (2016). Understanding Viruses: Philosophical Investigations. *Studies in History and Philosophy of Biological and Biomedical Sciences*. 59: 57-63. DOI: https://doi.org/10.1016/j.shpsc.2016.02.008

Reich, 1987 – *Reich, S., Almon, H., and Böger, P.* (1987) Comparing Short-Term Effects of Ammonia and Methylamine on Nitrogenase Activity in *Anabaena variabilis* (ATCC 29413). *Z. Naturforsch*. 42c: 902-906.

Reid et al., 2021 – *Reid, E., Kavamura, V., Abadie, M., Torres Ballasteros, A., Pawlett, M, Clark, I., Harris, J., Mauchline, T.* (2021). Inorganic Chemical Fertilizer Application to Wheat Reduces the Abundance of Putative Plant Growth-Promoting Rhizobacteria. *Front. Microbiol.* 11.

Ribeiro et al., 2018 – *Ribeiro, R., Besen, M., Figueroa, L., Iwasaki, G., Guginski-Piva, C., Sartor, L. and Piva, J.* (2018). Seed and leaf inoculation with Azospirillum brasilense and increasing nitrogen in wheat production. *Rev. Bras. Cienc. Agrar., Recife.* 13(3): e5550.

Rigg, 1838 – *Rigg, R.* (1838). On the Evolution of Nitrogen during the Growth of Plants, and the Sources from Whence They Derive That Element. *Philosophical Transactions of the Royal Society of London.* 128: 403-408. Published by: Royal Society. [Electronic resource]. URL: https://www.jstor.org/stable/108205

Rinsema, 1981 – *Rinsema, W.* (1981). Bemesting en meststoffen. Educaboek: Stam/Robijn Culemborg III.

Rippel, 1937 – *RippeI, A.* (1937). Der derzeitige Stand der Knöllchenbakterienfrage. Forschungsdienst, Berlin, Sonderh. 6: 215-225.

Rolvink, 2019 – *Rolvink, R.* (2019). Biologische voeding in kaart. [Electronic resource]. URL: https://www.consumentenbond.nl

Rosen, 2010 – *Rosen, J.* (2010). A review of the nutrition claims made by proponents of organic food. *Comprehensive reviews in food science and food safety.* 9.

Royal Brinkman, 2020 – Royal Brinkman (2020). [Electronic resource]. URL: https://royal brinkman.nl

Rusch, 1968 – *Rusch, H.P.* (1968). Bodenfruchtbarkeit. Organischer Landbau Verlag. Nederlandse vertaling door P. Vanhoof, 2014: Bodemvruchtbaarheid. [Electronic resource]. URL: https://www. dekoolstofkring.nl.

Sait, 2018 – *Sait, G.* (2018). The Bastardisation of our Food – The Daily Bread Story. [Electronic resource]. URL: https://blog.nutri-tech.com.au/the-bastardisation-of-our-food/

Santi et al., 2013 – *Santi, C., Bogusz, D. and Franche, C.* (2013). Biological nitrogen fixation in non-legume plants. *Ann. Bot.* 111: 743-767.

Schanderl, 1943 – *Schanderl, H.* (1943). Untersuchungen über den Stickstoffhaushalt von Nichtleguminosen und Leguminosen. *Planta: An International Journal of Plant Biology.* 33(3) (194305): 424-457.

Schanderl, 1947 – *Schanderl, H.* (1947). Botanische Bakteriologie und Stickstoffhaushalt der Pflanzen auf neuer Grundlage. Stuttgart Verlag Eugen Ulmer.

Schreiner, Skinner, 1912a – *Schreiner, O., Skinner, J.* (1912). Nitrogenous soil constituents and their bearing on soil fertility. US Department of agriculture.

Schreiner, Skinner, 1912b – *Schreiner, O., Skinner, J.* (1912). The effect of Guanidin on Plants. *Bulletin of the Torrey Botanical Club.* 39(11): 535-548.

Shiraishi et al., 2010 – *Shiraishi, A., Matsushita, N. and Hougetsu, T.* (2010). Nodulation in black locust by the Gammaproteo-bacteria Pseudomonas sp. and the Betaproteobacteria Burkholderia sp. *Systematic and Applied Microbiology.* 33(5): 269-274.

Sougoufara, 1990 – *Sougoufara, B., Danso, S.K.A., Diem, H.G., Dommergues, Y.R.* (1990). Estimating N_2-fixation and N derived from soil by Casurina equisetifolia using labelled 15N fertilizer: some problems and solutions. *Soil Biology and Biochemistry.* 22: 695-701.

Vanhoof, Nigten, 2020 – *Vanhoof, P., Nigten, A.* (2020). Drijfmest, Invloeden op emissies, en N-benutting op grasland. [Electronic resource]. URL: https://www.deVBBM.nl; https://www.NMV.nu

Ville, 1853 – *Ville, G.* (1853). Versuche über die Vegetation. *Journal für praktische Chemie.* 58(1): 10-15.

Visser, 2010 – *Visser, J.* (2010). Down to earth: a historical-sociological analysis of the rise and fall of 'industrial' agriculture and of the prospects for the re-rooting of agriculture from the factory to the local farmer and ecology. PhD thesis Wageningen University.

Voelcker, 1872 – *Voelcker, A.* (1872). On the Composition and Agricultural Value of Earth-Closet Manure. Journal of the Royal agricultural society of England.

Waart, 1998 – *Waart, S. de.* (1998). Biologisch: goed beter, best? Biologische en gangbare groenten en fruit vergeleken op de aspecten residuen, vitamine C, nitraat en drooggewicht: een overzicht. Louis Bolk instituut, the Netherlands.

Wabersich, 1967 – *Wabersich, R.* (1967). Der Bernburger Dauerdüngungsversuch. 1 Mitteilung: die Ertragsentwicklung. *Archives of Agronomy and Soil Science*. 11(7).

White, 2019 – *White, J.* (2019). How do plants extract nutrients from soil microbes? Department of Plant Biology Rutgers University, New Brunswick, New Jersey, 08901. (in Print).

White et al., 2018 – *White, J., Kingsley, K., Verma, S. and Kowalski, K.* (2018). Rhizophagy Cycle: An Oxidative Process in Plants for Nutrient Extraction from Symbiotic Microbes. *Microorganisms*. 6(95).

Wittwer, 1947 – *Wittwer, S.,* (1947). Vitamin C -nitrogen relations in peaches as influenced by fertilizer treatment. *Proc. Am. Soc. Hortic. Sci*. 49: 116.

Wittwer et al., 1945 – *Wittwer, S., Schroeder, S., and Albrecht, W.* (1945). Vegetable crops in relation to soil fertility. II. Vitamin C and nitrogen fertilizers. *Soil Science*. 59(45): 329-336.

Wunderlich et al., 2008 – *Wunderlich, S., Feldman, C., Kane, S., Hazhin, T.* (2008). Nutritional quality of organic, conventional, and seasonally grown broccoli using vitamin C as a marker. *International Journal of Food Sciences and Nutrition*. 59(1).

Yuan and Peng, 2017 – *Yuan, S. and Peng, S.* (2017). Exploring the Trends in Nitrogen Input and Nitrogen Use Efficiency for Agricultural Sustainability. *Sustainability*. 9: 1905. [Electronic resource]. URL: https://www.mdpi.com/journal/sustainability DOI: 10.3390/su9101905

Yutin et al., 2008 – *Yutin, N., Makarova, K., Mekhedov, S., Wolf, Y., and Koonin, E.* (2008). The deep archaeal roots of eukaryotes. *Mol. Biol. Evol*. 25(8): 1619-1630. Oxford university press.

Zuberer, 1998 – *Zuberer, D.A.* (1998). Biological dinitrogen fixation: introduction and nonsymbiotic. In: Sylvia, D.M., Fuhrmann, J.J., Hartel, P.G., Zuberer, D.A., eds. Principles and applications of soil microbiology. New Jersey: Prentice-Hall, 295-321.

Appendix 1

The discovery of the nitrogen fixing hairs on all plants by Jamieson.

Jamieson:

"Search for them [the hairs. author]*, though attended frequently with failure, at first, has, in every case, after persistency, been followed by success in finding them in one place or other, if not in the edges, or back, or ribs of the leaf, then on the young stalk, or the leaf scales, &c. So also, absolute failure to find them on some plants, such as legumes, was followed by success on searching at a certain stage of growth, i.e., the leaves just as they emerge from the buds—Later on they disappear, being absorbed into the leaf. The organs have now been found on a large number of the most unlikely plants (on which nothing akin to glandular hairs had been recognised), such as hard-leaved pines. In short, on no plant as yet thoroughly examined has the search been unsuccessful, and evidently therefore we have here not an occasional occurrence (as in the case of the so-called glandular hairs), but a general occurrence to ensure the provision of the substance most essential to life—viz. nitrogen to form albumen.*

This constant occurrence was a strong feature in the evidence. Had it not been found, in one form or other, on every plant examined, there would have been a weakness in the chain of evidence, more especially if the absence applied to a highly nitrogenous plant, such as any legume; and long and trying was the vain search for it on legumes. Had a legume been chosen first, as from its high nitrogen content it might probably have been, the discovery would almost certainly not have been made, and the investigation would probably have been given up. At last, on the edge of a leaf, a minute knob was observed which was unusual, and close examination brought out that it possessed the specific characters of an absorber, but in this case submerged in the fleshy leaf ; remains of numerous similar structures altogether submerged were then seen; it thus appeared that the examination must be made at an earlier stage. Seedlings were therefore raised and examined, when, instead of any difficulty in finding the absorbing organs, they now appeared at once and in abundance, standing up like a forest and in the usual typical form. This event was one of the most convincing features in the progress of the work—it gave confidence of being on the right track (..).

They [the hairs. Author] *have a definite and special character, a general resemblance in structure, and frequent resemblance in form, and although the form varies greatly in different plants, the specialised character always remains. The usual structure is a long blunt projection divided into* *sections, like a finger with its joints (as in Spergula), the lower sections being at first (and for some time) colourless, transparent, empty, and double walled ; the highest section, which is very often distended into a bulb or club-head-like form (as in legumes and geranium), is altogether different in appearance and distinctive in character from the lower sections, by containing yellowish-green matter resembling chlorophyll, but probably differing from chlorophyll ; it is the active and essential part of the organ, and it shows very marked changes during its period of activity or life. This is to say—the highest section of these structures, and it alone, is at first filled with this yellowish-green chlorophylllike matter, and, even when just fully formed, it shows no presence of albumen by the usual tests; gradually, however, that highest section, and that section alone, becomes charged with albumen, and ultimately is gorged with it ; the albumen then passes down through the open ends of the sections into the vascular system of the plant.*

These absorbers are found in all stages of growth in regard to albumen contents, i.e. absent, filling, filled, gorged, and emptied; frequently (as in the poplar and sycamore) they occur in groups, in which all these stages can be observed at one glance according to the varying age of the members of the group. Very often (as in potato) there are two forms on one plant, as if showing a reserve to ensure the provision of nitrogen, just as in plants there is frequently a, reserve method to secure reproduction (i.e. flowers and leaf buds, runners, &c.)". (Jamieson, 1910, page 95 and beyond).

On page 106 of his book you can find the results of a series of trials done by Jamieson:

ACTUAL GAIN OF NITROGEN FROM AIR.

No.	Plants Grown.	Actual Weight of Plants Grown.		Nitrogen Provided (in Seed, Soil, or Manure).	Nitrogen Found (in Plants and Soil).	Nitrogen Gained (in Plants and Soil).	Ratio of Gain. Nitrogen provided considered as 100.	Gain of Nitrogen in 100 parts Dry Plant.
		Fresh Weight (80% Water.	Dry Weight.					
		Grms.	Grms.	Grms.	Grms.	Grms.		Grms.
1.	Rape ..	20·20	4·04	1·3200	1·4408	—·1208	109	—·643
2.	Cress ..	15·08	3·01	1·3198	1·4286	—·1088	108	1·285
3.	Stellaria ..	15·14	3·02	—·1276	—·1440	—·0164	112	1·205
4.	Mimulus ..	19·53	3·90	—·1276	—·1429	—·0153	112	1·282
5.	Hydrocharis	1·90	—·38	—·0012	—·0092	—·0080	790	1·558
6.	Azol'a ..	8·65	1·83	—·0014	—·0263	—·0249	1843	1·395
7.	Potato ..	564·	112·80	27·2597	33·0557	5·7960	121	1·050
8.	Beet ..	438·	87·00	25·4280	27·0557	1·6277	106	1·670
9.	Petunia, Geranium, Tobacco	200·25	40·05	25·4799	29·6558	4·1759	118	2·033

In addition to the gain in the plant (stated

Appendix 2

Fertilizer trials in Bernburg, Germany: the effects of different fertilizers on the bacterial count, and the soil nitrogen content

Bodenkunde und Pflanzenernährung, 32. Band, Heft 1/2.

Untersuchungen über den Einfluß langjähriger einseitiger Düngungsmaßnahmen auf die Ausbildung und Nährstoffaufnahme der Wurzelknöllchen von Sojabohne.

Mitteilung Nr. 113 der Anhaltischen Versuchsstation Bernburg,
Staatliche Landwirtschaftliche Forschungs- und Untersuchungsanstalt.
Direktor: Dr. H. Lüdecke.

Von **H. Poschenrieder** (Berichterstatter) und **W. Lesch.**

Eingegangen: 4. September 1942.

Auf dem Bernburger Versuchsfeld mit seinem tiefgründigen, humosen und von Natur aus nährstoffreichen Lößboden läuft seit 1910 ein Dauerdüngungsversuch zu Kartoffeln. Diese Einfelderwirtschaft umfaßt 12 Teilstücke, die während der 32jährigen Versuchsdurchführung durchweg in jeweils bestimmter Richtung gedüngt wurden, so daß sich im Laufe der Jahre größere Unterschiede im Nährstoffgehalt der verschieden gedüngten Teilstücke herausbildeten. (Tabelle 1.)

Während die pH-Zahlen durchweg um den Neutralpunkt liegen und innerhalb der einzelnen Teilstücke so gut wie keine Reaktionsunterschiede erkennen lassen, kommen in den Neubauerzahlen die langjährigen einseitigen Düngungsmaßnahmen sowohl im Kali- als auch im Phosphorsäuregehalt entsprechend zum Ausdruck. Im Gehalt der Teilstücke an Gesamtstickstoff ergeben sich zwar ebenfalls größere Unterschiede, die jedoch der Größenordnung nach nicht immer mit der gegebenen Stickstoffdüngung in Einklang zu bringen sind. Bei der Überlegung, daß das Stickstoffkapital im Boden

Tabelle 1.
Ergebnisse der Bodenuntersuchung 1940.

Düngung	Teilstück	Reaktion pH(KCl)	Neubaueranalyse		Gesamtstickstoff	Bakterienzahl in Millionen in 1 g Boden
			mg P_2O_5	mg K_2O	%	
Ungedüngt . . .	U	7,1	1,6	20,9	0,066	9,86
Stickstoff	N	7,1	1,6	16,4	0,071	11,20
Phosphorsäure . .	P	7,2	8,8	14,9	0,061	8,00
Kali	K	7,0	1,8	31,4	0,056	9,60
Volldüngung ohne Stickstoff . .	PK	7,0	8,5	31,0	0,065	6,13
Volldüngung ohne Phosphorsäure	NK	7,3	2,4	31,4	0,087	4,80
Volldüngung ohne Kali	NP	7,0	8,6	13,4	0,051	19,20
Volldüngung mit schwefels. Ammoniak	NPK(NH_4)	7,2	9,2	30,4	0,079	24,80
Volldüngung mit Natronsalpeter . .	NPK(NO_3)	7,2	8,9	28,5	0,038	12,00
Stallmist	St	7,1	5,8	25,8	0,103	33,86
Stallmist mit Volldüngung ohne Stickstoff	St + PK	7,1	12,0	47,6	0,112	30,93
Stallmist mit Volldüngung . .	St + NPK	6,9	13,5	39,4	0,109	16,00

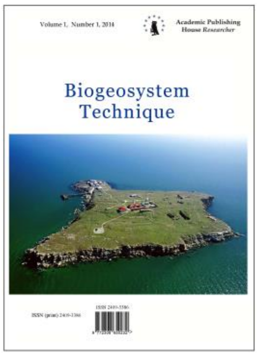
1

The highest bacterial count is in Farmyard manure, and Farmyard manure plus PK. The second group is NPK (NH_4 (which contains also sulphur)); NP, and FYM + NPK. The third group has a bacterial count comparable to the control (some with a somewhat higher, and some with even less bacterial count: N; P; K: NK; PK;. And NK and PK have the lowest bacterial count.

If we have a more close look at the three farmyard manure treatements (FYM; FYM + PK, and FYM + NPK we see that the last one has a low bacterial count compared to the two other treatments. So the influence of the extra mineral N on the bacterial count is strongly negative, comparable to the effect of N on nodulation.

The result of the soil nitrogen content measurements are also interesting for organic agriculture, because the highest soil nitrogen content is in the three farmyard manure treatments: Stallmist (FYM); Stallmist +PK; and Stallmist + NPK: 0,103; 0,112 and 0,109 respectively. Poschenrieder comments as follows:

“Die merklich höheren Stickstoffwerte der Stallmistteilstücke jedoch finden ihre Erklärung einerseits in der wesentlich niedrigeren Ausnutzung des Stallmiststickstoffs durch die hoheren Pflanzen. Aus diesem Grunde bleiben im Boden relativ grössere Mengen an Stickstoff zuruck. Anderseits unterliegen die im Stalldünger als Bestandteile des unverrotteten Düngers und der Mikroorganismen festgelegten Stickstoffverbindungen weniger der Auswaschung als die in mineralischer Form gegebenen Stickstoffmengen” (Poschenrieder, Lesch, 1942).



Published in the Slovak Republic
Biogeosystem Technique
Has been issued since 2014.
E-ISSN: 2413-7316
2021. 8(1): 34-44

DOI: 10.13187/bgt.2021.1.34
www.ejournal19.com

Human and Domesticated Species (Critical Review)

Valerii I. Glazko [a, b, *], Gleb Yu. Kosovsky [b], Tat'yana T. Glazko [a, b]

[a] Timiryazev Russian State Agrarian University – Moscow Agrarian Academy, Russian Federation
[b] Afanas'ev Research Institute of Fur-Bearing Animal Breeding and Rabbit Breeding, Russian Federation

Paper Review Summary:
Received: 2021, May 9
Received in revised form: 2021, June 21
Acceptance: 2021, June 30

Abstract

The agrarian civilization was formed due to the domestication of mainly five species of mammals (goat, sheep, cattle, pig, horse) and a limited number of plant species, the main of which are wheat and rice. This small number of species has remained the basis of the agrarian civilization to this day, despite the ongoing attempts to domesticate other wild species. By now, it has become clear that domestication is one of the variants of mutualistic relationships between humans and the corresponding species. It may well explain the limited number of species that have been successfully involved in domestication, since the possibilities of "symbiosis" with humans are highly species-specific. Hence, it becomes obvious that the loss of the diversity of the gene pools of agricultural species is fundamentally irreplaceable, as well as the degradation of soils, the biosphere and is a direct threat to the existence of agricultural civilization, since these species historically lay at the basis of its existence and development. From this point of view, the preservation of the biodiversity of agricultural species becomes a critical condition for the existence of an agrarian civilization in the future. Mechanisms of domestication are a key issue in understanding and managing the genetic resources of agricultural animal and plant species. The review examines the basis for the formation of an agrarian civilization, which is due to the activity of humans to involve other species in his niche, which contribute to increasing his adaptive and reproductive potential. A significant contribution to this process was made by factors related to human social activity, his cognitive functions and his ability to purposefully study and control various characteristics of the species involved in domestication. The main signs included in the "domestication syndrome" in animals are associated with their social activity and genomic instability. Their variability, which creates the basis for selection, depends on the diversity of the microbiota and the closely related virom. The formation of a mobilome in genomes based on virom creates the material basis for phenotypic diversity, which allows for targeted selection based on the adaptive potential of animals and plants to the niche created by man, as well as on various

* Corresponding author
E-mail addresses: vigvalery@gmail.com (V.I. Glazko)

phenotypic characteristics that increase the adaptive and reproductive potential of a person in the niche organized by him.

