Supplementary Materials

Decoupling livestock from land use through industrial feed production pathways

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Materials and methods

General description of the Model of Agricultural Production and its Impact on the Environment (MAgPIE)

The MAgPIE model is a mathematical programming model that is used to create long-term scenarios and assessments of global agriculture and land use. [1-7] It integrates several spatial scales (see Figure S1 for an overview of the model structure) and simulates the full food supply chain from nutrient, land and water use over crop and livestock production, food processing up to final consumption.

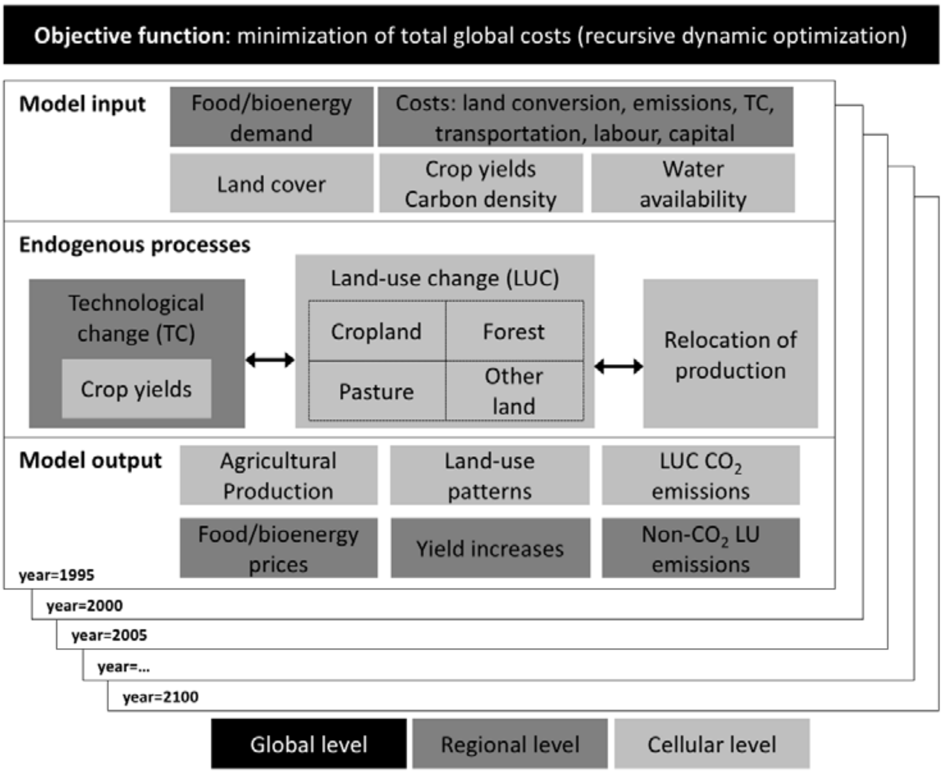


Figure S1. Schematic representation of the MAgPIE model.

For a given food demand (see Fig. S3 the model estimates cost-optimal production patterns, and simulates major dynamics of the agricultural sector, like trade, technological progress and land allocation according to the scarcity of suitable land, water and economic resources. [6]