Keywords: domestication, niche, social activity, microbiome, virom, mobile.

1. Введение

История доместикации животных и растений привлекает особое внимание биологов с тех пор, как Чарльз Дарвин впервые провел параллель между эволюцией в результате естественного отбора и при разведении домашнего скота, животных-компаньонов, культурных растений под влиянием человека. Одомашнивание растений и животных легло в основу аграрной цивилизации и ее глобального распространения, всех культурных инноваций человека, связанных с производством орудий труда, покорением огня или эволюцией вербального языка.

Одомашнивание растений или животных древними популяциями человека принципиально отличается от известных мутуализмов между разными видами, поскольку оно требовало преднамеренности и сознательного планирования, понимания поведения и репродуктивной биологии другого вида (Larson et al., 2014; Larson, Fuller, 2014). Таким образом, когнитивные потребности одомашнивания, ориентированного на человека, представляют собой явление, отличное от межвидовых взаимовлияний, развиваемых, в частности, социальными насекомыми.

Массовое одомашнивание растений и животных, как предполагается (McHugo et al., 2019), было вызвано значительными экологическими и климатическими изменениями, которые сопровождали глобальный переход от пика Последнего ледникового максимума (Last Glacial Maximum – LGM) примерно 21 тыс. лет назад к межледниковому периоду начала голоцена. Активизация стратегий одомашнивания могла привести к конкурентной борьбе, которая способствовала распространению растениеводства и животноводства. Демографическое давление растущей популяции людей также способствовало одомашниванию, усиливая отношения между людьми, растениями и животными. Причем сторонники модели, известной как ‘человеческая революция’, утверждают, что современное поведение человека возникло внезапно и почти одновременно во всем Старом Свете примерно за 40-50 тыс. лет до нашей эры. Предполагается, что этот фундаментальный поведенческий сдвиг сигнализирует о когнитивном прогрессе, возможной реорганизации мозга и происхождении языка. Самые ранние артефакты современного человека выявлены у *Homo sapiens sensu stricto*, представителей которого считают самыми древними в мире современными людьми (люди из Херто и Омо), найдены в Африке и прилегающем регионе Леванта. В этой связи модель ‘человеческой революции’ создает временные отличия между появлением анатомических современных характеристик и поведенческих особенностей, что создает впечатление, что самые ранние представители *Homo sapiens* вели себя примитивно.

2. Обсуждение и результаты

Этапы доместикации

Компоненты ‘человеческой революции’

Мнение о примитивном поведении *Homo sapiens* вызывает сомнение в его справедливости. Такой взгляд на события связан с глубоким евроцентрическим уклоном и неспособностью оценить глубину и широту археологических данных Африки. На самом деле многие компоненты ‘человеческой революции’, которые, как утверждается, появились 40-50 тыс. лет назад, были найдены в африканском среднем каменном веке десятками тысяч лет ранее (Mcbrearty, Brooks, 2000). Эти особенности включают в себя технологию изготовления клинка, костяных инструментов, расширение географического ареала, специализированная охота, использование водных ресурсов, торговля на больших расстояниях, систематическая обработка и использование пигмента, а также искусство и украшения. Эти элементы возникают не внезапно вместе, как предсказывает модель ‘человеческой революции’, а в местах, существенно разобщенных в пространстве и во времени. Это предполагает постепенную сборку пакета моделей современного поведения человека в Африке и его последующий экспорт в другие регионы Старого Света. Ископаемая летопись гоминид африканского среднего и раннего позднего плейстоцена достаточно непрерывна и в ней можно распознать ряд, вероятно, различных видов, которые являются возможными предками *Homo sapiens*. Появление в среднем периоде каменного века и первые признаки современного поведения человека совпадают с появлением артефактов, которые были приписаны *Homo helmei* (*Homo helmei* – название вида людей, предложенное для плейстоценовых гоминид, имеющих переходные характеристики между *Homo heidelbergensis* (Гейдельбе́ргский челове́к) и *Homo sapiens*, что позволяет предположить, что поведение *Homo helmei* отличается от поведения более ранних видов гоминид и очень похоже на поведение современных людей. Если принять тот факт, что по анатомическим и поведенческим признакам *Homo helmei* пересекается с *Homo sapiens*, происхождение нашего вида связано с появлением среднего периода каменного века примерно 200 тыс. лет назад.

К первым доместицированным животным относят домашнюю собаку (*Canis familiaris*). За появлением домашней собаки в археологических записях относительно скоро последовало одомашнивание сельскохозяйственных культур растений и домашнего скота, что позволило людям существенно увеличить количество пищи, которую они получали от охоты и собирательства. Следовательно, во время неолитического перехода—археологически задокументированного перехода от способов производства продуктов питания охотниками-собирателями к выращиванию растений и животноводству—все более сложные сельскохозяйственные общества развивались во многих местах по всей Евразии, Северной Африке и Южной и Центральной Америке (Diamond, 2002).

Результаты зооархеологических исследований в Юго-Западной Азии свидетельствуют о том, что овцы (*Ovis aries*), козы (*Capra hircus*), безгорбый европейский крупный рогатый скот (*Bos taurus*) и свиньи (*Sus scrofa*) были одними из первых доместицированных животных, которые подверглись одомашниванию 10-11 тыс. лет назад в регионе Плодородного Полумесяца (Larson et al., 2014; Conolly et al., 2011; Zeder, 2011). Примерно два тысячелетия спустя горбатый крупный рогатый скот (зебу - *Bos indicus*) был одомашнен, вероятно, сообществами в раннем неолите, расположенными в современном Белуджистане, Пакистане (Fuller, 2006). Свиньи, по-видимому, одомашнены примерно 8 тыс. лет назад в Восточной Азии из популяции дикого кабана, генетически отличной от популяции в Юго-Западной Азии (Frantz et al., 2015). Лошадь (*Equus caballus*) одомашнена в степях Центральной Азии примерно 5,5 тыс. лет назад (Outram et al., 2009), а курица (*Gallus gallus*) и кошка (*Felis catus*) прошли один и тот же путь около 4 тыс. лет назад в Юго-Восточной Азии и Северной Африке (Египет) соответственно (Linseele et al., 2007). Предполагается, что на время одомашнивания для ряда видов животных могли повлиять ключевые климатические события за последние 20 000 лет.

Общая ниша с человеком

В общем, доместикация – это процесс формирования общей ниши с человеком, что приводит к большому количеству изменений у всех участников (Zeder, 2015). Предполагается, что объединяющим показателем для доместицированных видов животных является то, что в настоящее время определяется как «синдром доместикации». Ключевым условием для доместикации животных человеком является такая характеристика, как предрасположенность к социализации, включающую пониженную агрессивность, социальную толерантность, исследовательские тенденции. Имеются данные о том, что социо-культурные особенности ниш, созданные человеком, вносят относительно больший вклад во внутривидовую дифференциацию культурных растений и доместицированных животных по сравнению с экологическими факторами (Colino-Rabanal et al., 2018). Интересно отметить, что предполагаемый предковый вид человека – бонобо (*Pan paniscus*), существенно отличается от, в частности, шимпанзе, своей толерантностью – способностью делится пищей с представителями других видов (Sánchez-Villagra, van Schaik, 2019).

Анализ накопленных данных позволяет прийти к выводу о том, что наиболее универсальными показателями синдрома доместикации являются изменения поведенческих характеристик (снижение агрессивности, повышение социализации (Wilkins, 2020) и существенное повышение фенотипической и популяционно-генетической изменчивости (Glazko et al., 2015).

Ключевое значение повышенной предрасположенности к социализации очень хорошо обоснована исследованиями бонобо (*Pan paniscus*). В отличие от своей сестринской группы, шимпанзе (*Pan troglodytes*), у бонобо проявляется несколько признаков, типичных для синдрома доместикации, включающие депигментацию губ, хвостовых окрасок, уменьшенный половой диморфизм, меньший размер черепа и, следовательно, мозга. Предполагается, что эти признаки развились как побочные продукты отбора на снижение агрессивности, обусловленного предпочтением самок спариваться с менее агрессивными самцами. Физиологическая реакция на социальный стресс также отличается у шимпанзе и бонобо: самцы шимпанзе проявляют тестостероновую реактивность, которая снижает потенциал толерантности, тогда как у бонобо повышается уровень кортизола. Кроме того, некоторые морфологические признаки бонобо могут рассматриваться как отражающие ювенильные паттерны развития. У бонобо, как и у некоторых наиболее изученных одомашненных животных (собаки, свиньи, лошади) "ювенилизация" проявляется в отдельных специфических чертах, а не "глобально".

Исследования в целом подтверждают идею о том, что бонобо более социально толерантны, чем шимпанзе (Sánchez-Villagra, van Schaik, 2019). Они охотнее, чем шимпанзе, устанавливают зрительный контакт или следят за происходящим взглядом, систематически более способны к совместному питанию, чем шимпанзе. Бонобо, в отличие от шимпанзе, охотно делятся пищей с незнакомцами, а также, в отличие от шимпанзе, проявляют заразительную зевоту в ответ на зевоту незнакомцев. Отмечено, что дикие бонобо делятся едой с членами других видов, что исключено для шимпанзе.

Таким образом, то, что бонобо вовлечены больше, чем шимпанзе, в проактивные (спонтанные, непроизвольные) просоциальные действия, которые характеризуют людей, является очевидной возможностью для поддержки современной гипотезы о самодоместикации человека на основе сформировавшейся в эволюции повышенной социальной толерантности.

К настоящему времени уже появились экспериментальные данные, поддерживающие гипотезу «самодоместикации» человека и роль темпов развития нервного гребня в одомашнивании животных (Zanella et al., 2019).

Предполагается (Progovac, Benítez-Burraco, 2019), что «самоодомашнивание» человека способствовало появлению менее агрессивного фенотипа, точнее фенотипа, склонного заменять физическую агрессию вербальной агрессией. В свою очередь, постепенный переход к вербальной агрессии и к более сложным формам вербального поведения способствовал социализации, причем два процесса включались во взаимно усиливающий цикл обратной связи, учитывая, что вербальное поведение влечет за собой не только меньшее насилие и лучшее выживание, но и больше возможностей для длительного взаимодействия и общения с большим количеством людей, в конечном счете, способствуя появлению более сложных форм языка. То есть, в случае «самодоместикации» половой отбор работал против признаков физической агрессии, а в случае ее замены словесными оскорблениями отбор работал в пользу вербальной агрессии. Напряженность между этими двумя, казалось бы, противоположными силами, разрешается/ослабляется тенденцией заменять физическую агрессию вербальной агрессией и вербальным поведением в более общем плане. Это также помогает разрешить парадокс гипотезы о самообучении, касающейся агрессии, точнее, почему агрессия у людей снижается только тогда, когда речь заходит о реактивной агрессии, но не тогда, когда речь заходит о проактивной агрессии, причем последняя демонстрирует рост с появлением современного языка. Авторы (Progovac, Benítez-Burraco, 2019) этих исследований полагают, что такая петля обратной связи была особенно важна в период формирования ранних сообществ *Homo sapiens*, поскольку позволяет предположить конкретные точки соприкосновения и взаимного усиления между двумя процессами – «самоодомашнивания» и ранней языковой эволюцией, включая снижение физической агрессии и влияния полового отбора.

Как отмечалось выше, общими характеристиками «синдрома доместикации» является повышенная фенотипическая изменчивость и повышенная предрасположенность к социализации. К настоящему времени выполнены попарные сравнения геномов основных доместицированных видов – базовых, для аграрной цивилизации – и их близкородственных диких видов: домашняя курица *Gallus gallus domesticus* и предковый вид *Gallus gallus* (Rubin et al., 2010); свиньи и дикие кабаны (Frantz et al., 2015; Paudel et al., 2013); предковый примитивный скот и современные породы крупного рогатого скота (Park et al., 2015); домашняя овца и муфлон, козы и безоаровый козел (Alberto et al., 2018); домашняя лошадь и лошадь Пржевальского (Der Sarkissian et al., 2015; Librado et al., 2017; Wutke et al., 2018);

домашний и дикий кролик (Carneiro et al., 2014; Irving-Pease et al., 2018) и ряда других видов. Основной вывод, который можно сделать на основании анализа результатов полногеномного секвенирования и сравнения мононуклеотидных отличий (Single Nucleotide Polymorphisms – SNP) и разнообразия изменчивости по копийности коротких геномных участков (Copy Number Variability – CNV) между доместцированными и близкородственными дикими видами, заключается в том, что, в основном, в геномных областях, в которых локализуются дифференцирующие эти виды SNP и CNV маркеры, локализованы гены, продукты которых связаны с развитием нервной и иммунной систем, а также характеристиками продуктивности животных сельскохозяйственных видов, причем вовлекаемые в эти процессы конкретные гены варьируют в зависимости от вида, то есть, сходные фенотипические решения достигаются с вовлечением разных генетических систем.

Участие в социализации генов хозяина, а также микробиома и вирома

В последние годы особое внимание привлечено к комплексу генов, мутации в которых приводит к формированию синдрома гиперсоциальности, связанному с описанным у человека синдромом Уильямса-Берена (WBS, синдром гиперсоциальности), вызванный соответственно гемиделецией или гемидупликацией 28 генов в области 7q11.23 (Zanella et al., 2019)). WBS является аутосомно-доминантной мутацией, вызванной геномными перестройками из-за больших регионоспецифичных изменений, присутствием Alu транспозонов (неавтономных коротких диспергированных ядерных элементов), которые могут приводить к неаллельной гомологичной рекомбинации в мейозе (Etokebe et al., 2008; Ferrero et al., 2010; Antonell et al., 2010). Частота встречаемости WBS в популяции составляет около 1/10000 для WBS гемизиготности и 1/20000 для WBS гемодупликациям. Делеция/дупликация критической области синдрома Уильямса-Берена (WBSCR) приводит к гемизиготности/гемодупликации 25-28 генов, объясняющая их фенотипические проявления (López-Tobón et al., 2020). Эта критическая область содержит гены, кодирующие ряд факторов регуляции транскрипции (Schubert, 2009). Предполагается, что изменчивость в их копийности может изменить баланс возбуждения/торможения (Makeyev, Bayarsaihan, 2011), что согласуется с многочисленными доказательствами, свидетельствующими о дисбалансе соотношения возбуждения/ингибирования кортикальных нейронов как основного субстрата развития сети генов, лежащих в основе социальной активности (Sohal, Rubenstein, 2019; Lopatina et al., 2018). Сравнительные исследования, посвященные механизмам, обусловливающим повышенную склонность собак к инициированию социальных контактов, по сравнению с серым волком, объясняли это поведение как тип поведенческой неотении, сохранение ювенильных черт у взрослого человека, что само по себе потенциально является результатом транскрипционной неотении в мозге (Somel et al., 2009).

Обнаружено, что структурные варианты в генах WBS лежат в основе стереотипной гиперсоциальности у домашних собак и лис (von Holdt et al., 2017; Kukekova et al., 2018).

Известно, что транскрипты WBSCR17 преимущественно экспрессируются в мозжечке, гиппокампе, таламусе и коре головного мозга крыс (Nakamura et al., 2005), причем исследования подтверждают его влияние на морфологию клеток и траффик через клеточные мембраны (Nakayama et al., 2012a, 2012b). WBSCR17 (N-ацетилгалактозаминилтрансфераза у человека GALNT17) высоко экспрессируется в коре головного мозга, участвует в функции лизосом, клеточной адгезии и формировании внеклеточного матрикса (Merla et al., 2002). В то же время, очевидно, что сама по себе социальная активность является сложным и, по своей сути, количественным признаком, имеющим генетическую и паратипическую компоненту в своей изменчивости. Так, исследования, выполненные на щенках 8-ми недельного возраста, позволяют предполагать, что примерно 40 % социальной активности определяется генетической компонентой. Однако существенная ее часть зависит от факторов окружающей среды, восприимчивость к которым тоже может иметь свою генетическую обусловленность, формирование которой еще менее доступно для анализа (Bray et al., 2021). Сложный характер наследования имеет и такая характеристика, тесно связанная с социальной активностью, как агрессивность. В частности, обнаружено, что экспрессия примерно 1200 генов отличается между породами крупного рогатого скота с высокой агрессивностью (порода Лидия, селекционируемая для коррид) и относительно дружелюбной (порода Вагью) (Eusebi et al., 2021).

Во многих работах представлены данные, свидетельствующие о том, что микробы, обитающие в желудочно-кишечном тракте, влияют на физиологию и работу мозга. Исследования показали, что микробиота желудочно-кишечного тракта может подавать сигналы в головной мозг через множество путей, включая иммунную активацию, выработку микробных метаболитов и пептидов, активацию блуждающего нерва и выработку различных нейромедиаторов и нейромодуляторов. В совокупности этот двунаправленный путь известен как ось микробиота-кишечник-мозг. В отсутствие микробиоты у мышей, получавших антибиотики и не имевших микробов, наблюдались изменения в ряде центральных физиологических процессов, таких как оборот нейромедиаторов, нейровоспаление, нейрогенез и морфология нейронов. Возможно, в результате этих неврологических изменений поведение животных с обедненной микробиотой особенно значительно отличается по социальному поведению от поведения грызунов, колонизированных бактериями. И наоборот, добавление животным определенных полезных бактерий (напр., *Bifidobacterium* и *Lactobacillus*) могут привести к заметному улучшению социального поведения как в раннем возрасте, так и во взрослом. В совокупности эти результаты свидетельствуют о том, что микробные сигналы важны для развития центральной нервной системы и программирования социального поведения в головном мозге (Cryan et al., 2019). Хотя исследования функциональных и экологических последствий для микробиоты кишечника у естественных популяций продолжаются, с эволюционной точки зрения остается неясным, почему и когда возникли взаимоотношения между микробами и структурами мозга, определяющими социальное поведение (Sherwin et al., 2019). Тем не менее, активно подвигающиеся исследования этой оси – кишечник – микробиота – мозг – позволяют рассчитывать на успешно развивающиеся методы бактериального лечения ряда заболеваний, одной из черт которых является десоциализация (Cryan et al., 2019). Так, например, получены данные, свидетельствующие о том, что люди с большими социальными взаимосвязями, как правило, имеют более разнообразный микробиом. Это позволяет предположить, что социальные взаимодействия могут формировать микробное сообщество кишечника человека. Напротив, тревога и стресс связаны с уменьшением разнообразия и изменением состава микробиома (Johnson, 2020). Накапливается информация о том, что белки эндогенных ретровирусов, экспрессия которых в норма подавлена, в некоторых случаях транслируются, что служит основой изменений поведенческих реакций и их патологий (Johansson et al., 2020).

В этой связи особый интерес представляет вопрос изменений микробиоты у доместицированных видов по сравнению с близкородственными дикими (Alessandri et al., 2019; Ikeda-Ohtsubo et al., 2018). Сформировано представление о том, что во время одомашнивания на изменчивость микробиоты кишечника также могли влиять близкие контакты с человеком (Milani et al., 2017).

Вирусные метагеномные исследования продемонстрировали также выраженное разнообразие эукариотических вирусов и бактериофагов (виром) в фекалиях домашних видов, что может быть тесно связано с особенностями диеты животных (Xie et al., 2019). Известно, что примерно половина генома у всех видов млекопитающих представлена вирусными и провирусными последовательностями и продуктами их рекомбинаций, в совокупности своей формирующий мобилом из мобильных генетических элементов или транспозонов (ТЕ) – генетического резерва внутри- и межгеномного обмена. Накапливается информация о том, что белки эндогенных ретровирусов, экспрессия которых в норма подавлена, в некоторых случаях транслируются, что служит основой изменений поведенческих реакций и их патологий (Johansson et al., 2020).

Выявлено межконтинентальное распространение ретровирусов, многочисленные случаи межвидовой передачи и появления у хозяев, представляющих по меньшей мере 11 отрядов млекопитающих, а также значительная роль рекомбинаций в диверсификации вирусных линий (Diehl et al., 2016). Известно, что ТЕ широко участвуют в эпигеномной изменчивости, влючая такие процессы, как изменения рисунка метилирования, модификации гистонов, формирование микроРНК, трансгенерационное наследование (Hosaka, Kakutani, 2018; Lanciano, Mirouze, 2018). Можно ожидать, что конструирование новых ниш, в которых участвуют человек и доместицируемые виды, способствует активации ТЕ и формированию новых регуляторных сетей на их основе (Cho et al., 2018; Colino-Rabanal et al., 2018; Shapiro, 2017).

3. Заключение

Накопленные данные позволяют сделать следующее заключение. Основа формирования аграрной цивилизации обусловлена активностью человека по вовлечению в свою нишу других видов, способствующих повышению его адаптивного и репродуктивного потенциала. Существенный вклад в этот процесс вносили факторы, связанные с социальной активностью человека, его когнитивными функциями и его способностью целенаправленно изучать и контролировать различные характеристики вовлекаемых в доместикацию видов. Основные признаки, входящие в «доместикационный синдром» у животных, ассоциированы с их социальной активностью и геномной нестабильностью, обеспечивающей появление широкого фенотипического разнообразия. Их изменчивость в определенной степени зависит от разнообразия микробиоты и тесно с ней связанного вирома, влияющих на различные физиологические системы млекопитающих, в том числе и на социальную активность. Специфика межвидовых сообществ такой ниши, определяемой человеком, изменчивость микробиома и вирома, зависящая, в частности, от особенностей источников питания, формирование на основе вирома мобилома в их геномах создает материальные основы для фенотипического разнообразия, позволяющего вести целенаправленный отбор по адаптивному потенциалу животных и растений к предлагаемой человеком нише, а также по разнообразным фенотипическим характеристикам, повышающим адаптивный и репродуктивный потенциал человека в организуемой им нише.

References

Alberto et al., 2018 – *Alberto, F.J., Boyer, F., Orozco-terWengel, P., Streeter, I., Servin, B., de Villemereuil, P., Benjelloun, B., Librado, P., Biscarini, F., Colli, L., Barbato, M., Zamani, W., Alberti, A., Engelen, S., Stella, A., Joost, S., Ajmone-Marsan, P., Negrini, R., Orlando, L., Rezaei, H.R., Naderi, S., Clarke, L., Flicek, P., Wincker, P., Coissac, E., Kijas, J., Tosser-Klopp, G., Chikhi, A., Bruford, M.W., Taberlet, P., Pompanon, F.* (2018). Convergent genomic signatures of domestication in sheep and goats. *Nat Commun.* 9(1): 813. DOI: 10.1038/s41467-018-03206-y

Alessandri et al., 2019 – *Alessandri, G., Milani, C., Mancabelli, L., Mangifesta, M., Lugli, G.A., Viappiani, A., Duranti, S., Turroni, F., Ossiprandi, M.C., van Sinderen, D., Ventura, M.* (2019). The impact of human-facilitated selection on the gut microbiota of domesticated mammals. *FEMS Microbiol Ecol.* 95(9): fiz121. DOI: 10.1093/femsec/fiz121

Antonell et al., 2010 – *Antonell, A., Del Campo, M., Magano, L.F., Kaufmann, L., de la Iglesia, J.M., Gallastegui, F., Flores, R., Schweigmann, U., Fauth, C., Kotzot, D., Pérez-Jurado, L.A.* (2010). Partial 7q11.23 deletions further implicate GTF2I and GTF2IRD1 as the main genes responsible for the Williams-Beuren syndrome neurocognitive profile. *J Med Genet.* 47(5): 312-320. DOI: 10.1136/jmg.2009.071712

Bray et al., 2021 – *Bray, E.E., Gnanadesikan, G.E., Horschler, D.J., Levy, K.M., Kennedy, B.S., Famula, T.R., MacLean. E.L.* (2021). Early-emerging and highly heritable sensitivity to human communication in dogs. *Curr Biol.* 26: S0960-9822(21) 00602-3. DOI: 10.1016/j.cub.2021.04.055

Carneiro et al., 2014 – *Carneiro, M., Rubin, C.J., Di Palma, F., Albert, F.W., Alföldi, J., Martinez Barrio, A., Pielberg, G., Rafati, N., Sayyab, S., Turner-Maier, J., Younis, S., Afonso, S., Aken, B., Alves, J.M., Barrell, D., Bolet, G., Boucher, S., Burbano, H.A., Campos, R., Chang, J.L., Duranthon, V., Fontanesi, L., Garreau, H., Heiman, D., Johnson, J., Mage, R.G., Peng, Z., Queney, G., Rogel-Gaillard, C., Ruffier, M., Searle, S., Villafuerte, R., Xiong, A., Young, S., Forsberg-Nilsson, K., Good, J.M., Lander, E.S., Ferrand, N., Lindblad-Toh, K., Andersson, L.* (2014). Rabbit genome analysis reveals a polygenic basis for phenotypic change during domestication. *Science.* 345(6200): 1074-1079. DOI: 10.1126/science.1253714

Cho et al., 2018 – *Cho, J.* (2018). Transposon-Derived Non-coding RNAs and Their Function in Plants. *Front. Plant Sci.* 9: 600. DOI: 10.3389/fpls.2018.00600

Colino-Rabanal et al., 2018 – *Colino-Rabanal, V.J., Rodríguez-Díaz, R., Blanco-Villegas, M.J., Peris, S.J., Lizana, M.* (2018). Human and ecological determinants of the spatial structure of local breed diversity. *Sci Rep.* 8: 6452. DOI: 10.1038/s41598-018-24641-3

Conolly et al., 2011 – *Conolly, J., Colledge, S., Dobney, K., Vigne, J.-D., Peters, J., Stopp, B., Manning, K., Shennan, S.* (2011). Meta-analysis of zooarchaeological data from SW Asia and SE

Europe provides insight into the origins and spread of animal husbandry. *J Archaeol Sci.* 38(3): 538-545. DOI: 10.1016/j.jas.2010.10.008

Cryan et al., 2019 – *Cryan, J.F., O'Riordan, K.J., Cowan, C.S.M., Sandhu, K.V., Bastiaanssen, T.F.S., Boehme, M., Codagnone, M.G., Cussotto, S., Fulling, C., Golubeva, A.V., Guzzetta, K.E., Jaggar, M., Long-Smith, C.M., Lyte, J.M., Martin, J.A., Molinero-Perez, A., Moloney, G., Morelli, E., Morillas, E., O'Connor, R., Cruz-Pereira, J.S., Peterson, V.L., Rea, K., Ritz, N.L., Sherwin, E., Spichak, S., Teichman, E.M., van de Wouw, M., Ventura-Silva, A.P., Wallace-Fitzsimons. S.E., Hyland, N., Clarke, G., Dinan, T.G.* (2019). The Microbiota-Gut-Brain Axis. *Physiol Rev.* 99(4): 1877-2013. DOI: 10.1152/physrev.00018.2018

Der Sarkissian et al., 2015 – *Der Sarkissian, C., Ermini, L., Schubert, M., Yang, M.A., Librado, P., Fumagalli, M., Jónsson, H., Bar-Gal, G.K., Albrechtsen, A., Vieira, F.G., Petersen, B., Ginolhac, A., Seguin-Orlando, A., Magnussen, K., Fages, A., Gamba, C., Lorente-Galdos, B., Polani, S., Steiner, C., Neuditschko, M., Jagannathan, V., Feh, C., Greenblatt, C.L., Ludwig, A., Abramson, N.I., Zimmermann, W., Schafberg, R., Tikhonov, A., Sicheritz-Ponten, T., Willerslev, E., Marques-Bonet, T., Ryder, O.A., McCue, M., Rieder, S., Leeb, T., Slatkin, M., Orlando, L.* (2015). Evolutionary Genomics and Conservation of the Endangered Przewalski's Horse. *Curr Biol.* 25(19): 2577-2583. DOI: 10.1016/j.cub.2015.08.032

Diamond, 2002 – *Diamond, J.* (2002). Evolution, consequences and future of plant and animal domestication. *Nature.* 418(6898): 700-707. DOI: 10.1038/nature01019

Diehl et al., 2016 – *Diehl, W.E., Patel, N., Halm, K., Johnson, W.E.* (2016). Tracking interspecies transmission and long-term evolution of an ancient retrovirus using the genomes of modern mammals. *Elife.* 5: e12704. DOI: 10.7554/eLife.12704

Etokebe et al., 2008 – *Etokebe, G.E., Axelsson, S., Svaerd, N.H., Storhaug, K., Dembić, Z.* (2008). Detection of Hemizygous Chromosomal Copy Number Variants in Williams-Beuren Syndrome (WBS) by Duplex Quantitative PCR Array: An Unusual Type of WBS Genetic Defect. *Int J Biomed Sci.* 4(3): 161-170.

Eusebi et al., 2021 – *Eusebi, P.G., Sevane, N., O'Rourke, T., Pizarro, M., Boeckx, C., Dunner, S.* (2021). Gene expression profiles underlying aggressive behavior in the prefrontal cortex of cattle. *BMC Genomics.* 22(1): 245. DOI: 10.1186/s12864-021-07505-5

Ferrero et al., 2010 – *Ferrero, G.B., Howald, C., Micale, L., Biamino, E., Augello, B., Fusco, C., Turturo, M.G., Forzano, S., Reymond, A., Merla, G.* (2010). An atypical 7q11.23 deletion in a normal IQ Williams-Beuren syndrome patient. *Eur J Hum Genet.* 18: 33-38. DOI: 10.1038/ejhg.2009.108

Frantz et al., 2015 – *Frantz, L.A., Schraiber, J.G., Madsen, O., Megens, H.J., Cagan, A., Bosse, M., Paudel, Y., Crooijmans, R.P., Larson, G., Groenen, M.A.* (2015). Evidence of long-term gene flow and selection during domestication from analyses of Eurasian wild and domestic pig genomes. *Nat Genet.* 47: 1141-1148. DOI: 10.1038/ng.3394

Fuller, 2006 – *Fuller, D.Q.* (2006). Agricultural origins and frontiers in South Asia: a working synthesis. *J World Prehist.* 20(1): 1-86. DOI: 10.1007/s10963-006-9006-8

Glazko et al., 2015 – *Glazko, V., Zybailov, B., Glazko, T.* (2015). Asking the Right Question about the Genetic Basis of Domestication: What is the Source of Genetic Diversity of Domesticated Species? *Adv. Genet. Eng.* 4: 2. DOI: http://dx.doi.org/10.4172/2169-0111.1000125

Hosaka, Kakutani, 2018 – *Hosaka, A., Kakutani, T.* (2018). Transposable elements, genome evolution and transgenerational epigenetic variation. *Curr Opin Genet Dev.* 49: 43-48. DOI: 10.1016/j.gde.2018.02.012

Ikeda-Ohtsubo et al., 2018 – *Ikeda-Ohtsubo, W., Brugman, S., Warden, C.H., Rebel, J.M.J., Folkerts, G., Pieterse, C.M.J.* (2018). How Can We Define "Optimal Microbiota?" A Comparative Review of Structure and Functions of Microbiota of Animals, Fish, and Plants in Agriculture. *Front Nutr.* 5: 90. DOI: 10.3389/fnut.2018.00090

Irving-Pease et al., 2018 – *Irving-Pease, E.K., Frantz, L.A.F., Sykes, N., Callou, C., Larson, G.* (2018). Rabbits and the Specious Origins of Domestication. *Trends Ecol Evol.* 33(3): 149-152. DOI: 10.1016/j.tree.2017.12.009

Johansson et al., 2020 – *Johansson, E.M., Bouchet, D., Tamouza, R., Ellul, P., Morr, A.S., Avignone, E., Germi, R., Leboyer, M., Perron, H., Groc, L.* (2020). Human endogenous retroviral protein triggers deficit in glutamate synapse maturation and behaviors associated with psychosis. *Sci Adv.* 6(29): eabc0708. DOI: 10.1126/sciadv.abc0708

Johnson, 2020 – *Johnson, K.* (2020). Gut microbiome composition and diversity are related to human personality traits. *Human Microbiome Journal*. 15: 100069. DOI: https://doi.org/10.1016/j.humic.2019.100069

Kukekova et al., 2018 – *Kukekova, A.V., Johnson, J.L., Xiang, X., Feng, S., Liu, S., Rando, H.M., Kharlamova, A.V., Herbeck, Y., Serdyukova, N.A., Xiong, Z., Beklemischeva, V., Koepfli, K.P., Gulevich, R.G., Vladimirova, A.V., Hekman, J.P., Perelman, P.L., Graphodatsky, A.S., O'Brien, S.J., Wang, X., Clark, A.G., Acland, G.M., Trut, L.N., Zhang, G.* (2018). Red fox genome assembly identifies genomic regions associated with tame and aggressive behaviours. *Nat Ecol Evol*. 2(9): 1479-1491. DOI: 10.1038/s41559-018-0611-6

Lanciano, Mirouze, 2018 – *Lanciano, S., Mirouze, M.* (2018). Transposable elements: all mobile, all different, some stress responsive, some adaptive? *Opinion in Genetics and Development*. 49: 106-114. DOI: https://doi.org/10.1016/j.gde.2018.04.002

Larson, Fuller, 2014 – *Larson, G., Fuller, D.Q.* (2014). The evolution of animal domestication. *Annu Rev Ecol Evol Syst*. 45(1): 115-136. DOI: 10.1146/annurev-ecolsys-110512-135813

Larson et al., 2014 – *Larson, G., Piperno, D.R., Allaby, R.G., Purugganan, M.D., Andersson, L., Arroyo-Kalin, M., Barton, L., Climer Vigueira, C., Denham, T., Dobney, K., Doust, A.N., Gepts, P., Gilbert, M.T., Gremillion, K.J., Lucas, L., Lukens, L., Marshall, F.B., Olsen, K.M., Pires, J.C., Richerson, P.J., Rubio de Casas, R., Sanjur, O.I., Thomas, M.G., Fuller, D.Q.* (2014). Current perspectives and the future of domestication studies. *Proc Natl Acad Sci U S A*. 111(17): 6139-46. DOI: 10.1073/pnas.1323964111

Librado et al., 2017 – *Librado, P., Gamba, C., Gaunitz, C., Der Sarkissian, C., Pruvost, M., Albrechtsen, A., Fages, A., Khan, N., Schubert, M., Jagannathan, V., Serres-Armero, A., Kuderna, L.F.K., Povolotskaya, I.S., Seguin-Orlando, A., Lepetz, S., Neuditschko, M., Thèves, C., Alquraishi, S., Alfarhan, A.H., Al-Rasheid, K., Rieder, S., Samashev, Z., Francfort, H.P., Benecke, N., Hofreiter, M., Ludwig, A., Keyser, C., Marques-Bonet, T., Ludes, B., Crubézy, E., Leeb, T., Willerslev, E., Orlando, L.* (2017). Ancient genomic changes associated with domestication of the horse. *Science*. 356(6336): 442-445. DOI: 10.1126/science.aam5298

Linseele et al., 2007 – *Linseele, V., Van Neer, W., Hendrickx, S.* (2007). Evidence for early cat taming in Egypt. *J Archaeol Sci*. 34(12): 2081-2090. DOI: 10.1016/j.jas.2007.02.019

Lopatina et al., 2018 – *Lopatina, O.L., Komleva, Y.K., Gorina, Y.V., Olovyannikova, R.Y., Trufanova, L.V., Hashimoto, T., Takahashi, T., Kikuchi, M., Minabe, Y., Higashida, H., Salmina, A.B.* (2018). Oxytocin and excitation/inhibition balance in social recognition. *Neuropeptides*. 72: 1-11. DOI: 10.1016/j.npep.2018.09.003

López-Tobón et al., 2020 – *López-Tobón, A., Trattaro, S., Testa, G.* (2020) The sociability spectrum: evidence from reciprocal genetic copy number variations. *Mol Autism*. 11(1):50. DOI: 10.1186/s13229-020-00347-0

Makeyev, Bayarsaihan, 2011 – *Makeyev, A.V., Bayarsaihan, D.* (2011). Molecular basis of Williams-Beuren syndrome: TFII-I regulated targets involved in craniofacial development. *Cleft Palate Craniofac J*. 48(1):109-16. DOI: 10.1597/09-093

Mcbrearty, Brooks, 2000 – *Mcbrearty, S., Brooks, A.S.* (2000). The revolution that wasn't: a new interpretation of the origin of modern human behavior. *Journal of Human Evolution*. 39(5): 453-563. DOI: 10.1006/jhev.2000.0435

McHugo et al., 2019 – *McHugo, G.P., Dover, M.J., MacHugh, D.E.* (2019). Unlocking the origins and biology of domestic animals using ancient DNA and paleogenomics. *BMC Biol*. 17(1): 98. DOI: 10.1186/s12915-019-0724-7

Merla et al., 2002 – *Merla, G., Ucla, C., Guipponi, M., Reymond, A.* (2002). Identification of additional transcripts in the Williams-Beuren syndrome critical region. *Hum Genet*. 110(5): 429-438. DOI: 10.1007/s00439-002-0710-x

Milani et al., 2017 – *Milani, C., Mangifesta, M., Mancabelli, L. Lugli G.A., James, K., Duranti, S., Turroni, F., Ferrario C., Ossiprandi, M.C., van Sinderen, D, & Ventura, M.* (2017). Unveiling bifidobacterial biogeography across the mammalian branch of the tree of life. *The ISME J*. 11: 2834-2847. DOI: https://doi.org/10.1038/ismej.2017.138

Nakayama et al., 2012a – *Nakamura, N., Toba, S., Hirai, M., Morishita, S., Mikami, T., Konishi, M., Itoh, N., Kurosaka, A.* (2005). Cloning and expression of a brain-specific putative UDP-GalNAc: polypeptide N-acetylgalactosaminyltransferase gene. *Biol Pharm Bull*. 28(3): 429-433. DOI: 10.1248/bpb.28.429

Nakayama et al., 2012b – *Nakayama, Y., Nakamura, N., Oki, S., Wakabayashi, M., Ishihama, Y., Miyake, A., Itoh, N., Kurosaka, A.* (2012). A putative polypeptide N-acetylgalactosaminyltransferase/Williams-Beuren syndrome chromosome region 17 (WBSCR17) regulates lamellipodium formation and macropinocytosis. *J Biol Chem*. 287(38): 32222-35. DOI: 10.1074/jbc.M112.370932