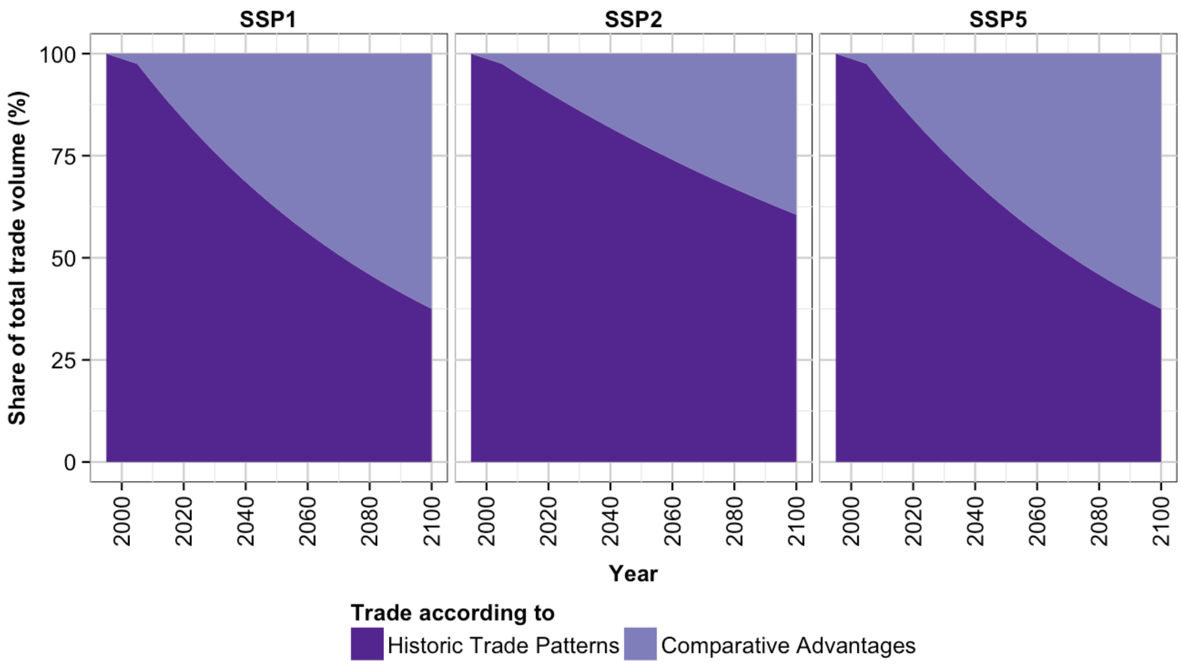


Figure S2. Agricultural Trade scenarios based on Schmitz et al., (2012). [8] There are two trade pools in the MAgPIE model, one with trade according to historic trade patterns, another one with free trade according to comparative advantages. In SSP1 and SSP5 barriers for international trade decline by 1% per year (Policy scenario as described in Schmitz et al., (2012), reflecting globalized agricultural markets in these scenarios. In SSP2, trade barriers decline only by 0.5% per year, reflecting rather regionalized trade of agricultural goods.

As it treats agricultural products not only as economic values but also as physical goods, MAgPIE can perform analysis of material flows. The products in the model comprise 17 crop groups, each with individual above – and belowground crop residues, 5 livestock production types (i.e. beef cattle, dairy cattle, pigs, broiler and laying hen), 8 types of conversion by-products originating from food processing, as well as grazed pasture and scavenging. Products can be used for food, feed, other use (comprising material use and waste in the production chain) and seed, where applicable. Crop residues can be recycled to soils, burned in the fields, used as feed, or used as material. [2] The demand for food enters the model as an exogenous trajectory. [2, 9]

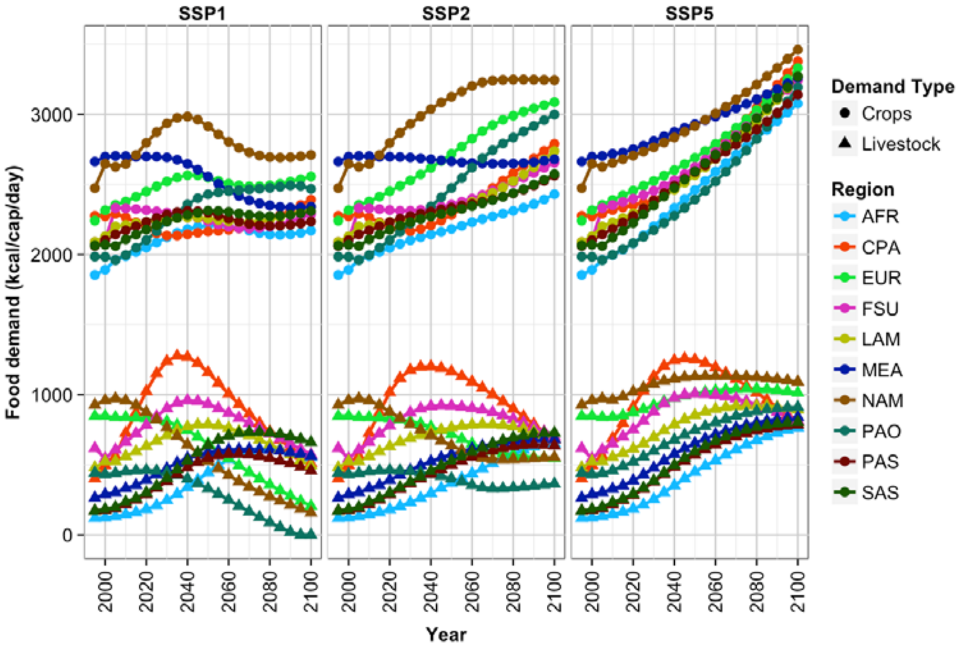


Figure S3. Time-series of regional per capita food demand in SSP1, SSP2 and SSP5**.** The values are estimated using the methodology described in Bodirsky et al [10] based on SSP population and GDP projections. SSP1 and SSP2 use a different demand system than SSP5, leading to higher demand for the latter. Additionally, it was assumed that per-capita calories in SSP1 fall to a maximum of 3000 kcal per capita per day in 2100, and that the share of livestock products in rich countries does not fall below 15% in SSP2 and SSP5.

Demand for material consumption and production waste is assumed to grow over time in proportion to food demand, while the demand for seed is a fixed share of crop production. [2] The demand for feed depends on the quantity of livestock production, as well as regional and livestock system-specific feed baskets. [11] Supply of livestock products (ruminant meat, whole-milk, pork, poultry meat and eggs) is provided by five animal food systems (beef cattle, dairy cattle, pigs, broiler and laying hen). The regional and system-specific feed baskets were derived as follows: In a first step, regional feed energy requirements were calculated based on specific feed energy requirements per product [12] for each animal food system and animal group (i.e. reproducers, producers and replacing animals) that entail the minimum requirements for maintenance, growth, lactation, reproduction and other basic biological functions of the animals. In addition, they comprise a general allowance for basic activity, temperature effects and expenditures for grazing. In a second step, these regional feed energy requirements are settled by regional feed energy supply comprising concentrate feed, conversion by-products (both in accordance with the Commodity Balance Sheets (CBS) from [13]), crop residues [2], and grazed biomass. As there are little data available on the amount of grazed biomass, grazed biomass was assumed to provide the remainder to fulfill the energy requirements of ruminants. The feed was distributed between the animal groups based on constraints regarding the nutrient density of resulting feed baskets. Future development of livestock productivity is driven by exogenous projections (Figure S4), and determines feed energy requirements and feed basket composition, leading to a higher share of concentrates with higher yields.