Outram et al., 2009 – *Outram, A.K., Stear, N.A., Bendrey, R., Olsen, S., Kasparov, A., Zaibert, V., Thorpe, N., Evershed, R.P.* (2009). The earliest horse harnessing and milking. *Science*. 323(5919): 1332-5. DOI: 10.1126/science.1168594

Park et al., 2015 – *Park, S.D., Magee, D.A., McGettigan, P.A., Teasdale, M.D., Edwards, C.J., Lohan, A.J., Murphy, A., Braud, M., Donoghue, M.T., Liu, Y., Chamberlain, A.T., Rue-Albrecht, K., Schroeder, S., Spillane, C., Tai, S., Bradley, D.G., Sonstegard, T.S., Loftus, B.J., MacHugh, D.E.* (2015). Genome sequencing of the extinct Eurasian wild aurochs, *Bos primigenius*, illuminates the phylogeography and evolution of cattle. *Genome Biol*. 16:234. DOI: 10.1186/s13059-015-0790-2

Paudel et al., 2013 – *Paudel, Y., Madsen, O., Megens, H.J., Frantz, L.A., Bosse, M., Bastiaansen, J.W., Crooijmans, R.P., Groenen, M.A.* (2013). Evolutionary dynamics of copy number variation in pig genomes in the context of adaptation and domestication. *BMC Genomics*. 14:449. DOI: 10.1186/1471-2164-14-449

Progovac, Benítez-Burraco, 2019 – *Progovac, L., Benítez-Burraco, A.* (2019). From Physical Aggression to Verbal Behavior: Language Evolution and Self-Domestication Feedback Loop. *Front. Psychol*. 10: 2807. DOI: 10.3389/fpsyg.2019.02807

Rubin et al., 2010 – *Rubin, C.J., Zody, M.C., Eriksson, J., Meadows, J.R., Sherwood, E., Webster, M.T., Jiang, L., Ingman, M., Sharpe, T., Ka, S., Hallböök, F., Besnier, F., Carlborg, O., Bed'hom, B., Tixier-Boichard, M., Jensen, P., Siegel, P., Lindblad-Toh, K., Andersson, L.* (2010). Whole-genome resequencing reveals loci under selection during chicken domestication. *Nature*. 464(7288): 587-591. DOI: 10.1038/nature08832

Sánchez-Villagra, van Schaik, 2019 – *Sánchez-Villagra, M.R., van Schaik, C.P.* (2019). Evaluating the self-domestication hypothesis of human evolution. *Evol Anthropol*. 28(3): 133-143. DOI: 10.1002/evan.21777

Schubert, 2009 – *Schubert, C.* (2009). The genomic basis of the Williams-Beuren syndrome. *Cell Mol Life Sci*. 66(7): 1178-1197. DOI: 10.1007/s00018-008-8401-y

Shapiro, 2017 – *Shapiro, J.A.* (2017). Living Organisms Author Their Read-Write Genomes in Evolution. *Biology*. 6(4): 42. DOI: 10.3390/biology6040042

Sherwin et al., 2019 – *Sherwin, E., Bordenstein, S.R., Quinn, J.L., Dinan, T.G., Cryan, J.F.* (2019). Microbiota and the social brain. *Science*. 366(6465): eaar2016. DOI: 10.1126/science.aar2016

Sohal, Rubenstein, 2019 – *Sohal, V.S., Rubenstein, J.L.R.* (2019). Excitation-inhibition balance as a framework for investigating mechanisms in neuropsychiatric disorders. *Mol Psychiatry*. 24(9): 1248-1257. DOI: 10.1038/s41380-019-0426-0.

Somel et al., 2009 – *Somel, M., Franz, H., Yan, Z., Lorenc, A., Guo, S., Giger, T., Kelso, J., Nickel, B., Dannemann, M., Bahn, S., Webster, M.J., Weickert, C.S., Lachmann, M., Pääbo, S., Khaitovich, P. (*2009). Transcriptional neoteny in the human brain. *Proc Natl Acad Sci USA*. 106(14): 5743-5748. DOI: 10.1073/pnas.0900544106

von Holdt et al., 2017 – *von Holdt, B.M., Shuldiner, E., Koch, I.J., Kartzinel, R.Y., Hogan, A., Brubaker, L., Wanser, S., Stahler, D., Wynne, C.D.L., Ostrander, E.A., Sinsheimer, J.S., Udell, M.A.R.* (2017). Structural variants in genes associated with human Williams-Beuren syndrome underlie stereotypical hypersociability in domestic dogs. *Sci Adv*. 3(7): e1700398. DOI: 10.1126/sciadv.1700398

Wilkins, 2020 – *Wilkins, A.S.* (2020). A striking example of developmental bias in an evolutionary process: The "domestication syndrome". *Evol Dev*. 22(1-2): 143-153. DOI: 10.1111/ede.12319

Wutke et al., 2018 – *Wutke, S., Sandoval-Castellanos, E., Benecke, N., Döhle, H.J., Friederich, S., Gonzalez, J., Hofreiter, M., Lõugas, L., Magnell, O., Malaspinas, A.S., Morales-Muñiz, A., Orlando, L., Reissmann, M., Trinks, A., Ludwig, A.* (2018). Decline of genetic diversity in ancient domestic stallions in Europe. *Sci Adv*. 4(4): eaap9691. DOI: 10.1126/sciadv.aap9691

Xie et al., 2019 – *Xie, X.T., Kropinski, A.M., Tapscott, B., Weese, J.S., Turner, P.V.* (2019). Prevalence of fecal viruses and bacteriophage in Canadian farmed mink (Neovison vison). *Microbiologyopen*. 8(1): e00622. DOI: 10.1002/mbo3.622

Zanella et al., 2019 – *Zanella, M., Vitriolo, A., Andirko, A., Martins, P.T., Sturm, S., O'Rourke, T., Laugsch, M., Malerba, N., Skaros, A., Trattaro, S., Germain, P.L., Mihailovic, M., Merla, G., Rada-Iglesias, A., Boeckx, C., Testa, G.* (2019). Dosage analysis of the 7q11.23 Williams region identifies BAZ1B as a major human gene patterning the modern human face and underlying self-domestication. *Sci Adv*. 5(12): eaaw7908. DOI: 10.1126/sciadv.aaw7908.

Zeder, 2011 – *Zeder, M.A.* (2011). The origins of agriculture in the Near East. *Curr Anthropol*. 52(S4): S221-SS35. DOI: 10.1086/659307

Zeder, 2015 – *Zeder, M.A.* (2015). Core questions in domestication research. *Proc Natl Acad Sci USA*. 112(11): 3191-3198. DOI: 10.1073/pnas.1501711112

Человек и доместицированные виды (обзор)

Валерий Иванович Глазко [a, b, *], Глеб Юрьевич Косовский [b], Татьяна Теодоровна Глазко [a, b]

[a] Российский государственный аграрный университет – МСХА им. К.А. Тимирязева, Российская Федерация

[b] НИИ пушного звероводства и кролиководства им. В.А. Афанасьева, Российская Федерация

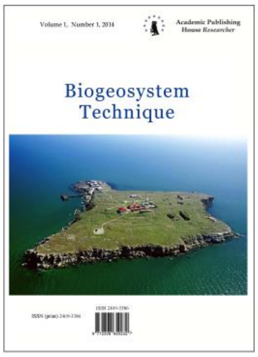
Аннотация. Аграрная цивилизация была сформирована благодаря доместикации главным образом пяти видов млекопитающих (козы, овцы, крупный рогатый скот, свиньи, лошади) и ограниченного количества видов растений, главные из которых – пшеница и рис. Это небольшое количество видов осталось базой аграрной цивилизации и до сих пор, не смотря на продолжающиеся попытки доместикации других диких видов. К настоящему времени стало понятно, что доместикация является одним из вариантов мутуалистических взаимоотношений между человеком и соответствующими видами. Это может хорошо объяснять ограниченность количества видов, успешно вовлекавшихся в доместикацию, поскольку возможности «симбиоза» с человеком высоко видоспецифичны. Отсюда становится очевидным, что утрата разнообразия генофондов сельскохозяйственных видов принципиально невосполнима, так же как и деградация почв, биосферы и является прямой угрозой для существования аграрной цивилизации, поскольку именно эти виды исторически лежали в основе ее существования и развития. С этой точки зрения сохранение биоразнообразия сельскохозяйственных видов становится критическим условием для существования аграрной цивилизации в будущем. Механизмы доместикации – ключевой вопрос в понимании и управлении генетическими ресурсами сельскохозяйственных видов животных и растений. В обзоре рассматривается основа формирования аграрной цивилизации, которая обусловлена активностью человека по вовлечению в свою нишу других видов, способствующих повышению его адаптивного и репродуктивного потенциала. Существенный вклад в этот процесс вносили факторы, связанные с социальной активностью человека, его когнитивными функциями и его способностью целенаправленно изучать и контролировать различные характеристики вовлекаемых в доместикацию видов. Основные признаки, входящие в «доместикационный синдром» у животных, ассоциированы с их социальной активностью и геномной нестабильностью. Их изменчивость, создающая основы для отбора, зависит от разнообразия микробиоты и тесно с ней связанного вирома. Формирование на основе вирома мобилома в геномах создает материальные основы для фенотипического разнообразия, позволяющего вести целенаправленный отбор по адаптивному потенциалу животных и растений к предлагаемой человеком нише, а также по разнообразным фенотипическим характеристикам, повышающим адаптивный и репродуктивный потенциал человека в организуемой им нише.

Ключевые слова: доместикация, ниша, социальная активность, микробиом, вирусом, мобилом.

[*] Корреспондирующий автор
Адреса электронной почты: vigvalery@gmail.com (В.И. Глазко)

Published in the Slovak Republic
Biogeosystem Technique
Has been issued since 2014.
E-ISSN: 2413-7316
2021. 8(1): 45-67

DOI: 10.13187/bgt.2021.1.45
www.ejournal19.com



Is There a Nitrogen Deficiency in Organic Farming, and are the Yields in Organic Agriculture Lagging Due To Nitrogen Deficiency? And Can Conventional Agriculture Learn from the Mistakes of Organic Agriculture? (Critical Review)

Anton O. Nigten [a, *]

[a] Salt of the Earth, Wageningen, Netherlands

Paper Review Summary:
Received: 2021, February 20
Received in revised form: 2021, June 22
Acceptance: 2021, July 2

Abstract

In organic agriculture the yields are roughly 25 % lower than in conventional agriculture. And the quality differences are only small, save for pesticide residuals which are in general lower in organic products. Some scientists think the lower yields are caused by the slow mineralization of organic nitrogen. But in this article is demonstrated that the quality of the animal dung and the warm compost hinders the uptake of organic nitrogen and other organic nutrients. The dung and the compost in organic agriculture today are not treated well and because of this the plants are hindered in their growth. Their symbionts in the soil can't assist them in collecting the organically bound nutrients, because they are lacking or silenced. Above that the dung and the warm compost have lost lots of nutrients in the stables, during composting and while at the piles. Vermicomposts on the other side have less losses, better microbes and no growth limiting poisonous organic compounds. Compared to vermicompost, animal dung and warm compost give lower yields, inferior growth qualities and less resistance to pests and diseases. The adding of earth into the dung or the compost has comparable positive effects as vermicompost. The improperly treated dung and warm compost lead to crops which contain too much non protein nitrogen and probably also non protein sulphur. And the crops are not in balance for their other cations and anions. Just like the crops in conventional agriculture. Through this cows for instance don't become old and they produce a poor quality urine and poop. And the milk is also of poorer quality. So there is a lot to improve. In organic agriculture and in conventional agriculture.

Keywords: nitrogen deficiency, organic farming, organic agriculture, conventional agriculture.

1. Introduction

Already more than 200 years there is a clash between organic agriculture and chemical agriculture. It started around 1820 with the mineral theory (Sprengel) against the humus theory (Thaer). Or, in other words, chemical oriented scientists versus organic theorists. Thaer versus Sprengel and Liebig as mentioned… or Stoklasa, Frank, and Schanderl who studied the nitrogen

* Corresponding author
E-mail address: aonigten@hotmail.com (A.O. Nigten)

assimilation by all plants versus the proponents of inorganic nitrogen who denied that assimilation by non leguminous crops; Jamieson versus Lawes in the phosphate battle; Hopkins – representative of the fertilizer industry – versus Howard and Lady Balfour, the two proponents of organic agriculture…

At the Wageningen agricultural university the last fifty years the conflict has hardened. The scientists who are convinced that artificial fertilizers are unmissable have done everything to stop organic research at this university. They used all means to oppose the organic research. That led to personal dramas. But a real scientific debate was not possible. At the background the fertilizer industry had a big influence through their funds and textbooks. There are at the moment at either side no people who are able to bridge the gap. The arguments of the scientists on the side of conventional agriculture circle around two themes: a. the yields of organic agriculture are too low to feed the world, and, b. the claim that the products of organic agriculture are healthier is not proven. The arguments on the other side, from the proponents of organic agriculture, emphasize the risks of artificial fertilizers, pesticides and genetic engineering for the environment, for nature and for men. Both sides have strong and valid arguments.

But both groups have more in common than they suppose. In order to bridge the gap the presuppositions of the two approaches have to be clarified. Both groups for example have only very summary ideas about what quality really is, and even here there is no agreement. The organic proponents for instance claim lower levels of nitrate in their products. But the proponents of conventional agriculture today say – in Western Europe - that nitrate in food is no longer a problem. And they deny or ignore simply that the nutrient density of the crops has gone down. But the nutrient density of organic crops is almost the same as that of the conventional agriculture. and contain also, like conventional agriculture, too little trace elements (Swoboda[*], 2016; Dangour et al, 2009; Fan et al., 2008 ; Dimkpa, Bindrapan, 2016). A real stalemate.

Organic agriculture is not really ‘organic’ because in most cases the mineralization of its nutrients is at the moment the only way in which the plants can get in their nitrogen, sulphur, and other nutrients. As salt ions. Plants should be given the possibility to get their nutrients in an organic form. Through their symbionts, or directly by themselves (see part one)..

In this article some new arguments for a real organic agriculture will be worked out which can help to overcome the standstill. What we need is more reflection on the presumptions of conventional and of today organic agriculture and a thorough paradigm shift based on that reflection.

2. Discussion and results

Nitrogen in organic agriculture.

Yields.

The yields in organic agriculture are roughly 25 % lower than in conventional agriculture. But that is indeed a rough figure because if we zoom in, we see that there are big differences. And those differences are related to the following factors: the type of crop; annual versus perennial crops; and the regions where the crops are grown. The differences are small for fruit and oilseeds. Vegetables, potatoes and some grains, on the other hand, show large yield differences. The differences are smaller in developed countries than in underdeveloped countries. And there are even more nuances, such as with legumes that supply their own nitrogen, or the influence of the craftsmanship of the farmers (Seufert et al., 2012).

Mineralization?

Various authors have concluded that the differences in organic yields compared with conventional agriculture can be explained by a shortage of plant available mineral nitrogen (Seufert et al., 2012; Opdebeeck et al., 2004[†]). Sometimes a shortage of phosphorus is also mentioned to explain

[*] “Micronutrient deficiency is a common constraint in organic farming worldwide” (Swoboda, 2016)

[†] In 2019 I have done a preliminary investigation on this subject, based on the data of Opdebeeck et al. and on a great trial with potatoes by The Louis Bolk institute. This institute is founded in the Netherlands for research in organic agriculture. My report is written in dutch and contains a lot of empirical data (Nigten, 2019).

the differences in yield. In general, there is sufficient organic nitrogen present with good fertilization*, but it is not released quickly enough – not at the right time and not in the right amounts.

'The organic nitrogen in green manures, compost and animal manure is not transformed quickly enough from organic nitrogen into a mineral form, so growth is slow to start and the crop cannot fully grow out or continue to mature. As a result, the yields and the protein content lag behind', according to both authors.

The question is whether this statement is correct. At first sight, the facts seem to be correct. Yes, many organic soils contain more than enough organic nitrogen in case of regular fertilization (Lawes, Gilbert, 1858; Poschenrieder, Lesch, 1942; Hopkins, 1956). Nevertheless, the yields and the protein contents in organic agriculture are lagging behind. But is this because of a too slow mineralization? That is still the question. In fact, my central question. There are at least five other ways in which plants can get their nitrogen (see part one). But why do plants on most organic farms not use these possibilities? That is the real issue.

Let us look at it in more details.

Vermicompost, cow dung and warm compost.

A comparison between vermicompost, farmyard manure and/or warm compost shows that there are big differences in composition, performance and microbial life:

"Earthworms vermicompost is proving to be a highly nutritive 'organic fertilizer' and more powerful 'growth promoter' over the conventional composts and a 'protective' farm input (increasing the physical, chemical & biological properties of soil, restoring & improving its natural fertility) against the 'destructive' chemical fertilizers which have destroyed the soil properties and decreased its natural fertility over the years. Vermicompost is rich in NKP (nitrogen 2-3 %, potassium 1.85-2.25 % and phosphorus 1.55-2.25 %), micronutrients, beneficial soil microbes and also contain 'plant growth hormones & enzymes'. It is scientifically proving as 'miracle growth promoter & also plant protector' from pests and diseases. Vermicompost retains nutrients for long time and while the conventional compost fails to deliver the required amount of macro and micronutrients including the vital NKP to plants in shorter time, the vermicompost does" (Sinha et al., 2009).

So vermicompost delivers the required amounts of nutrients in time.

This was verified by: Bhatia, 2000; Bhatia et al., 2000; Sinha and Bharambe, 2007; Krunal, 2009, and by Dalsukh, 2009.

---

* E. Hennig calculated that in a humus-rich soil where sufficient cobalt is present, enormous amounts of organic nitrogen are found in the soil: "*A soil with 4 % organic matter contains 120,000 kg (..) of organic matter in the top 20 cm per hectare. It is stated in the professional literature that humus contains about 5 % nitrogen. Based on this five percent, the 120 tons of organic matter have 6,000 kg of organically bound nitrogen. (...) In one hectare of land there is an amount of nitrogen in the top 20 cm that is more than enough to fertilize this one hectare with approximately 400 kg N for 55 years* "(Hennig 1996: 141-142). That must of course be: 15 years. 15 x 400 = 6000. I suspect that something went wrong with the translation. In calculating the amount of organic matter, Hennig assumed that a cubic decimeter of earth weighs 1.5 kg. Checking with moist loamy sandy soil from my garden shows that a liter of this soil weighs 1140 to 1200 g. But maybe it was too loose because I was scooping it up. Clay soil will be heavier.

Van Kessel calculated that a soil with 3 % organic matter contains 9000 kg N/ha in the top 30 cm. So with 4% organic matter it contains 12.000 kg N/Ha in the top 30 cm (Van Kessel, 2008). The difference is that Van Kessel states that the C: N ratio in soil organic matter is 10: 1 while Hennig says it is 20: 1. To decide how much nitrogen is in the top soil only measurements can give a definite answer. But a figure between 6000 and 9000 kg Nitrogen per hectare means there is a lot which the plants – at least theoretically – can get. And a consumption of 400 kg N/year is pretty much, 100-200 kg is enough for most crops. For some crops more is needed (and lost).