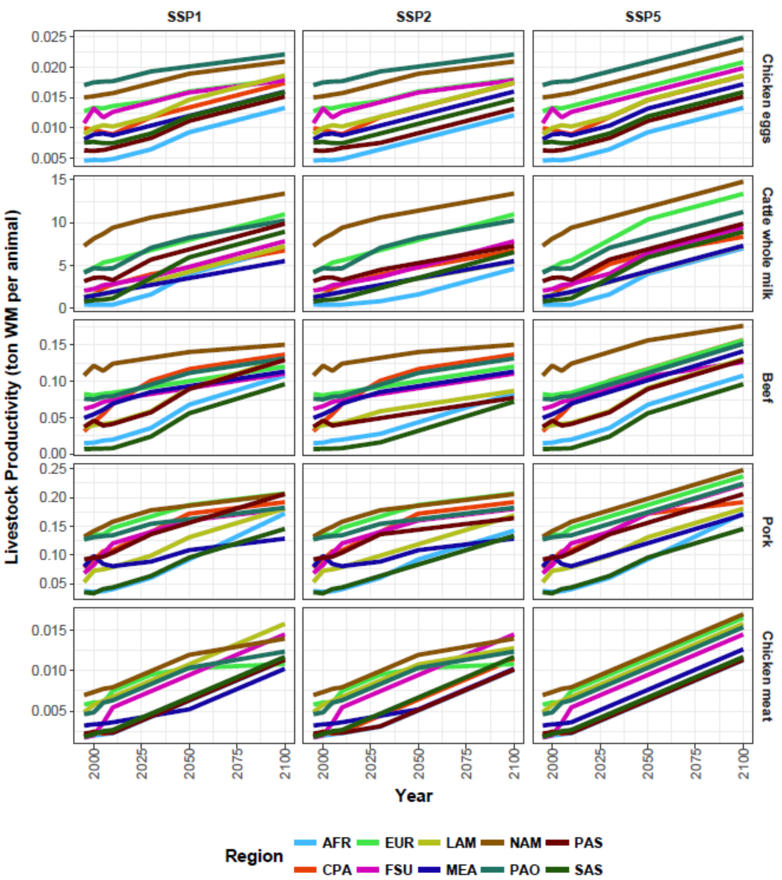


Figure S4. Time-series of regional livestock productivity in SSP1, SSP2 and SSP5. Future scenarios of livestock productivity are derived based on informed guesses, taking into account past productivity improvements, GDP projections, cultural particularities, and the general SSP story line. Livestock productivity scenarios determine feed energy requirements and feed basket composition.

All demand categories are calculated on the level of ten world regions (Figure S5), and can be settled through domestic production or international trade.

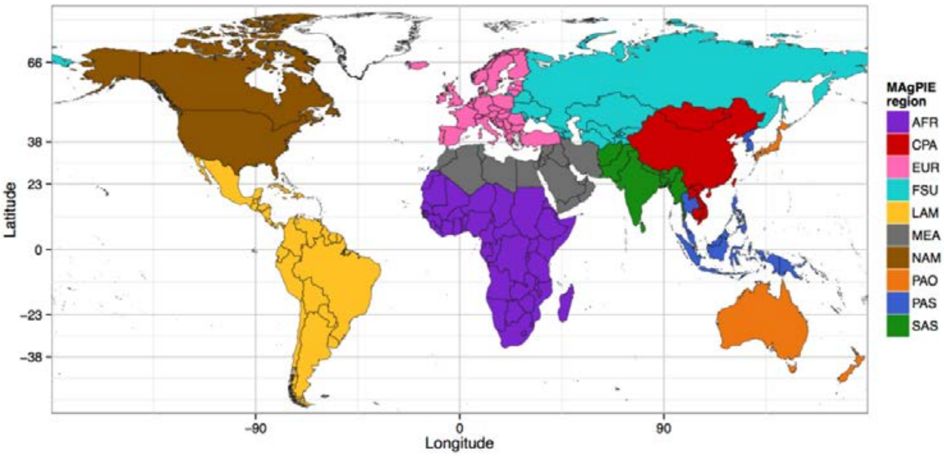


Figure S5. Map of economic world regions in the MAgPIE model.

To account for trade barriers, the regions have to produce a certain share of their demand domestically. [8] The production of crops requires financial resources as well as land, water and fertilizer. [14] Also, spatial production patterns have to account for good-practice crop rotations, which are enforced by crop-specific constraints. Cropland expansion leads to additional costs and is limited by biophysical constraints as well as by competing land use activities. Additionally to land-expansion, the model can also invest into yield-increasing research and technology. [15] Crop growth functions connect crop harvest to the production of above – and belowground residues. [2] Similarly, the production of conversion by-products depends upon fixed conversion factors multiplied with the regional crop supply. [2] Finally, livestock production requires financial resources, feed and water. For meeting the demand, the model endogenously decides, based on cost-effectiveness, about the level of intensification (i.e. yield-increasing technological change), land expansion (i.e. land-use change) and production relocation (i.e. intra-regionally and interregionally through international trade). [8, 15-17] The decision criterion is cost minimization. Major cost types in MAgPIE are factor requirement costs (e.g. capital, labour and fertilizer), land conversion costs, transportation costs to the closest market and investment costs for yield-increasing technological change (TC). The model is solved in a recursive dynamic mode with a time step length of five years on a timescale from 1995 to 2100.

Biophysical information on initial land-cover, crop yields, carbon densities and water availability is spatially explicit (0.5 degree longitude/latitude). Due to computational constraints, spatially explicit data is aggregated to 200 simulation units for the optimization process. For each of the ten world regions in MAgPIE, the clustering algorithm combines grid cells to simulation units based on the similarity of data. [18] Land types in MAgPIE comprise cropland, pasture, forest, other land (e.g. non-forest natural vegetation, abandoned agricultural land, deserts) and urban land (static over time). [19] In 2005, the global land area (12907 Mha in total) consists of 1471 Mha cropland, 3129 Mha pasture, 4208 Mha forest and 4135 Mha other land (e.g. urban areas) (Fig. S6, Supplementary Materials excel spreadsheet).

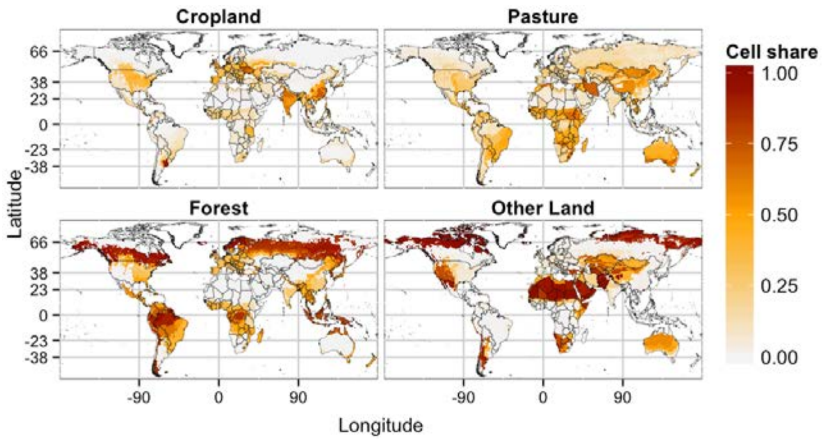


Figure S6. Spatially explicit land use / land cover in MAgPIE in 2005. For each grid cell, the cell shares of the four land types add up to 1.

Cropland covers cultivation of 16 food/feed crop types (e.g. temperate and tropical cereals, maize, rice, oilseeds, roots), both rainfed and irrigated systems, and two 2nd generation bioenergy crop types (i.e. grassy and woody). Biophysical yields for these crop types as well as carbon densities of natural vegetation and water availability for irrigation are derived by a dynamic global vegetation, hydrology and crop growth model, the Lund-Potsdam-Jena model for managed Land (LPJmL) [20, 21], and calibrated to observed yields from. [13]

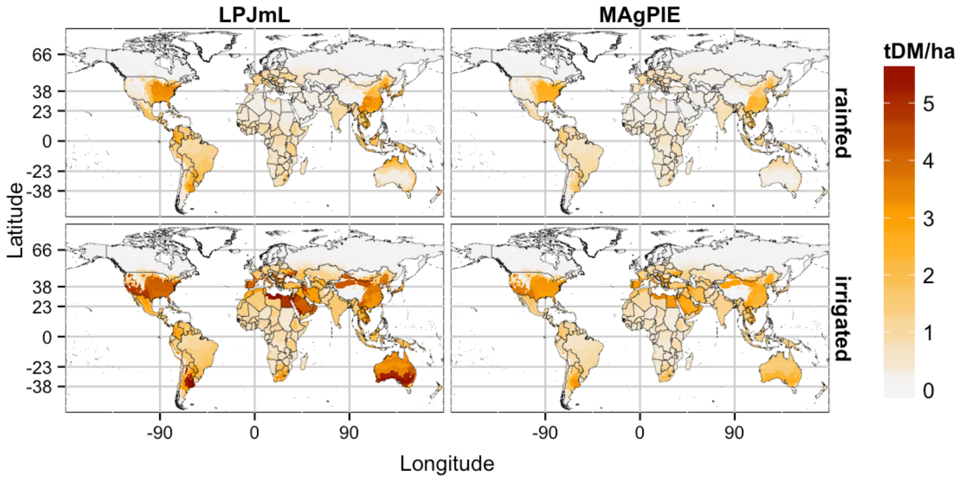


Figure S7. Tropical cereal yields in rain fed and irrigated production systems as derived by LPJmL, and how these yields are adjusted before entering the MAgPIE model.