Hopkins gives the following data for phosphor, potassium (and nitrogen) in an average soil: "*Here are some figures from Cornell University in the U.S.A. In the soil studied, the figures for total phosphoric acid in the top four feet of soil showed a content that could, if it were all available, support normal rotation cropping for 367 years. For potash, the calculated period was 1,435 years. Speaking more generally about the same matter in this country, the late Sir Daniel Hall stated,'Roughly speaking, an average soil contains enough plant food for a hundred full crops*.' (Hopkins, 1956). Hopkins gives also the amount of nitrogen per acre in Prairieland in the USA before the farmers came: "*When the prairie land was ploughed, its nitrogen content was 6,940 pounds per acre*" (Hopkins, 1956). And ploughing alone gave a loss of 68 pounds per year per acre.

The research by Agarwal and Sinha demonstrates the following differences for the three main elements between warm compost from cow manure and vermicompost (Table 1).

Table 1. Content of nutrients in warm compost from cow manure and/or of worms

Nutrient	Warm compost from cow dung	Vermicompost
Nitrogen	0.4 – 1.0 %	2.5 – 3.0 %
Phosphor	0.4 – 0.8 %	1.8 – 2.9 %
Potassium	0.8 – 1.2 %	1.4 – 2.0 %

Important other nutrients contained in vermicompost are compared with nutrients in conventional anaerobic and aerobic composts in Table 2. The three composts were made from the same basic material: food and garden waste, but the differences were substantial, except for potassium.

The extremely high levels of iron and magnesium probably come from the earth particles of the weeds. In India, the grounds of the Deccan plateau for instance are very rich in iron and magnesium, and worms also "digest" the soil, depending on the type of worms used.

Table 2. Content of nutrients in vermicompost, aerobic compost, and anaerobic compost (Sinha et al., 2009)

Nutrient	Vermicompost	Aerobic compost	Anaerobic compost
1) Nitrogen(N)	9.500	6.000	5.700
2) Phosphorous (P)	0.137	0.039	0.050
3) Potassium (K)	0.176	0.152	0.177
4) Iron (Fe)	19.730	15.450	17.240
5) Magnesium (Mg)	4.900	1.680	2.908
6) Manganese (Mn)	0.016	0.005	0.006
7) Calcium (Ca)	0.276	0.173	0.119
(Sinha et al., 2009).			

The yields varied correspondingly to the kind of compost. The major differences are presented in Table 3.

Table 3. Agricultural effects of worm compost, cow manure compost and fertilizer on the growth and yield of wheat*

Treatment	Input/Hectare	Yield/Hectare
1) Control	(No Input)	1.52 t/ha
2) Worm compost (vermicompost) (VC)	2.5 t VC/ha	4.01 t/ha
3) Cow dung Compost (CDC)	10 t CDC/ha	3.32 t/ha
4) Fertilizer (CF)	NPK(120:60:40) kg/ha	3.42 t/ha

*Keys: N = Urea; P = Single Super Phosphate; K = Murete of Potash (kg/ha) (Sinha et al., 2009).

With cattle dung compost applied at 10 t/ha (4 times of vermicompost), the yield was just over 3,3 t/ha which is about 18 % less than that with vermicompost and that too after using 400% more conventional [cattle dung] compost.

Application of vermicompost had other agronomic, economic and environmental benefits. It significantly 'reduced the demand of water for irrigation' by nearly 30-40 %. Test results indicated 'better availability of essential micronutrients and useful microbes' in vermicompost applied soils. A remarkably significant observation was 'less incidences of pests and disease' attacks in vermicompost applied crops.

Agronomic impacts of earthworms and vermicompost vis-a-vis cattle dung compost and chemical fertilizers on growth and yield of potted wheat crops are shown in Table 4 (Sinha et al., 2009).

Table 4. Growth and yield of potted wheat under different fertilization

Biometrical data (average)	Control	Treatment 1 Earthworms **and** vermicompost	Treatment 2 Chemical fertilizer	Treatment 3 Cattle dung compost
1. Percentage of germinated seeds	50	90	60	56
2. Height of plant (cm)	34.16	85.22	39.97	37.3
3. Ear length (cm)	4.82	8.77	5.45	5.1
4. Number of seed grains per ear (pcs)	11.80	31.10	19.90	17.4
5. Number of tillers per plant (pcs)	1	2-30	1-20	1-2

In an overview study on vermicompost, Pathma et al. wrote in 2012:

"Worm composting is the best alternative to traditional composting. It goes 2-5 times faster. The material becomes more homogeneous. And the microbes in worm compost and warm compost differ widely.

Worm compost is also much richer in bacteria and fungi than warm compost. And it contains exactly the bacteria that belong to the rhizosphere[*]*. They bind carbon to the metal ions.*

Worm compost contains more humic and fulvic acids. These bind the released nutrients better, making worm compost less saline than warm compost.

The nitrogen present in the given material is made available faster and better. There are more nitrogen-binding bacteria and more growth-promoting substances.

Soil fertilized with worm compost showed better plant growth compared to soil fertilized with chemical fertilizer or cow manure. The quality of fruit and vegetables was better, with less heavy metals and less nitrate than with mineral fertilization" (Pathma, Sakthivel, 2012).

The importance of the right microbes is also proven by the use of vermicompost tea. Small amounts of these teas are enough for good results.

The work of the following three authors gives a good overview of the worm compost research (Sinha et al., 2009; Pathma, Sakthivel, 2012; Chaudhuri et al., 2016).

[*] Husssain came to the same conclusion: "vermicompost enhances the activities of beneficial microbes in the soil (Arancon et al., 2005; Yardim et al., 2006; Cardoza, 2011; Singh et al., 2013; Xiao et al., 2016; Hussain et al., 2020).

Chaudhuri et al. have researched the results of different dosages of vermicompost in pineapple cultivation. Among other things, they showed that loamy sandy soil turned into loamy clay soil in a short period of two years at a dosage of 20 ton of worm compost per hectare per year.

"A 20 % decrease in sand content and 10% and 30% increase in silt and clay contents respectively were noted in Plot T3 among different treatment plots. (..) Highest silt (27.5 %) and clay (32.5 %) and lowest sand (40 %) in fact were recorded in Plot T3" (Chaudhuri et al., 2016).

Chaudhuri et al. also found that the amounts of carbon and nitrogen in the soil increased with higher applications of worm compost. After application of the 30 t/ha of worm compost per year, the amount of organic carbon increased by 16 % and the total amount of nitrogen doubled.

Vermicompost tea.

My colleague, Mr. Kennes, sprayed in 2020 vermicompost tea over pastures, over corn for silage, and over fodder beet fields. Mr Kennes applied a mixture of vermicompost in water in concentration of 1% in the dose of 80 l/ha. Then the growth of corn, which had almost stopped as a consequence of the extreme drought of that season, restored. The pastures became green again. The same effect was seen in the fields with corn, and with fodder beets. The growth of the corn and the fodder beets went on till very late in the year. The fodder beets were harvested in December. The beets had even then no fungi nor deficiency symptoms.

Mr Kennes' results are very similar to those of Mr. Dixon who managed to overcome the 1826 drought without much difficulty. He had made compost from slurry, peat, swamp soil and ditch dredge. In 1839, Mr. Dixon was awarded a prize of the Royal Agricultural Society of England for his essay on his experiences with this method of composting:

"The full effects of this practice I first experienced in the dry season of 1826: I had some clovers which had been manured the previous winter; my land was soon covered with crop, and that so vigorous a one, that the hot weather did not overpower it. My cows, that summer, were tied up during the day-time, and in the night they were turned out into the pastures ; most of the stock in my district were much distressed from over-heat as well as from being short of food for some weeks ; milk yielded little butter, scarcely any for a time was offered in our large market-town : —no doubt that year will be remembered by many gentlemen on the Agricultural Society's committee. I, however, was under no difficulties on account of the season: my clovers produced plenty of food for my cattle, and in return they yielded as much milk and butter as I ever recollect from the same number" (Dixon[*], 1839).

Future research.

To my opinion there is only one explanation possible: the vermicompost tea contains the microbes which the land and the crops are missing. If this is correct, then it is really amazing that such small amounts of microbes make the difference. Not only for the growth of the crops, but also for protection aganist the heat and the drought and for the resistance against pests and diseases.

And are the microbes from the 'slurry, peat, swamp soil and ditch dredge' of Dixon comparable to these in the vermicompost tea? Further on in the third article about nitrogen we will see that mixing earth with manure or plant residuals gives very good results, because the nutrients are not lost.

Here is a challenge for science. Do microbes in vermicompost, vermicompost tea or in with earth and plant residuals enriched manure and compost really make the difference? Or is another explanation possible or necessary?

Summarizing.

Warm compost, farmyard manure and slurry are not (yet) suitable as food for the symbionts in the soil and for the plants for various reasons. With vermicompost, and with farmyard manure, slurry and plant residuals or compost mixed with earth, you can avoid these problems.

We see that the yields with organic material (i.e. vermicompost, or with earth enriched manure and compost) can be the same or higher than with artificial fertilizers. Also the quality of the plants, expressed as growth characteristics, is better with vermicompost.

---

[*] Mr. Dixon made two other remarks, worth to mention. 1. If you use 'most foul or weedy mould' plus liquid manure, the urine kills all wire worms, slugs and destroying insects, and weed seeds as well. 2. He planned to build a movable railway for the transport of the liquid manure to the fields, to make the compost heaps on the spot. In this way he hoped to save a lot of money for transport.

That needs more explanation because we have all learned – especially in organic agriculture – that warm compost, farmyard manure and slurry are 'the ideal plant foods'. Only in Biodynamic agriculture, based on the teachings of Rudolf Steiner, the use of slurry is forbidden. Also Rusch, the cofounder of organic agriculture in Switzerland and Austria, had serious doubts about the use of slurry. He also advised the use of fresh materials (dung and plant residuals) ón or just under the earth surface. (Rusch, 1968).

For farmyard manure and slurry, I want to explain why this is usually not (yet) a suitable food for soil life.

Farmyard manure and slurry not (yet) a suitable food for soil life.

The urine of the cow

The cow's urine may be too rich in nitrogen because the animal is being fed a too protein-rich diet, or because the nitrogen in, for example, the grass or the concentrate has not been sufficiently converted into real protein. The feed then contains much nitrate, ammonium and other forms of nitrogen like urea and nitric oxide, not converted into protein. These compounds ensure that the plants don't grow well, and become out of balance. And consequently, the animal's digestion is not optimal. The excess of protein and/or inorganic non protein nitrogen does not end up in the milk as milk protein or in the meat, but is converted into urea by the liver, or directly removed as sodium nitrate (Swerczek, 2007). We find this urea – and probably also part of the sodium nitrate – In the milk on the one hand and in the urine on the other. Or it accumulates as sodium urate in the (toe) joints (gout) or the skin. This may also contribute to arthritis and hoof and skin problems. According to Bredon and Dugmore, an excess of phosphorus over calcium can also lead to hoof problems (Bredon, Dugmore, 1985).

Nitrate can also be converted by the body via nitrite, into nitrosamine or nitric oxide (NO). Neither is good for health. Nitrosamines are carcinogenic, and too much nitric oxide leads to disruptions in various biological control systems of animals and man. Nitric oxide participates in the pathogenesis of Alzheimer disease, but 'many questions need still to be elucidated' (Kadowaki et al., 2005). Nitric oxide is an intercellular signaling substance and a neurotransmitter. NO is also an important steering element for plants (Visser, 2010).

Not only cows and humans have health problems as a consequence of high NPN and high protein levels in their feed:

"Vegetables have been a significant part of human diet since time immemorial. Besides adding the elegance and attractiveness to a meal, vegetables are abundant source of vital minerals, vitamins and several biologically active compounds essential for maintaining human health. Most importantly, vegetables account for 70-85 % of total human nitrate intake [..]— ubiquitous within food and physiological systems (..)*; after ingestion, the ingested nitrate gets converted into nitrite by microflora in the oral cavity and in the gastrointestinal tract. This results in increased oxidation of hemoglobin to methemoglobin, leading to methaemiglobinnemia. Simultaneously, increased production of free oxide radical and free radical nitrate oxide** occurs. These radicles predispose persons for carcinogenic and other effects. The other effects observed were increased infant mortality, abortions, birth defects, recurrent diarrhea, recurrent stomatitis, early onset of hypertensions, histopathological changes in cardiac muscles, alveoli of lungs and adrenal glands, recurrent respiratory tract infection in children, hypothyroidism and diabetes. Recent ongoing studies indicated that nitrate ingestion adversely affects the immune system of the body as well".* (Umar et al., 2013, Foreword).

"It has been suggested by McCall and Willumsen (1998) *that high rates of nitrate application lead to increase in plant nitrate content without any increase in the yield. Therefore, farmers who apply excessive fertilizers to ensure that nitrogen is not limiting for plant growth, may increase the nitrate content of crops to the levels potentially toxic to humans, without any increase in yield. (..) Moreover, there is an upper limit to the levels of N-metabolizing enzymes that the plant can accommodate* (Anjana, 2007)*. Therefore, the plant continued nitrate uptake due to its abundant availability in the soil but was not able to assimilate it. As a result, accumulation of nitrate in the plant to unsafe limits occurred*" (Anjana, Umar, 2018).

Consequences.

* This should be: nitrogen oxide, NO_x. Nitrate oxide does not exist (the author).

So you can have healthy urine or unhealthy urine (and everything in between). The first is relatively low in nitrogen in the form of urea, uric acid, hippuric acid and creatinine. The nitrogen absorbed by the body of the cow is in this case efficiently converted into milk or meat proteins. The unhealthy urine on the other hand is excessively rich in these nitrogen compounds or even in protein, due to inefficient digestion. Once the cow has urinated, the decomposition of the organic nitrogen in the urine begins, unless it is bound to earth particles and humus. The latter happens automatically when the cow walks outside (and the soil is rich in humic acids, and/or clay particles. Even sand can bind nitrogen (Frank, 1888).

If it is not bound and/or if the pH is too high, urea is converted into ammonia and carbon dioxide by bacteria that release the enzyme urease. That should be avoided as much as possible because you want to keep all the nitrogen and the carbon. The creatinine, which is also a constituent of the urine, can be absorbed directly by the plant roots (Schreiner, Skinner, 1912).

I have not come across much research into the question whether the plants can also directly absorb urea, uric acid or hippuric acid.

Dimkpa et al. suggest that plants can take up urea, but they need enough nickel for the conversion of urea in ammonium:

"*Prior studies with soybean showed that a Ni deficiency-induced regulation of the activity of urease negatively impacted N metabolism in the plant, leading to urea accumulation and necrosis of shoot* (Polaccao et al., 1999; Sirko, Brodzik, 2000)*"* (Dimkpa, Bindrapan, 2016). (..).

... Urea can be assimilated exclusively by urease in higher plants. Moreover, urease is the only nickel-containing metalloenzyme yet identified in plants (..) The importance of nickel for urease activity was demonstrated by the observation that urea-grown nickel-deprived rice (Oriza sativa) plants showed reduced growth and accumulated large amounts of urea due to reduced urease activity[*]*"* (Sirko, Brodzik, 2000).

But there is another conclusion in the article of Gerendás et al. (1998) which is important for the discussion about the risks of ammonia, nitrate and urea fertilizers:

"*Although the growth of plants with NH_4NO_3 was not affected by the Nickel supply, they accumulated endogenous urea due to arginase action in conjunction with low urease activity. It is clear that the Nickel status of a plant has significant consequences for the relative suitability of urea and NH_4NO_3 as nitrogen sources*" (Gerendás et al., 1998).

So not only urea can lead to urea accumulation in plants but also NH_4NO_3, ammonium nitrate.

Many farmers in the Netherlands use 'Kalkammonsalpeter' as a nitrogen fertilizer. Kalkammonsalpeter is $CaCO_3NH_4NO_3$. Sometimes it also contains magnesium. So the risk is that our crops also compose urea from NH_4NO_3. In order to find out if this is a serious risk we should measure the amount of urea in our crops. In our soils is nickel, but I don't know if our crops absorb it sufficiently. Remember that potassium and ammonium both hinder the uptake of many cations.

According to the overview of Schreiner and Skinner (1912) urea is harmful for higher plants, but not for all of them. Hippuric acid is slightly harmful, and uric acid is beneficial.

Cyanobacteria can also absorb urea directly, in addition to nitrate and nitrite (Herrero and Flores, 2019).

These cyanobacteria can then be "digested" by the plant roots as White describes. So also indirectly, the urea can be absorbed by the plants in the form of organic (bacterial) nitrogen. A safe way.

If the urine of the cows is too rich in nitrogen, it can cause burn spots in the grassland.

Poor digestion.

[*] And this has also consequences for human health, because the stomach bacterium Helicobacter pylorum, which is a normal habitant in our stomachs, can become dangerous if there is too much urea in the food: "*One of the most frequently mentioned examples in the recent literature is the urease from Helicobacter pylori because of its essential role in the pathogenesis of this microorganism and the high prevalence of this human pathogen*" (Eaton et al., 1991). So plants can accumulate nitrate, ammonia and urea. All three are u health burden for animals and men. One of the risks is the growth of pathogenic helicobacter pylorum. Ammonia in the intestines is also a risk for our brains (Galland, 2014).

If the digestion of the cow is not optimal, even more nitrogen losses will occur: namely via the cow's mouth. Through its respiration, the cow loses not only methane – about 100 to 160 kg/year* – but also ammonia (Regnault, Reiset, 1849; Reiset, 1863; Frere, 1863), and hydrogen sulfide (Voisin, 1963). Various measurements taken in the 19th century showed an average release of about 10 kg of ammoniacal nitrogen per cow per year via the mouth. In the 19th century there were many outbreaks of diseases among cows. Almost every few years (see the different editions of the 'Journal of the Royal agricultural society of England': 1839 – 1880) thousands of cows and sheep died from these diseases. With protein- or NPN rich feeding, as happens today, there will be even more ammonia from the mouths.

The harmful compounds – too much protein; NPN and NPS – are all released in the rumen where the wrong bacteria start working if the correct elements and trace elements for the conversion of nitrogen and sulphur into bacterial protein are missing or insufficient. Phosphine exhalation via the mouth was not measured in the 19th century. Nor today.

The correct balance of the macro elements is also important: not too much potassium, and sufficient sodium, calcium and magnesium. You get wrong bacteria if the rumen environment is not optimal, due to, for example, poor quality (silage) grass with high levels of potassium, or an excess of protein or NPN / NPS from roughage and concentrate in the rumen. The bacteria that break down the cellulose and hemicellulose into, for example, acetic acid and some propionic acid should prevail, not the bacteria that feed on an overdose of protein, nitrates, ammonium or urea and other forms of non-protein-bound nitrogen, or on non-protein sulfur and phosphor, without sufficient magnesium and trace elements to convert these compounds in bacterial protein. They also lower the pH. At too low a pH, you get proportionally more and more propionic acid and lactic acid according to Kaufman et al. (1980). Also, the conversion of acetic acid to methane should not get the upper hand, because that is a pure loss from the cow's point of view. If the rumen wall absorbs acetic acid well, it is converted into milk fat. According to Kaufman et al., the optimal absorption of the acids has everything to do with the right pH. If the acid concentration is too high, the pH shifts further down. Too much concentrate/protein and NPN, NPS lowers the pH.

A cow with poor digestion tries to get rid of the harmful compounds in every possible way: via the urine; through exhalation, through the milk and through the dung, and also via the skin†. Or the cow will pile them up somewhere in the body after neutralization with cations, if the discharge through these five routes is stagnant or inadequate. This occurs, for example, in the form of protein accumulation in the connective tissue (Wendt, 1983) or as sodium urate between the (toe) joints and in the skin. According to Swerczek, an American veterinarian, many cows have the potassium nitrate syndrome. And he describes in detail which symptoms are part of this syndrome. Grass tetany, he says, is the extreme form of this syndrome, and many cows don't get tetany but are bearers of its preceding stages. These animals are seriously weakened (Swerczek, 2007). Colleagues told me that in the slaughterhouses in the Netherlands there arrive almost no cows with a healthy liver.