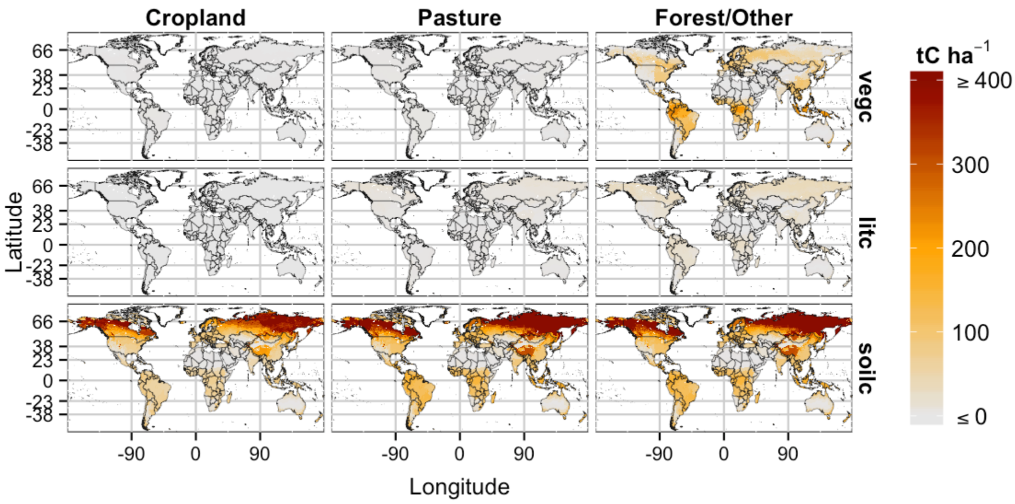


Figure S8. Spatially explicit carbon density (tC ha^{-1}) based on LPJmL for four land types and the three carbon pools vegetation, litter and soil (vegc, litc, soilc).

LPJmL simulations of crop yields assume that all crops are grown in all grid cells to assess the possible crop productivity also in areas currently not under cultivation to inform possible shifts in cropping areas. In seven individual LPJmL runs, crop yields are derived for seven different management intensity levels. LPJmL represents potential crop yields, while MAgPIE aims to represent actual crop yields. Due to endogenous investments in yield-increasing technological change in the MAgPIE model, MAgPIE yields can exceed LPJmL yields in future time steps. [15]

MAgPIE was extended by a Nr flow module [2] that transforms all biomass flows in the model into reactive nitrogen (Nr) flows. The closed budget approach and a consistent connection of all Nr flows guarantees that the sum of Nr fixation, Nr release and inflows from other sectors corresponds to the sum of Nr losses and Nr flows to other sectors. Similarly, intermediary closed budgets are also used on a regional level for cropland soils, distribution and processing of agricultural products, livestock feeding, manure management, and the household sector. While certain processes in MAgPIE like crop production and water use are simulated spatially explicit on the level of clustered 0.5° grid cells, Nr flows and Nr budgets are calculated on the level of 10 world regions. [2]

Nr in harvested crops (*H*), aboveground (*RA*) and belowground (*RB*) crop residues are estimated based on crop-specific crop growth functions and Nr contents of the individual plant components. The Nr derived from biological fixation within plants (*B1*) is estimated based on plant-specific shares of plant Nr derived from atmospheric fixation, while biological fixation by free-living microorganisms (*B2*) is estimated based on typical rates of Nr fixation per cultivated area. All belowground crop residues are assumed to be recycled to the field (*RRB*). In contrast, aboveground crop residues have to settle first the demand for feed from the livestock sector; moreover, a fixed share of crop residues is assumed to be used for material purposes or is lost when residues are burned on the field. Only the remaining residues are recycled on fields (*RRA*). The Nr which is released on croplands when soil organic matter depletes after the opening of new cropland (*SOM*) is calculated on the basis of model-endogenous land-use change activity, spatial-explicit carbon contents of natural soils, typical climate-specific shares of lost soil carbon under cropland management, and a fixed carbon to nitrogen (C:N) ratio in soils. Atmospheric deposition (*D*) is estimated based on current deposition rates and is assumed to grow proportional to volatilized Nr losses. The Nr input of seeds (*S*) to cropland soils is based on regional plant-specific shares of the production that are used for seed. [2]

To estimate the amount of manure recycled to croplands (*RM*), a budget approach is used. The Nr in animal feed (*A*) is estimated by assigning Nr contents to all feed items. The Nr in livestock products (*FA*) and slaughter waste (*SW*) is subtracted from the Nr in animal feed (*A*) to derive the amount of excreted Nr. While the feed from pasture is assumed to be excreted back on pastureland (*RP1*), other excrements are distributed between different animal waste management systems according to regional shares that change over time with scenario assumptions. Depending on their management, different shares of the nutrients are lost to volatilisation and denitrification (*LA*); the remainder is recycled as organic fertilizers on cropland (*RM*) and pasture (*RP2*). [1, 2]

Central to the model are regional nutrient budgets for cropland soils, which are used to determine the future requirements of fertilizers. Different Nr inputs to croplands have different fertilizer equivalents, as they are to a different degree subject to losses on the field. Homogeneous Nr inputs (e.g. Haber-Bosch fertilizer) that can be easily distributed over the field have high equivalence values, while inhomogeneous Nr inputs, whose quantity, spatial distribution and timing cannot be controlled (e.g. atmospheric deposition) have low equivalence values. Using a simplified approach, we distinguish only two categories in MAgPIE: Firstly, Nr inputs from seed (*S*) and biological fixation within the plant (*B1*) are not taken up from the soil, and hence are not subject to losses by volatilisation, leaching or denitrification. Nr from these inputs is therefore assumed to be fully incorporated into plant tissue. Second, we assume that all other Nr inputs are subject to losses prior to plant uptake and that only a share of the applied Nr inputs are withdrawn by the plant roots and incorporated into plant biomass. This share, named as soil Nr uptake efficiency (*SNUPE*), is a regional scenario parameter which reflects next to the climatic and biophysical conditions mostly the fertilization technology of farmers (Fig. S9).