Calcification is also a form of accumulation. Calcification in humans usually involves calcium phosphate. That is not surprising. The amount of phosphates in our diet is almost three times as high as the RDA of 600 mg/day (Seelig, 1981; Itkonen, 2015). To neutralize these phosphates, the body extracts calcium from the bones and teeth. As a result, these bones and teeth gradually weaken, which in turn leads to osteoporosis and weak teeth. Research on phosphate poisoning is now starting to gain momentum (Brown, Razzaque, 2015). We should check whether dairy cattle also suffer from too much phosphate. We fertilize our crops intensively with the salts of nitrogen, phosphorus and potassium – NPK. It should come as no surprise that these three elements are very often in excess. In any case, phosphate salts are harmful to plants, as Jamieson and his colleagues in the period 1880-1910 have shown in **the "phosphate battle"**. They were able to prove that turnips grew better and healthier on natural rock phosphate than on phosphate salts (Jamieson, 1910). Based on tests and scientific arguments during thirty years, Jamieson et al. won the

* 100 kg/year is based on research of Smink et al., (2003). Smink et al. based this figure on 1993 research by van Amstel. So this is older research. According to an overview by Rotgers, it is higher. Between 133 and 162 kg/year, depending on the calculation method (Rotgers, 2017).

† We know from sportsmen who eat extra proteins that part of the resulting ammonia can leave the body through the skin. Their sportswear smells like a slurry pit. So I suppose that the same happens with cows. Maybe this attracts the flies in the sheds.

phosphate war, but the fertilizer industry, with superphosphate, ultimately won decisively over science. Their economic power was too big to stop them.

The same for potassium. These are five conclusions from the evaluation by Khan et al. (2013) regarding potassium chloride:

"- Based on an evaluation of more than 2,100 field trials, Khan et al. finds that potassium chloride fertilization very often does not contribute to an increase in yield.

- Based on 1400 field trials, Khan and his colleagues conclude that the potassium chloride fertilization is harmful to the crop, the soil and the consumer.

- The higher the potassium / calcium ratio, the less root nodules and root nodules bacteria form on the roots of leguminous crops;

- Fertilization with potassium chloride is more harmful than fertilization with patentkali ($K_2SO_4.MgSO_4$), or potassium sulphate;

- High chloride levels in the soil promote the uptake of cadmium by plants" (Khan et al., 2013).

Theel did in 1933 measurements on hay, collected from all over Germany, and concluded that, compared to the amounts in the hay in 1880 and before, which Wolff had collected, potassium, sulphur and chloride, had almost doubled (Theel, 1933).

Arzet demonstrated in 1972 that potassium in animal feed had gone up further, and magnesium had gone down further since 1870 (Arzet, 1972).

Theel and Arzet didn't look at sodium, but from the data of David Thomas for vegetables in the UK we know that sodium also has gone down. Based on a comparison of 27 vegetables from 1940 and 1991, Thomas accounted that the vegetables in 1991 had 49 % less sodium. K/Na was 10 in 1940, and 17 in 1991[*]. In 1991, phosphorus was 9 % higher. Magnesium was 24 % less and calcium 46 % less. Potassium had gone down with 16 %[†]. In 1991, copper was 76 % less. And between 1978 and 1991 zinc had gone down with 59%, in no more than 13 years (Thomas, 2003).

More sodium in grass helps to convert non protein nitrogen into real proteins (Chiy, Phillips, 1993). Maybe in other plants this will happen too.

As a consequence, the potassium magnesium ratio in for example grass and grass silage became steadily higher (Figure 1).

The golden rule is that all aggressive anions – acid residues with nitrogen; phosphorus; chlorine or sulfur – above the physiological levels are, if not used for protein building, bound by the body to the cations of calcium, sodium and magnesium[‡]. In this way, they are neutralized. These cations are taken from the body-stores and replenished from the diet, if they are sufficiently available. And these neutralized compounds are quickly drained through the urine if the liver and kidneys are working properly. If the liver and the kidneys cannot keep up or are fattened or calcified, these substances accumulate in the body.

Calcification occurs in the most diverse places in the body and causes blockages, such as arteriosclerosis in the blood vessels or calcification of the connective tissue (calcinosis) or the brain. One of the most risky forms of calcification is that of the mitochondria. Kapustin relates elevated levels of calcium and phosphorus in the blood to mitochondrial damage and increased superoxide production in vascular cells (Kapustin et al., 2011).

[*] Today in 71 raw Dutch vegetables the potassium/sodium ratio is on average 16.8. I calculated this on the basis of Food data of RIVM. RIVM is the Dutch State Agency responsible for public health and Environment. And in a potato trial in 2012 all the potatoes of the thirteen different fertilizing schemes – organic and inorganic – had a potassium / sodium ratio of 225 to 1 (My calculations, based on data from Rietberg and van der Burgt, 2012).

[†] In a Finnish study was also shown that potassium had gone down (especially in cereals) in 30 years from 1970 to 2000, like most (trace) elements. Despite this the total potassium consumption from cereal and vegetable foods between 1970s and 2000s had gone up with 15 %. (Ekholm et al., 2007). A scientific mystery.

[‡] It is striking that potassium is never mentioned as an acid residue binder in the relevant professional literature. The research by Frassetto et al. (1998) does point to a different role of potassium: potassium bicarbonate acts as acid binder. However, the bicarbonate HCO_3^- is the acid binder: $HCO_3^- + H + > CO_2 + H_2O$. Both disappear through the breath.

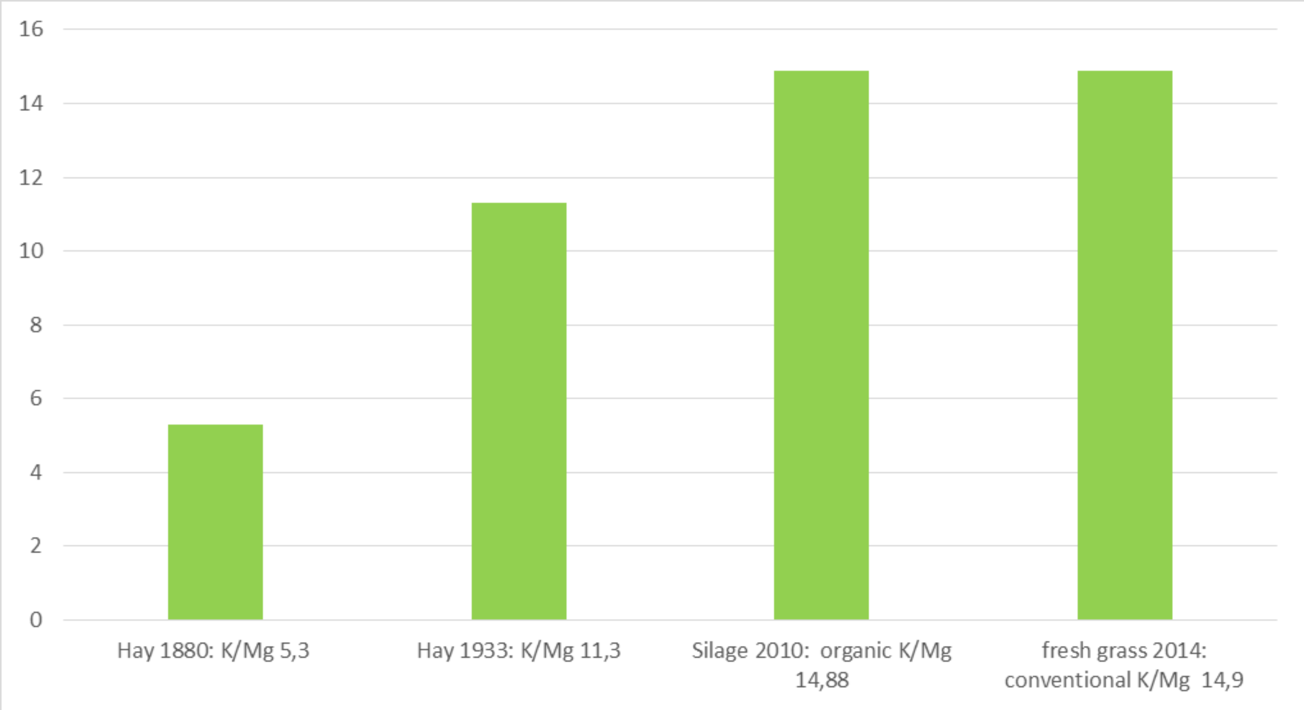


Fig. 1. K/Mg ratio in Hay and silage (1880–2010)

If the mitochondria calcify, the oxygen-rich combustion can no longer work properly, and the cell becomes a cancer cell that switches to lactic acid fermentation. Calcification is caused by too low magnesium levels in the food / feed. Then potassium and magnesium disappear from the cells, and their place is occupied by calcium, sodium and protons (Schroll, 2002). The cells calcify, become saline and acidify. All in one. The cell pumps don't work properly anymore.

Mitochondrial calcification is probably the missing link in Otto Warburg's work. Warburg stated in 1956 that the main cause for the development of cancer cells must be sought in mitochondrial dysregulation. He thought the cause of this were carcinogenic compounds such as arsenic acid, radiation, urethane and hydrogen sulfide* (Warburg, 1956). However, Warburg ignored the "normal dysregulation" of the mitochondria through calcification, which is a result of insufficient magnesium levels in our foods and drinks.

To my knowledge, the extent to which salinization† and acidification of the cell also play a role in causing cancer has never been investigated.

Calcifications often happen after the consumption of too much phosphate or oxalate, in combination with too little magnesium.

Sulfur can accumulate as sulfur stones (cistin), as homocysteine‡ or as hydrogen sulphide. Too much sulfur in cows leads to brain damage (polioencephalomalacia), or it causes strokes (Kobayashi, 1957) and hydrogen sulphide disregulates the cell mitochondria (see Warburg here above).

* It is interesting that Warburg mentions hydrogen sulphide. Voisin warned already in 1963 for the risks of this compound. Hydrogen sulphide is one of the compounds which are released when the digestion is disturbed. Maybe the same is true for urethane. And arsenicum was very often used as a pesticide in agriculture in the past.

† It is more correct to say sodium accumulation, because I don't know if also chloride goes into the cell.

‡ On Wikipedia you can find a whole range of symptoms and conditions under the keyword Homocystinuria. In that case, the blood and urine always contain too much homocysteine. This homocysteine normally has to be converted into cisteine or back into methionine, but this conversion can stagnate due to a lack of magnesium and vitamins B9, B12 and / or B6. "Hyperhomocisteinemia was detected in 69.8% of all the [elderly] subjects evaluated. The study showed that 76.2% of the men and 66.4% of the women had high Hyperhomocisteinemia levels (Janson et al., 2002). High Hyperhomocisteinemia has a strong correlation with vascular diseases.

Nitrogen stacks as ammonium, sodium nitrate*, NO, proteins / beta-amyloids† or urea and urates, etc.

When the whole body is calcified, it is called calcinosis universalis, general calcification. I found a too visual description of this phenomenon in Roovers (1937). Sodium citrate (citras natricus) (oral) in combination with calcium (Calcium Sandoz: intravenous) turned out to be a well working medicine in the case study of a five-year-old girl described by this medical doctor.

Carson's research (Carson, 1998) shows that bacteria are almost always hidden in the accumulated calcium compounds. The so-called nanobacteria. Roovers' description indirectly confirms this. The girl who suffered from calcinosis universalis had regular temperature increases before her treatment. Often 38 degrees, which can indicate chronic, low-grade inflammation – Inflammation that does not get resolved, but continues to persist. Five weeks after starting the treatment, the girl developed a high fever for many weeks. During the fever, "*large and fluctuating swellings, red and warm to the touch, developed. The swellings contained calcium grit*"‡. After months, the definitive recovery started. X-rays showed that the calcium deposits had disappeared from her entire body.

Maybe we can use this therapy of Roovers against calcification today: the girl – five years old – got 3-4 g sodium citrate per day. That is a lot for a girl of say 18 kg (165 mg sodium citrate/kg body mass). An adult of 70 kg should get more – 11 to 15 g. That seems to me very much, so for safety reasons a medical doctor should give say 8 g/day to start with. Plus 5 cm^3 (or some more) calcium Sandoz intravenous as Roovers gave the girl. More recently, Schmiedl et al advised potassium citrate and magnesium citrate for the treatment of calcifications:

"*…the combination of potassium citrate and magnesium citrate, which shows enormous anticalcification efficacy, deserves high priority in clinical trials aimed at evaluating strategies for the prevention of stones*" (Schmiedl et al., 1998).

My assessment is that first, there are the calcium phosphate accumulations, and then the growth of the harmful nanobacteria. In other words, first the environment changes, then micro-organisms that feed on it and are harmful to humans. But according to Kajander et al. the nanobacteria produce the calcifications as an envelope 'at physiological phosphate and calcium concentrations' (Kajander and Ciftcioglu, 1998). But they forgot to look at the magnesium level. Calcification offers a nice test case to find out if magnesium is the missing link. From the literature, we know that magnesium prevents calcification (Seelig, 1980; Driessens and Verbeeck, 1988). And magnesium – especially magnesium chloride - improves our natural resistance (Neveu et al., 2009).

The faeces of the cow.

Poor digestion leads not only to nitrogen-rich urine and smelly breath, but also to faeces that are abnormal. On many dairy farms in the Netherlands, the cows are permanently suffering from diarrhea. As far as our measurements in 2019 were correct, there are in this respect no big differences between organic and conventional dairy farms, but these measurements were based on only one year with great variations in the weather: a severe drought, followed by much rain. The grass was as a consequence extremely rich in crude protein (Vanhoof, Nigten, 2020).

Measurements by Wigle Vriezinga show that the dung often contains a lot of undigested fibers and resistant starch. That starch, together with unused acetic acid in the manure, leads to

---

*The presence of sodium nitrate in the milk would also explain why an increased amount of sodium in the milk is indicative of mastitis. The nitrate is of course a fantastic food source for bacteria. I suppose, sodium is not the mastitis-causing element. The nitrate is. According to Nele nitrate in milk can vary between 20 µg/100 g and 1240 µg/100 g (Nele, 2006). The maximum is sixty times more than the minimum.

† Amyloid.. a protein that is deposited in the brain, the liver, kidneys, spleen, or other tissues in certain diseases. "Studies have shown that amyloid deposition is associated with mitochondrial dysfunction and a resulting generation of reactive oxygen species (ROS)". Source: Wikipedia, english, keyword: amyloid. Wikpedia refers to a study of (Kadowaki et al., 2005). The authors link ROS and NO to Alzheimer disease: "These findings suggest that ROS and NO may be important mediators of Ab [= amyloid Beta] -induced neuronal cell death in the development of AD" (Alzheimer disease).

‡ The words chalk grit and calcification are misleading in that the reader quickly thinks that it is pure calcium. However, these are calcium compounds, mostly calcium phosphate and calcium oxalate. And the acid residue is leading in our diet. There are also situations where it is the other way around. Then a cow gets, for example, too much calcium in the form of calcium carbonate in the dry period. Then the body extracts phosphorus from the feed or the bones to neutralize the calcium.

methane formation (oral statement by Sentobin, Vogelsang), which means that the slurry pits which are covered with low grade methane emission deposits (foams) can explode. As has happened sometimes in the Netherlands. A few cows disappeared into the slurry pit and died.

The undigested fibers lead to a crust on the slurry. Both the foams and the crust prevent gas exchange from the pit into the air.

Characteristic of good cowpats is that – in the pasture – dung flies come immediately, and dung beetles and dung worms etc. etc. And that is again a feast for the meadow birds (de Ark, 2020; Wesseling, 2019). Jelmer Buijs' research states that residuals of insecticides, vermicides, cleaning products and other poisons ensure that no insects, or very few, live around the slurry pit and that the cowpats are no longer attractive to insects (Buijs and Samwel Mantingh, 2019). This connection is disputed by Rotgers (2019). Perhaps the quality of the dung is the missing link…

From the research of Buijs (Buijs and Samwel Mantingh, 2019) we learn that there are many, many residuals of pesticides, cleaning products and veterinary medicines in the dung, the fodder, the concentrates, and the soil, even in organic dairy farms (but there on average less than on conventional dairy farms). They have proven that there is a strong relation between these residuals and the missing beetles and insects in the pastures:

"With the collected information we cannot conclude otherwise than that the ecosystem of the livestock farms is seriously threatened by the multiplicity of pesticides that are present there. This was further confirmed by the fact that in fresh manure of the cows no, or hardly any, Coleoptera (beetles) were found on most farms. In the manure of farms where concentrated feed and hay with relative high concentrations of insecticides were used, the occurrence of beetles in fresh manure was significantly lower" (Buijs, Samwel Mantingh, 2019).

And look at the pastures where the cows defecate and draw your own conclusions. Usually, the cowpats remain completely untouched by insects, beetles and worms. You can also see that the cows almost always avoid the grass around the pats. If you travel crisscross through the Netherlands by train, you can see this clearly. Because you sit higher than the surrounding pastures, you see cow pats everywhere with lush growing grass that is not eaten.

Summarizing

The fodder and the concentrate of cows contain too much protein and/or NPN and NPS (Schmack, 2020; Swerczek, 2007). And are not in balance for its cations too. Potassium is high and sodium, calcium and magnesium are low. Silicon is probably also too low, but it is mostly not measured at all. The K/Mg ratio has gone up since 1880. The same for the K/Na ratio.

The cows are unhealthy because of this low fodder and concentrate quality. There is an accumulation of nitrogen and probably also sulphur compounds, and a calcification of the soft tissues. Swerczek gave it a name: the potassium nitrate syndrome. But the stocking of risky compounds is wider than that. Also in humans, we see comparable health problems.

Cows try to get rid of the accumulations. Partly they end in the urine and the poop. Both become also unhealthy and out of balance. They rot and stink. The urine and the poop are above that enriched with pesticide, insecticide, vermicide and antiobiotic residues. Through this, the number of insects and meadow birds has gone down dramatically.

Artificial fertilizers give comparable problems and losses. Superphosphate weakens the plants just as potassium chloride. Ammonia, nitrate and urea accumulate in the plants and this attracts pests and diseases. Probably the same applies for sulphur. The sulphur in hay in 1933 had almost doubled since 1880 (Theel, 1933).

The reasons for lower yields and less quality in organic agriculture.

So here we have the reasons why the yields in organic agriculture are suboptimal, and the crops not that healthy:

- There is often too much protein, and/or NPN and NPS (and NPP?) in the fodder and the concentrates for the cows, pigs, chickens etc. This ends in part in the deep litter manure and the slurry, together with putrefactive bacteria and their toxins;

- A lot of nitrogen, carbon, sulphur, phosphor and other nutrients are lost from the slurry pits and deep litter stables, and during warm composting;

- There is imbalance of the macro-elements in the feeds: too much inorganic nitrogen, sulphur and potassium, and probably also too much phosphor. And too little sodium, magnesium, calcium and silicon. Especially the healing role of sodium and magnesium, as well as the protective role of silicon in roughage and concentrate, is heavily underestimated;

- This results in farmyard manure and slurry which is also not in balance and not healthy. With too much NPN, NPS and potassium. They contain putrefactive bacteria and other harmful microbes. And these produce rotting products and toxins. The rotting compounds, and putrefactive bacteria and their toxins inhibit the growth of the plants and suppress the symbiotic bacteria, viruses and fungi;
- And warm compost also has the wrong bacteria, and it has lost a lot of nitrogen, carbon, phosphor, potassium and other macro- and trace elements during processing. Heating leads to wrong microbes, mineralization and big losses.
- A number of the organic farmers use antibiotics, cleaning agents, insecticides and vermicides for their animals and in the stables, because the animals health is suboptimal, as the fodder and concentrates are out of balance. The animals get fertility and birth problems, abomasum twisting, mastitis and foot diseases, and attract flies and intestinal worms, which have to be controlled with antibiotics, fungicides, insecticides and vermicides. The residuals of these products end in the dung and from there present in the soil and the grasses, and other fodder products.