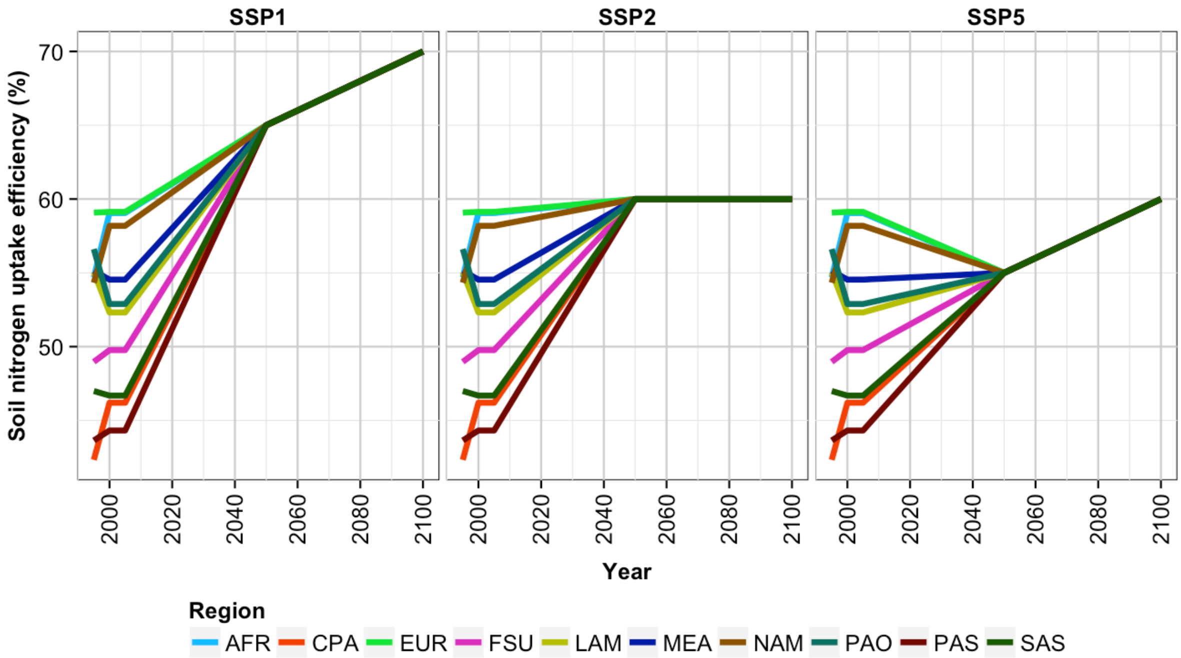


Figure S9. Time-series of regional soil nitrogen uptake efficiency in SSP1, SSP2 and SSP5, based on [1]. The soil nitrogen uptake efficiency reflects the fertilization technology of farmers. Thus, the soil nitrogen uptake efficiency determines, together with the fertilization requirements for the production of crops, the application of inorganic nitrogen fertilizer. First, all available organic nitrogen fertilizer is used to fulfil the fertilization requirements. Subsequently, the remainder needs to be balanced out by the application of inorganic nitrogen fertilizer. Accordingly, the soil nitrogen uptake efficiency influences nitrogen pollution and N_2O emissions from soils. As the challenges for climate change mitigation increase from SSP1 to SSP2 to SSP5, the soil nitrogen uptake efficiency is high in SSP1 (low challenges), medium in SSP2 (medium challenges) and low in SSP5 (high challenges).

As we do not consider the differences in fertilizer equivalences between individual soil Nr inputs like manure and inorganic fertilizers, a high value for *SNUPE* also implies that inhomogeneous fertilizers can be better integrated into crop fertilization, e.g. by improved monitoring or by manure processing.

To calculate the application of inorganic fertilizers (*F*), we estimate the fertilization requirements to obtain a certain production under a given fertilization technology level. The Nr withdrawals from crop production (*H*+*RA*+*RB*) and the exogenous soil Nr uptake efficiency (*SNUPE*) determine the required Nr inputs from organic or inorganic fertilizers:

$$H + RA + RB = SNUPE^{*}(F + RM + RRA + RRB + D + B2 + SOM) + 1^{*}(S + B1) \quad \text{(Eq. 1)}$$

Based on the availability of organic fertilizers, it is ultimately determined which amount of inorganic fertilizers is needed to balance out the budget. [2] As the cropland budget is aggregated over crop-types, nutrient transfers within crop rotations are accounted for. The difference between inputs and withdrawals represents the field losses (*LF*) by denitrification, leaching and volatilisation:

$$LF = F + RM + RRA + RRB + D + B2 + SOM + RH + RS + S + B1 - H - RA - RB \quad \text{(Eq. 2)}$$

Agricultural nitrous oxide (N_2O) emissions from agricultural soils and manure management are estimated in MAgPIE with the emission factors of the IPCC guidelines for national greenhouse gas inventories [22]. Methane (CH_4) emissions from rice, enteric fermentation and manure management are estimated based on the methodology of IPCC. [23]

CO_2 emissions from land-use change in MAgPIE reflect differences in carbon stocks between time steps. If, for instance, forest is converted to cropland within the same simulation unit, the carbon stock of this unit decreases according to the difference in carbon density of forest and cropland (Fig. S10). In case agricultural land is abandoned (other land pool), ecological succession leads to regrowth of natural vegetation carbon stocks along sigmoid growth curves [3]. Regrowth of carbon stocks in MAgPIE is constrained by the LPJmL natural vegetation carbon density. If the vegetation carbon density of re-growing vegetation passes a threshold of 20 tC/ha, the respective area is shifted to the forest land pool. [24, 25]