Together all these factors hinder the uptake of organic nitrogen by the plants, and block the nitrogen fixation in the soil and above the ground on the leaves and stems. So in this situation the plants can only grow on 'mineralized' nitrogen – ammonia and nitrate – and mineralized phosphor, potassium, calcium, sodium, sulphur and magnesium etc.

The release of the nutrients from manure and compost starts already during the winter, even when the soil is cold. And the microbes eat them (Krasil'nikov, 1958). But when the manures and composts are given in the early spring, the regular practice in the Netherlands, the mineralization must still start and the toxins and growth inhibiting rotting products hinder the plants. When the soil is cold, nitrate is taken up before potassium can be taken up, and potassium is taken up before magnesium is taken up. The putrefactive bacteria produce ammonia and nitrate and rotting products and toxins from organic materials, which hinder the growth (Schreiner, Skinner, 1912). The plants can't get help from their symbionts to get organic nutrients.

Domagała-Świątkiewicz and Gąstoł compared a.o. ammonia and nitrate in organic and conventional agriculture in Poland. (Domagała-Świątkiewicz, Gąstoł, 2012). Both are lower in organically grown products than in conventional products in this Poland study. But still considerable. I reviewed their results in: Nigten, 2019. Some studies which compare the nitrate level in organic and conventional products conclude that nitrate in organic products is the same as in conventional products, or slightly lower (de Waart, 1998). According to Dangour et al. nitrate is definitely lower in organic products (Dangour et al., 2009).

With artificial fertilizers – ammonia, urea, nitrate, potassium chloride and superphosphate – you avoid the barriers consisting of rotting bacteria, their toxins and growth inhibiting organic compounds, but these artificial fertilizers also result in crops which are not in balance and unhealthy. So the only way out is the production of amino acids, proteins, nucleic acids and organically bound cations and anions from the manure and from the organic residuals from plants, plus a plant friendly rhizobiome.

Mixing earth with manure and/or plant residuals, and conversion of manure and plant residuals by worms into vermicompost give good solutions.

Urea madness.

In 2016 Mr Schmack, published a book, titled "Die beschädigte Kuh im Harnstoffwahnsinn" (The damaged cow by the urea madness), which has been translated into Dutch and published by "At the Origin" (Schmack, 2020). The word "urea" refers to the fact that the amount of urea in the milk is always used as a measure of whether the animals are getting adequate protein. And by urea madness, Schmack means that the farmers on average maintain a much too high urea number in their milk as the standard. The maximum urea content is, according to Schmack, 10 mg/100 ml milk or less. Almost all farm scores are higher, too much higher. At current German feed recommendations, the urea number in Germany is 25 mg / 100 ml of milk. And 35 mg for high yielding cows (Schmack, 2020).

The underlying "theory" is that the cows must get a lot of protein in order to be able to give a lot of milk. At Wageningen University, the research into milk and animal feed, and the suppliers of animal feed, are vigorously perpetuating this myth. This costs farmers a lot of money and has seriously affected the health of the dairy herds. And what does this mean for the quality of the milk and meat? See for the quality of milk Appendix 1.

My assessment of the risks of ammonia, nitrate and urea that humans ingest through vegetables, fruits, potatoes and animal products is in line with Schmack's findings. However, the damage these compounds cause to cows is much greater and much more systematic, according to Schmack's research, than I had already feared. Systematic means that almost no dairy farm avoids it. Schmack then points out that this primarily leads to seriously damaged livers and kidneys. But also the other known livestock diseases, such as fertility problems, abomasum twisting, udder inflammation, paralysis, and leg problems can be traced directly to this excess of nitrogen that the animals ingest. Schmack considers the role of bacteria, viruses and fungi in the development of livestock diseases to be secondary."80 % of livestock diseases can be explained by the excess of protein in the feed", according to Schmack (2020).

Schmack's assessment is entirely consistent with that of an American veterinarian, Swerczek (2007). But Schmack has worked them out even more thoroughly at a detailed level.

It won't be much better in humans. The Dutch Kidney Foundation reports the following:

"1.7 million Dutch people[*] have chronic kidney damage. People with chronic kidney damage are at an increased risk of kidney failure and cardiovascular disease. For example, someone aged 55 with a severely reduced kidney function may die 12 years earlier than average".

The Dutch Liver Patients Association writes:

"About 250,000 people in the Netherlands have to deal with an acute or chronic disease of the liver or bile ducts. This can be caused by:

- *a viral infection,*
- *an autoimmune disease,*
- *lifestyle,*
- *hereditary burden,*
- *or, for example, long-term exposure to alcohol or drugs".*

Ammonium poisoning does not occur in the story of the Liver Patient Association. The amounts of nitrate, ammonium, nitric oxide, nitrite, nitrosamines and urea in the food are also not measured by the RIVM. All these compounds contribute to kidney and liver poisoning.

According to a UMC[†] Utrecht employee, fatty liver disease is much more common than chronic kidney damage:

"(..) fatty liver disease occurs in about 90 % of overweight people, which is equivalent to a quarter of the world's population[‡]"

With fatty liver you are not yet sick, according to the employee. But it can end in liver damage: fatty liver => hepatitis => cirrhosis.

I have not been able to find hard figures in the Netherlands about how many people actually have liver disease. An important reason for this is that livers can recover and that they can continue to function on a small amount of healthy tissue, while a big part is already sick....until the moment the livers can no longer function on the small healthy part, and then the damage is irreparable.

A study on liver diseases by Anthony Williams shows a much more serious picture as far as the liver is concerned. Williams says that nine out of ten livers in humans are sick. And also that many other systems in our body are diseased by a sick liver. But nitrate and ammonia are almost lacking in his work. It is all about fats (Williams, 2018).

Galland has demonstrated that "*Bacterial enzymes may produce neurotoxic metabolites such as D-lactic acid and ammonia*". And he concludes: "*The only mechanisms with a high level of proof in humans are the neurotoxic effects of ammonia in HE [hepatic encephalopathy[§]] and of D-lactic acid in short bowel syndrome"*. (Galland, 2014).

---

[*] The Netherlands has a population of 17,777,085. So almost 10 percent has a chronic kidney damage.

[†] UMC is the University Medical Center in Utrecht, the Netherlands.

[‡] And these are the data for obesity in the Netherlands: More than half of the adults in the Netherlands are overweight (BMI (=Body Mass Index) higher than 25). This has emerged from research by the Central Bureau of Statistics, 12.7 percent of all people – four years and older – even have some form of obesity (BMI over 30). (CBS, 2019).

[§] Hepatic encephalopathy gives these symptoms: "The mildest form of hepatic encephalopathy is (..) experienced as forgetfulness, mild confusion, and irritability. The first stage of hepatic encephalopathy is characterised by an inverted sleep-wake pattern (sleeping by day, being awake at night). The second stage is

The ammonia in Gallands article is produced by bacteria, but we may suppose that ammonia from plants or animal products has the same effect. The quantity is of course decisive.

3. Conclusion

The yields in organic agriculture are roughly 25% lower than in conventional agriculture. On most organic farms, mineralized nitrogen and sulphur are the only nitrogen and sulphur compounds for the plants to grow on, and there is not enough of it for the plants to create a good yield. The plants are unable to take the organic nitrogen and sulphur etc. from the soil. Both are normally sufficiently available.

Comparisons between warm compost, cow dung compost and vermicompost show that only with vermicompost the plants get enough nitrogen in the right form. And the plant characteristics during growth are proven to be better with vermicompost. The bacteria in vermicompost are the bacteria that belong to the rhizosphere, which is not the case for the microbes in warm compost and cow dung (compost).

Vermicompost contains more humic acids. These bind the released nutrients better, making vermicompost less saline than warm compost and cow dung. The nitrogen and other nutrients present in vermicompost are available faster and better.

Stimulated by vermicompost, the earthworms in the soil can break down the loamy sand particles into loamy silt and clay.

And vermicompost tea can help to overcome severe droughts. The same for cattle manure mixed with plant residuals and soil.

The urine and poop of the cows is very often not healthy because of too much protein, and/or non protein nitrogen and non protein sulphur in their feeds. These compounds – nitrate, ammonia, urea, hydrogen sulphide etc. – disturb the health of plants, animals and men after accumulation in these organisms. In animals and humans, there is also another kind of accumulation, which disturbs their health: calcification of the soft tissues of their bodies.

Very often the level of potassium in plants is much too high, and the sodium and magnesium level much too low. Higher sodium gives lower potassium, plus higher magnesium and calcium as well. Sodium in grass helps to convert non protein nitrogen into real proteins. But most crops are in disbalance for their cations. And ammonium and potassium hinder the uptake of silicon and other cations by plants, which lowers their natural resistance further.

During the ‘phosphate battle’ Scottish scientists have proven that superphosphate weakens the plants grown on this salt.

In organic agriculture, just as in conventional agriculture, plants and animals are not healthy because their ‘food’ contains too much NPN, and is not in balance for their cations and trace elements. Their crops and fodder grow, like in conventional agriculture, on inorganic salt ions. Many organic farmers have to use pesticides and antibiotics to repress these diseases. So todays organic farming has much in common with conventional farming.

The inorganic nitrogen is not a safe plant food, and that plants can uptake organic nitrogen from the soil or nitrogen from the air. But the results of Lawes, Poschenrieder and Lesch, and Hopkins demonstrated that the uptake of organic nitrogen from farmyard manure is often a problem, even if there is enough organic nitrogen in the soil. Giving warm compost does not solve the problem either, as the comparisons with vermicompost show.

But at the same time not all organic nitrogen compounds are a good food for plants. Many rotting N containing products from manure, slurry and warm compost also disturb or slow down the growth of plants. The putrefactive bacteria, which are producing the rotting, are overruling the

marked by lethargy and personality changes. The third stage is marked by worsened confusion. The fourth stage is marked by a progression to coma. More severe forms of hepatic encephalopathy lead to a worsening level of consciousness, from lethargy to somnolence and eventually coma. In the intermediate stages, a characteristic jerking movement of the limbs is observed (..); this disappears as the somnolence worsens. There is disorientation and amnesia, and uninhibited behaviour may occur. In the third stage, neurological examination may reveal clonus and positive Babinski sign. Coma and seizures represent the most advanced stage; cerebral oedema (swelling of the brain tissue) leads to death”. Source: wikipedia english. Keyword: hepatic encephalopathy.

symbiotic bacteria. Especially when the slurry, the manure or the warm compost is given in spring, while it is still rotting.

In the next article about nitrogen, we will show the simple solutions for these problems in organic and conventional agriculture.

References

Anjana, 2007 – *Anjana, A.* (2007). Factors contributing to nitrogen use efficiency in Triticum aestivum L. and Spinacia oleracea L. Ph.D. Thesis.

Anjana and Umar, 2018 – *Anjana, A. and Umar, S.* (2018). Nitrogenous Fertilizers – Boon or Bane? *SDRP Journal of Plant Science*. 2(2).

Arancon et al., 2005 – *Arancon, N.Q., Galvis, P.A., Edwards, C.A.* (2005). Suppression of insect pest populations and damage to plants by vermicomposts. *Bioresource Tech*. 96(10): 1137-1142.

Ark, 2020 – *Ark de*. (2020). Poep moet leven. [Electronic resource]. URL: https://www.ark.eu

Arzet, 1972 – *Arzet, H*. (1972). Änderungen des Kaliums- und Magnesiumgehaltes einiger wirtschaftseigen Futtermittel in den letzten 100 Jahren. *Universität Stuttgart Hohenheim*.

Barnett, 1954 – *Barnett, L.B*. (1954). New Concepts in Bone Healing. *Journal of Applied Nutrition*. 7: 318-323.

Bhatia, 2000 – *Bhatia, S*. (2000). Earthworm and Sustainable Agriculture: Study of the Role of Earthworm in Production of Wheat Crop. Ph.D Thesis Awarded by University of Rajasthan, Jaipur, India.

Bhatia et al., 2000 – *Bhatia, S., Sinha, R.K. and Sharma, R*. (2000). Seeking Alternatives to Chemical Fertilisers for Sustainable Agriculture: A Study on the Impact of Vermiculture on the Growth and Yield of Potted Wheat Crops (Triticum aestivum Linn). International J. of Environmental Education & Information, University of Salford, UK, 19(4): 295-304.

Bredon, Dugmore, 1985 – *Bredon, R.M. and Dugmore, T.J. et al*. (1985). Dairying in Kwazulu – Natal. Mineral & Vitamin Nutrition Of Dairy Cattle. *Department: agriculture and rural development. Province of Kwazulu-Natal*.

Brown, Razzaque, 2015 – *Brown, R.B., Razzaque, M.S*. (2015). Phosphate toxicity: a stealth bio-chemical stress factor? *Medical Molecular Morphology*. 49(1): 1-4. DOI: 10.1007/s00795-015-0122-3

Buijs, Samwel Mantingh, 2019 – *Buijs, J. and Samwel Mantingh M*. (2019). Een onderzoek naar mogelijke relaties tussen de afname van weidevogels en de aanwezigheid van bestrijdingsmiddelen op veehouderijbedrijven in Gelderland. Buijs Agro-Services.

Cardoza, 2011 – *Cardoza, Y.J*. (2011). Arabidopsis thaliana resistance to insects, mediated by an earthworm-produced organic soil amendment. *Pest Manag. Sci*. 67(2): 233-238.

Carson, 1998 – *Carson, D.A*. (1998). An infectious origin of extraskeletal calcification. *Proceedings of the National Academy of Sciences of the United States of America*. 95(14): 7846-7847.

CBS, 2019 – CBS. (2019). Obesity in the Netherlands. CBS: Central Bureau of Statistics, in the Netherlands. [Electronic resource]. URL: https://www.cbs.nl

Chaudhuri et al., 2016 – *Chaudhuri, P.S., Paul, T.K., Dey, A., Datta, M. and Dey, S.K.* (2016). Effects of rubber leaf litter vermicompost on earthworm population and yield of pineapple (Ananas comosus) in West Tripura, India. *International Journal of Recycling of Organic Waste in Agriculture*. 5(2): 93-103.

Chiy and Phillips, 1993 – *Chiy, Pc., Phillips, C*. (1993). Sodium fertilizer application to pasture. 1. Direct and residual effects on pasture production and composition. *Grass and Forage Science*. 48(2): 189-202.

Dalsukh, 2009 – *Dalsukh, V*. (2009). Study of Aerobic, Anaerobic and Vermicomposting Systems for Food and Garden Wastes and the Agronomic Impacts of Composts on Corn and Wheat Crops; Report of 40 CP Honours Project for the Partial Fulfillment of Master of Environmental Engineering Degree, Griffith University, Australia.

Dangour et al., 2009 – *Dangour, A., Dodhia, S., Hayter, A., Aikenhead, A., Allen, E., Lock, K., Uauy, R*. (2009). Nutritional quality of organic foods: a systematic review. *Am J Clin Nutr*. 90(3): 680-85.

Denisova, Booth, 2005 – *Denisova N.A., Booth, S.L*. (2015). Vitamin K and Sphingolipid Metabolism: Evidence to Date. *Nutr Rev*. 63(4): 110-121.

Dimkpa, indrapan, 2016 – *Dimkpa, C.O., Bindrapan, P.S.* (2016). Fortification of micronutrients for efficient agronomic production: a review. *Agron. Sustain. Dev.* 36: 7.

Dixon, 1839 – *Dixon, J.* (1839). An essay of making compost heaps from liquids and other substances. Written on the evidence of many years' experience. *The Journal of the Royal Agricultural society of England.* 135-140.

Domagała-Świątkiewicz, Gąstoł, 2012 – *Domagała-Świątkiewicz, I., Gąstoł, M.* (2012). Comparative study on mineral content of organic and conventional carrot, celery and red beet juices. *Agricultural University in Kraków. Acta Sci. Pol., Hortorum Cultus.* 11(2): 173-183.

Driessens and Verbeeck, 1988 – *Driessens, F.C., Verbeeck, R.M.* (1988). On the prevention and treatment of calcification disorders of old age. *Medical Hypotheses.* 25(3): 131-137.

Eaton et al., 1991 – *Eaton, K.A., Brooks, C.L., Morgan, D.R., Krakowka, S.* (1991). Essential role of urease in pathogenesis of gastritis induced by Helicobacter pylori in gnotobiotic piglets. *Infect. Immun.* 59: 2470-2475.

Ekholm, et al., 2007 – *Ekholm, P., Reinivuo, H., Mattila, P., vPakkala, H., Koponen, J., Happonen, A., Hellström, J., Ovaskainen, M-L.* (2007). Changes in the mineral and trace element contents of cereals, fruits and vegetables in Finland. *Journal of Food Composition and Analysis.* 20: 487-495.

Fan et al., 2008 – *Fan, M., Zhao, F., Fairweather-Tait, S., Poulton, P.R., Dunham, S.J., McGrath, S.P.* (2008). Evidence of decreasing mineral density in wheat grain over the last 160 years. *Journal of Trace Elements in Medicine and Biology* 22: 315-324.

Frank, 1888 – *Frank, A.B.* (1888). Ernährung der Pflanze mit Stickstoff und über den Kreislauf desselben in der Landwirthschaft. Verlag von Paul Parey, berlin.

Frassetto et al., 1998 – *Frassetto, L.A., Todd, K.M., Curtis Morris R.Jr., Sebastian, A.* (1998). Estimation of net endogenous noncarbonic acid production in humans from diet potassium and protein contents. *J Clin Nutr.* 68: 576-83.

Frere, 1863 – *Frere, P.H.* (1863). On M. J. Reiset's Agricultural Experiments. *The Journal of the Royal Agricultural society of England*, Part one, XXIV.

Galland, 2014 – *Galland, L.* (2014). The Gut Microbiome and the Brain. Journal of medicinal food. *J Med Food.* 17(12): 1261-1272.

Gerendás et al., 1998 – *Gerenda´s, J., Zhu, Z., Sattelmacher, B.* (1998). Influence of N and Ni supply on nitrogen metabolism and urease activity in rice (Oryza sativa L.). *Journal of Experimental Botany.* 49(326): 1545-1554.

Hennig, 1996 – *Hennig, E.* (1996). De geheimen van een vruchtbare bodem. *Agriton Noordwolde Zuid.*

Herrero, Flores, 2019 – *Herrero, A., Flores, E.* (2019). Genetic responses to carbon and nitrogen availability in Anabaena. *Environmental Microbiology.* 21(1): 1-17.

Hopkins, 1956 – *Hopkins, D.P.* (1956). Chemicals, Humus and the soil. A simple presentation of contemporary knowledge and opinions about fertilizers, manures, and soil fertility. Ed. Faber & Faber LTD, London.

Hussain et al., 2020 – *Hussain, N., Abbasi, T. and Abbasi, S.A.* (2020). Evaluating the fertilizer and pesticidal value of vermicompost generated from a toxic and allelopathic weed ipomoea. *Journal of the Saudi Society of Agricultural Sciences.* 19: 43-50.

Itkonen, 2015 – *Itkonen, S.*(2015). Dietary Phosphorus Bioavailability and Associations with Vascular Calcification in a Middle-Aged Finnish Population. Dissertation 2015. University of Helsinki. Ed. Helsingin Yliopisto. Hansaprint.

Jamieson, 1910 – *Jamieson, Th.* (1910). History of the progress of agricultural science in Great Britain. Printed and published by Messrs C. & R. Anderson. "North British agriculturist", Edinburgh

Janson et al, 2002 – *Janson, J.J., Galarza, C.R., Muru´ a, A., Quintana, I., Przygoda, P.A., Waisman, G., Camera, L., Kordich, L., Morales, M., Mayorga, L.M., and Camera, M.I. (2002).* Prevalence of Hyperhomocysteinemia in an Elderly Population. American Journal of Hypertension 2002; 15:394–397.

Kadowaki et al., 2005 – *Kadowaki, H., Nishitoh, H., Urano, F., Sadamitsu, C., Matsuzawa, A., Takeda, K. Masutani, H., Yodoi, J., Urano, Y., Nagano, T., and Ichijo, H.* (2005). Amyloid b induces neuronal cell death through ROS-mediated ASK1 activation. Cell Death Differ. 12(1): 19-24. doi: 10.1038/sj.cdd.4401528.

Kajander, Ciftcioglu, 1998 – *Kajander, E.O., Ciftcioglu, N.* (1998). Nanobacteria: An alternative mechanism for pathogenic intra and extracellular calcification and stone formation. *Proc Natl Acad Sci USA*. 95(14): 8274-8279. PMCID: PMC20966 Medical Sciences.

Kapustin et al., 2011 – *Kapustin, A., Galkin, A., Furmanik, M., Alvarez-Hernandez, D., Shanahan, C.* (2011). Elevated calcium and phosphate impair mitochondrial function in calcifying human vascular smooth muscle cell. Published by the BMJ Publishing Group Limited.

Kaufman et al., 1980 – *Kaufman, W., Hagemeister, H. and Dirksen, G.* (1980). Adaptation to changes in dietary composition, level and frequency of feeding. In: Digestive physiology and metabolism in ruminants. Westport, Ct. Avi publishing 1980. P 587.

Kessel Van, 2008 – *Kessel Van, C.* (2008). Nitrogen transformations and turnover in soils amended with organic sources. University of California, Davis, California, USA. Chapter 4.2 in: Guidelines on Nitrogen Management in Agricultural Systems. International Atomic Energy Agency, Vienna, 2008.

Khan et al., 2013 – *Khan, S.A., Mulvaney, R.L., Ellsworth, T.R.* (2013). The potassium paradox: Implications for soil fertility, crop production and human health. *Renewable Agriculture and Food Systems*. 29(1): 3-27. DOI: 10.1017/S1742170513000318

Kobayashi, 1957 – *Kobayashi, J.* (1957). On geographical relationship between the chemical nature of river water and death-rate from apoplexy. *Berichte d Ohara Inst f landwirtsch Biologie*. 11:12-21.

Krasil'nikov, 1958 – *Krasil'nikov, N.* (1958). Soil microorganisms and higher plants. Academy of Sciences of the USSR Publishing, Moscow.

Krunal, 2009 - *Krunal, C.* (2009). A Comprehensive Study of Vermiculture Technology: Potential for its Application in Solid Waste and Wastewater Management, Soil Remediation and Fertility Improvement for Increased Crop Production; Report of 40 CP Honours Project for the Partial Fulfillment of Master of Environmental Engineering Degree; Griffith University, Australia.

Lawes and Gilbert, 1858 – *Lawes, J.B. and Gilbert, J.H.* (1858). XXV. Report of Experiments with different Manures on Permanent Meadow Land. Part one. *Journal of the Royal agricultural society of England*.

Masterjohn, 2008 – *Masterjohn, C.* (2008). On the Trail of the Elusive X-Factor: A Sixty-Two-Year-Old Mystery Finally Solved. [Electronic resource]. URL: https://www.Westonaprice.org/health-topics/abcs-of-nutrition/.

McCall, Willumsen, 1998 – *McCall, D. Willumsen, J.* (1998). Effects of nitrate, ammonium and chloride application on the yield and nitrate content of soil-grown lettuce. *The Journal of Horticultural Science and Biotechnology*. 73: 698-703.

Nele, 2006 – *Nele, G.* (2006). Belang van mineralen en vitaminen in de melkveevoeding. *Katholieke hogeschool Kempen in Vlaanderen*.

Neveu et al., 2009 – *Neveu, A., Billi, M., Farma, J.I.* (2009). Le chlorure de magnésium dans les maladies infectieuses. Sciences libres. Bayonne.

Nigten, 2019 – *Nigten, A.O.* (2019). Het stikstofvraagstuk in de land- en tuinbouw. De valkuil van de ammonium- en nitraatmeststoffen. Uitgegeven in eigen beheer. [The nitrogen issue in agriculture and horticulture. The pitfall of ammonium and nitrate fertilizers. (in Print).

Nigten, 2019 – *Nigten, A.O.* (2019). How Healthy are Our Vegetables? Contours of a New Fertilizing Paradigm. Minerals and non Protein Nitrogen in Vegetables, Grown Organically and Respectively Conventionally. A Quality Assessment (Review). *Biogeosystem Technique*. 6(1): 3-22. DOI: 10.13187/bgt.2019.1.3

Opdebeeck et al., 2004 – *Opdebeeck, H.G., Verhelst, G., De Marez, E., Tejeda, H., Van Hyfte, M.* (2004). Use of Natural Chilean Nitrate in Organic Farming. Ed. Chamoson, Switzerland. [Electronic resource]. URL: https://earthwiseagriculture.net

Pathma, Sakthivel, 2012 – *Pathma, J., Sakthivel, N.* (2012). Microbial diversity of vermicompost bacteria that exhibit useful agricultural traits and waste management potential. *SpringerPlus*. 1: 26. [Electronic resource]. URL: http://www.springerplus.com/content/1/1/26

Polaccao et al., 1999 – *Polaccao, J., Freyermuth, S., Gerendas, J., Cianzio, S.* (1999). Soybean genes involved in nickel insertion into urease. *J Exp Bot*. 50: 1149-1156. DOI: 10.1093/jxb/50.336.1149

Poschenrieder, Lesch, 1942 – *Poschenrieder, H., Lesch, W.* (1942). Untersuchungen über den Einfluss langjähriger einseitigen Düngungsmassnahmen auf die Ausbildung und Nährstoffaufnahme der Würzelknollchen von Sojabohne. *Bodenkunde und Pflanzenernährung*. 32. Band. Heft I/2.

Regnault, Reiset, 1849 – *Regnault, H.V., Reiset, J.* (1849). Recherches chimiques sur la respiration des animaux des diverses classes. Paris – Bachelier.

Reiset, 1863 – *Reiset, J.* (1863). Recherches pratiques et expérimentales sur l'agronomie. Édition: J B Baillierre.

Rietberg, van der Burgt, 2012 – *Rietberg, P., Van der Burgt, G-J.* (2012). Mest Als Kans 2012: Activiteiten en resultaten Bedrijfsoptimalisatie bemesting. Editor: Louis Bolk institution.

Roovers, 1937 – *Roovers, J.* (1937). Calcinosis universalis. Nederlandsch tijdschrift voor geneeskunde 22 mei 1937.

Rotgers, 2017 – *Rotgers, G.R.* (2017). Stoot Nederlandse melkkoe meeste of minste methaan uit binnen Europa? 4 nov 2017 – Onderzoek en beleid. [Electronic resource]. URL: https://www.v-focus.nl › 2017/11 › stoot-nederlandse-mel

Rotgers, 2019 – *Rotgers, G.R.* (2019). Minder weidevogels door insecticiden landbouw? *STAF Research - Stichting agrifacts*. [Electronic resource]. URL: https://stichtingagrifacts.nl› uploads› 2019/04› To.

Rusch, 1968 – *Rusch, H.P.* (1968). Bodenfruchtbarkeit. Organischer Landbau Verlag. Nederlandse vertaling door P. Vanhoof, 2014: Bodemvruchtbaarheid. [Electronic resource]. URL: https://www.dekoolstofkring.nl.

Schmack, 2020 – *Schmack, K-H.* (2020). De beschadigde koe door de ureumgekte. Uitgeverij Bij de Oorsprong, Dalfsen. [Electronic resource]. URL: https://www.bijdeoorsprong.nl

Schmiedl et al., 1998 – *Schmiedl, A., Schwille, P.O., Bergé, B., Markovic, M., Dvorak, O.* (1998). Reappraisal of the quantity and nature of renal calcifications and mineral metabolism in the magnesium-deficient rat. Effects of treatment with potassium citrate or the combination magnesium citrate and potassium citrate. *Urol Int*. 61(2): 76-85.

Seufert et al., 2012 – *Seufert, V., Ramankutty, N., Foley, J.A.* (2012). Comparing the yields of organic and conventional Agriculture. *Nature*. 485: 229-232. DOI: 10.1038/nature11069

Schreiner, Skinner, 1912 – *Schreiner, O. and Skinner, J.J.* (1912). Nitrogenous soil constituents and their bearing on soil fertility. US Department of agriculture. Washington: Government printing office.

Schroll, 2002 – *Schroll, A.* (2002). Importance of magnesium for the electrolyte homeostasis - an overview. *Deutsches Herzzentrum München, KIinik für Herz – und Gefaßchirugie, Lothstr*. 1(1): D-80335 München, Germany. [Electronic resource]. URL: https://www.mgwater.com

Seelig, 1980 – *Seelig, M.S.* (1980). Magnesium deficiency in the pathogenesis of disease. Early Roots of Cardiovascular, Skeletal and Renal Abnormalities Chapter 11: Skeletal and renal effects of magnesium deficiency. Magnesium, Bone Wasting, and Mineralization. Goldwater Memorial Hospital, New York University. [Electronic resource]. URL: https://www.mgwater.com

Seelig, 1981 – *Seelig, M.S.* (1981). Magnesium Requirements in Human Nutrition. *Contemporary Nutrition*. 7(1). [Electronic resource]. URL: https://www.mgwater.com

Singh et al., 2013 – *Singh, R., Singh, R., Soni, S.K., Singh, S.P., Chauhan, U.K., Kalra, A.* (2013). Vermicompost from biodegraded distillation waste improves soil properties and essential oil yield of Pogostemon cablin (patchouli) Benth. *Appl. Soil Ecol*. 70: 48-56.

Sinha, Bharambe, 2007 – *Sinha, R.K., Bharambe, G.* (2007). Studies on Agronomic Impacts of Vermicompost Vis-à-vis Conventional Compost and Chemical Fertilizers on Corn Crops. CESR Sponsored Project; Griffith University, Brisbane, Australia.

Sinha et al., 2009 – *Sinha, R.K., Herat, S., Valani, D., Chauhan, K.* (2009). Special Issue on Vermiculture and sustainable agriculture. *American-Eurasian J. Agric. & Environ. Sci.* 5(S): 01-55.

Sirko, Brodzik, 2000 – *Sirko, A., Brodzik, R.* (2000). Plant ureases: roles and regulation. *Acta Biochim Polon*. 47: 1189-1195.

Smink et al., 2003 – *Smink, W., Veen, W. A. G., Dijkstra, J., Williams, B.A., Boer, H.* (2003). Methaanreductie melkvee: een onderzoeksproject naar de inschatting van de methaanproductie vanuit de voeding en naar de reductiemogelijkheden via de voeding van melkkoeien. Feed Innovation Services. [Electronic resource]. URL: https://edepot.wur.nl/177082

Smith, 1993 – *Smith, B.L.* (1993). Organic Foods vs Supermarket Foods: Element Levels. *Journal of applied nutrition*. 45(1).

Swerczek, 2007 – *Swerczek, T.W.* (2018). Nitrate Toxicity, Sodium Deficiency and the Grass Tetany Syndrome – long version [Electronic resource]. URL: http://www.growersmineral.com/grass tetany.

Swoboda, 2016 – *Swoboda, P.* (2016). Rock dust as agricultural soil amendment: a review. Master's Thesis at the Institute of Systems Sciences, Innovation and Sustainability Research. University of Graz.

Theel, 1933 – *Theel, E.* (1933). Über den Mineralstoffgehalt deutscher Futtermittel. Ergebnisse an Wiesenheu. Publisher: Landwirtschaftliche Hochschule Berlin.

Thomas, 2003 – *Thomas, D.* (2003). A study on the mineral depletion of the foods available to us as a nation over the period 1940 to 1991. *Nutrition and health (Berkhamsted, Hertfordshire)*. 17(2): 85-115.

Tsaioun, 1999 – *Tsaioun, K.I.* (1999). Vitamin K-dependent Proteins in the Developing and Aging Nervous System. *Nutrition Review*. 57(8).

Umar et al., 2013 – *Umar, S., Anjum, N.A., Khan, N.A.* (2013). Nitrate in leafy vegetables. Toxicity and safety measures. I.K. International Publishing House Pvt. Ltd. New Delhi.

Vanhoof and Nigten, 2020 – *Vanhoof, P. and Nigten, A.* (2020). Drijfmest, Invloeden op emissies, en N-benutting op grasland. [Electronic resource]. URL: https://www.deVBBM.nl; https://www.NMV.nu. [in Dutch]

Veeteelt, 2021 – *Veeteelt* (2021). Nederlandse melkveehouders leverden in 2020 melk af met gemiddeld 21,6 milligram ureum per 100 gram melk.

Visser, 2010 – *Visser, J.* (2010). Down to earth: a historical-sociological analysis of the rise and fall of 'industrial' agriculture and of the prospects for the re-rooting of agriculture from the factory to the local farmer and ecology. PhD thesis Wageningen University. [Electronic resource]. URL: https://research.wur.nl › *publications*

Voisin, 1963 – *Voisin, A.* (1963). Grass tetany. Translated from the French by C. Herriott. C. Thomas – Publisher Bannerstone House 301-327. East Lawrence avenue, Springfield, Illinois, USA.

Waart, 1998 – *Waart, S. de.* (1998). Biologisch: goed beter, best? Biologische en gangbare groenten en fruit vergeleken op de aspecten residuen, vitamine C, nitraat en drooggewicht: een overzicht. Louis Bolk instituut, the Netherlands.

Warburg, 1956 – *Warburg, O.* (1956). On the Origin of Cancer Cells. *Science*. 123(3191): 309-314.

Wendt, 1983 – *Wendt, L.* (1983). Gesund werden durch Abbau von Eiweißüberschüssen. Schnitzer Verlag, St Georgen/Schwarzwald.

Wesseling, 2019 – *Wesseling, M.* (2019). Grote grazers moeten gezonder gaan poepen, want 'poep geeft leven'. *Trouw*, 17 augustus. [Electronic resource]. URL: https://www.trouw.nl/nieuws/grote-grazers-moeten-gezonder-gaan-poepen-want-poep-geeft-leven~bdc905b0/Newspaper

Williams, 2018 – *Williams, A.* (2018). Medical medium liver rescue. Hay house inc. California.

Xiao et al., 2016 – *Xiao, Z., Liu, M., Jiang, L., Chen, X., Griffiths, B.S., Li, H., Hu, F.* (2016). Vermicompost increases defense against root-knot nematode Meloidogyne incognita) in tomato plants. *Appl. Soil Ecol.* 105: 177-186.

Yardim et al., 2006 – *Yardim, E.N., Arancon, N.Q., Edwards, C.A., Oliver, T.J.* (2006). Suppression of tomato hornworm (Manduca quinquemaculata) and cucumber beetles (Acalymma vittatum and Diabotrica undecimpunctata) populations and damage by vermicomposts. *Pedobiologia* 50: 23-29.

Appendix 1

Milk quality has decreased

Cow milk in the Netherlands is no longer suitable for calves (Table 5). According to Agrifirm*, Sprayfo and the Flemish ministry of Agriculture, the quality of cow milk is low.

Table 5. Cow milk no longer suitability for calves in the Netherlands

The milk is too fat (it contains two times more fat than the calf needs). The calves are at risc of getting diarrhea.They can't metabolize the fat properly.
The cow milk contains 1,5 times the amount of protein a calf needs. Too much urea in the milk is a risc†.
The magnesium content should be twice as high (Flemish Ministry of agriculture)
The copper content is 8 times too low
The selenium content is 16 times too low
The iron content is 16 times too low
The manganese content is 33 times too low
The vitamin B1 content is too low;
The vitamin A is 2,5 times too low
The vitamin D3 is 10,3 times too low
The vitamin E content is 15 times too low according to the Flemish Ministry of Agriculture.

The exact amount of urea in the milk – much too high according to Schmack (Schmack, 2020) – is not listed in this overview, nor the amount of nitrate in the milk which can vary sixty fold (Nele, 2006).

According to a study of Masterjohn also the amount of vitamin K2 – not mentioned here above – has gone down:

"*Commercial butter is only a moderate source of vitamin K_2. After analyzing over 20,000 samples of butter sent to him from around the world, however, Price found that the Activator X [= vit K_2] concentration varied 50-fold. Vitamin K-rich cereal grasses, especially wheat grass, and alfalfa in a lush green state of growth produced the highest amounts of Activator X, but the soil in which the pasture was grown also profoundly influenced the quality of the butter. The concentrations were lowest in the eastern and far western states* [in the USA] *where the soil had been tilled the longest, and were highest in Deaf Smith County, Texas, where excavations proved the roots of the wheat grass to pass down six feet or more through three feet of top soil into deposits of glacial pebbles cemented together with calcium carbonate. It was this amazingly vitamin-rich butter that had such dramatic curative properties when combined with*

* Agrifirm – a dutch company that trades in seeds, animal feed and fertilizers - published data on cow milk quality in order to promote the use of artificial milk for calves which they are selling themselvs. They got the data from one of the biggest Dutch Milk dairies "Friesland Campina'. After critical questions of farmers and others they removed the data from their website. Sprayfo is another seller of artifical milk for calves.

† The average of the last seven years (2013 – 2020) of the Urea number (urea in mg/100 gram milk) in Dutch milk was 22,5 (Veeteelt, 2021). Schmack advizes max 10 for urea in milk (Schmack, 2020).

high-vitamin cod liver oil and nutrient-dense meals of whole milk, whole grains, organ meats, bone broths, fruits and vegetables" (Masterjohn, 2008).

The butter of Deaf Smith county was send all over the USA because of its health promoting qualities.

The groundwater of Deaf Smith county is very high in iodine, magnesium and trace elements. Farmers used this groundwater to irrigate their crops. The bones and teeth of this area contained five times more magnesium than the bones and teeth elsewere in Texas. In this area people had no health problems with bones and teeth (Barnett, 1954).

In the same article Masterjohn describes the many functions of vitamin K_2 in our body. Their role in our brain is one of them:

"*Vitamin K_2 supports the enzymes within the brain that produce an important class of lipids called sulfatides. The levels of vitamin K_2, vitamin K-dependent proteins and sulfatides in the brain decline with age; the decline of these levels is in turn associated with age-related neurological degeneration*[*] *Comparisons of human autopsies associate the early stages of Alzheimer's disease with up to 93 percent lower sulfatide levels in the brain*" (Masterjohn, 2008).

Too high levels of sulfatides are also a risk. But vitamin K_2 regulates the sulfatide concentrations in the brain (Tsaioun, 1999).

[*] Masterjohn refers to the following article for this conclusion: Denisova, Booth, 2005.