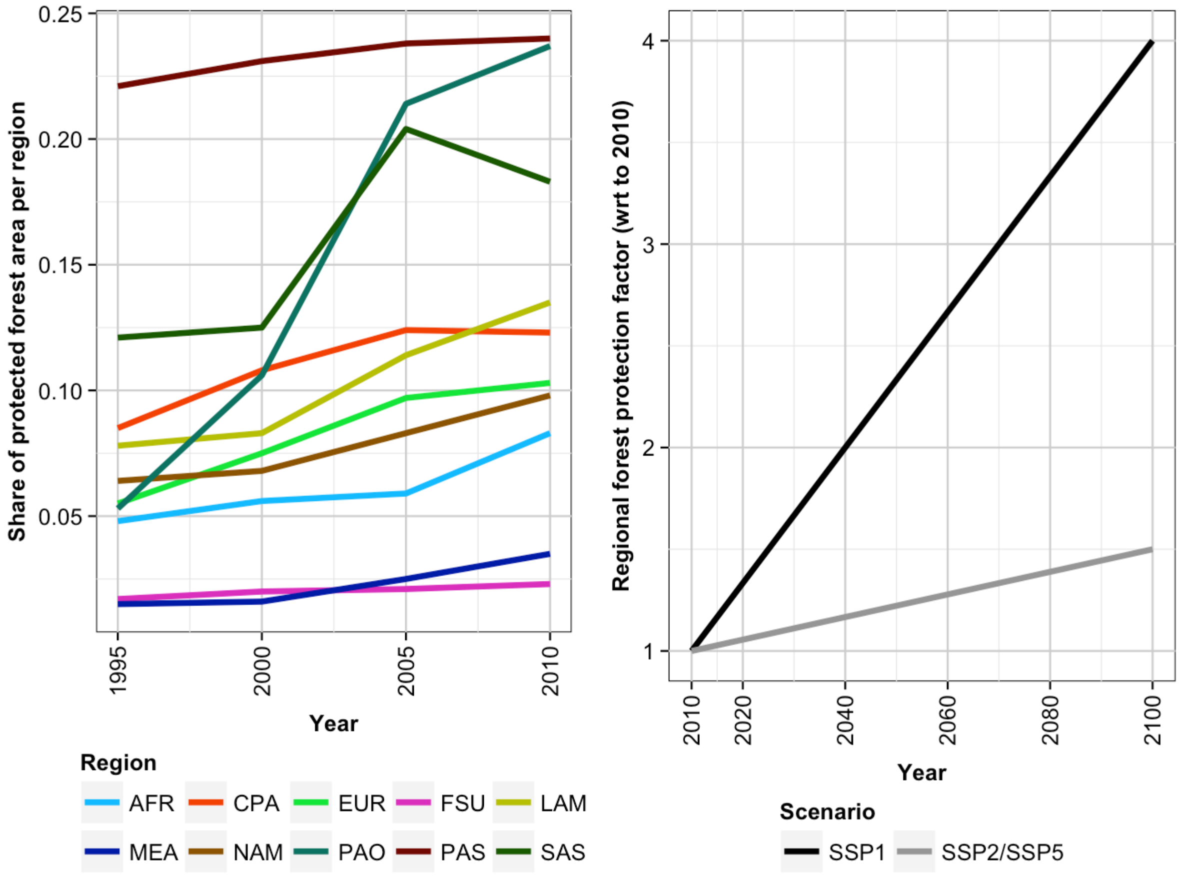


Figure S10. Left: Shares of protected forest for each region (in terms of regional forest area). Values are based on the global forest resources assessment report. [26] Right: Assumed increase of regional forest protection shares in SSP1, SSP2 and SSP5 with respect to 2010. Forest protection takes out land from the pool of available land for agricultural expansion. Thus, forest protection increases competition for land in MAgPIE.

Model results

Feed basket: The altered feed baskets for the five livestock categories, namely, beef cattle, dairy cattle, pigs, broiler chickens and laying hen are found in Figure S11-S15.

Impacts on agricultural production, land-use dynamics and nitrogen flows: A detailed resume of the model results obtained for all 48 scenarios in terms of land use, nitrogen budget and greenhouse gas emissions is available in the Supplementary Materials excel spreadsheet.

Economic assessment of the technological pathways for production of Microbial Protein

General assumptions

To establish whether the production of MP can become economically competitive to crop-based production of protein, the economic feasibility of five MP production pathways was investigated. General assumptions for the process requirement are reported in Table S4.

Process requirements and costs: capital investments and annuity

The capital investments were calculated based on the annuity method assuming loan repayments on an annual basis. The calculated annual repayment was subsequently normalized to the specific costs per ton MP produced ($/ton MP). The following assumptions were made:

- Capital investment of US$75 million (US$50 – US$125 million);
- Equipment lifetime of 25 years;
- Repayment period of 25 years;
- Interest rate of 5% (3 – 7%);
- MP production of 25,000 ton MP per year (expressed in 100% dry weight).

Table S9 reports the annuity per ton MP produced above described assumptions (i.e. $203/ton MP). Shifting the required capital investment to US$50, US$100 and US$125 million and interest rate between 3 to 7%, would result in a minimum and maximum annuity of $112 to $401/ton MP, respectively.

Process requirements and operational costs: energy and carbon sources

Hydrogen

Table S5 shows the levelized costs per kg hydrogen produced as well as the hydrogen production costs per ton MP produced. An 80% hydrogen conversion into MP was assumed, with the other 20% presumed being lost due to gas transfer limitations. The reminder hydrogen could in theory be converted into heat and power using a Combined Heat Power (CHP) unit. The latter would provide sufficient energy for the drying step, an option that was not considered here however (table S9).

Methane

A low cost estimate (\$0.17 / Nm^3) applicable to the USA market, and a high cost estimate (\$0.33 / Nm^3), applicable to the European market were considered for natural gas requirements. An 80% methane conversion into MP was assumed similarly to hydrogen above and the potential to recover heat and power via a CHP unit was not considered (Table S9).

Raw sugar

Table S5 shows price of raw sugar per ton raw sugar purchased, whereas in Table S10 the costs are expressed as the cost per ton MP produced.

Other process requirements and related operational costs

Nitrogen and phosphate

The amount of nitrogen and phosphate required and their associated operational costs are presented in table S6. The production of MP also requires the use of trace elements and micro-nutrients and pH regulation dosing acid, which were found to be marginal and as such have been implicitly included in the overhead costs (table S9).

Oxygen

Oxygen is required for microbial growth, independently of the carbon and energy sources. Table S7 shows the estimated resources required to generate industry-grade pure oxygen; 80% oxygen utilization efficiency was assumed while the reminder 20% was considered lost due to gas transfer limitations, similar to the hydrogen and methane utilization rates.

Pumping and mixing

Fermentation in stirred tank reactor requires energy for gassing, mixing and pumping. Energy requirements for gas fermentation are higher than for sugar substrate since gaseous substrate necessitates strong mixing to facilitate gas-to-liquid transfer and obtain high volumetric productivity. Pumping energy is comparable across substrates and an average mixing energy between 0.2 – 3 kWh/m^3, with 0.8 kWh/m^3 considered in our assessment. [27] In our assessment, a rather conservative value of 1.5 kWh/m^3 for mixing and pumping was considered as energy demand for all substrates.

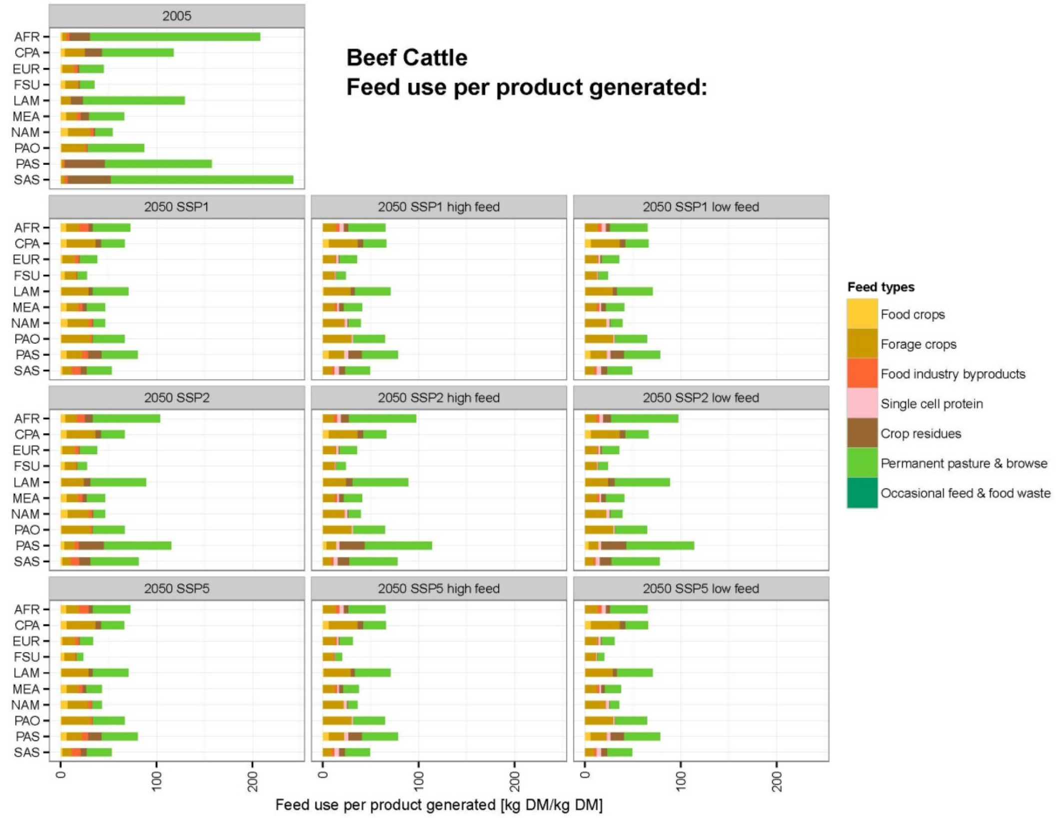
Dewatering, drying and sterilization

After the fermentation step, produced undergoes heat-treatment to lyse cells and increase protein accessibility, reduce nucleic acid content and obtain a dry sterilized product. [28] Prior to heat-treatment, water is removed by centrifugation (or other conventional dewatering methods) leaving around 25% dry solids content to reduce energy consumption for heat-treatment. Drying is achieved via spray-drying with integrated fluidized bed, a common practice in the food processing industry.[29] The operational costs of drying and sterilization are mainly related to the energy consumption of the process (table S8).

Overhead

The overhead cost was assumed to be equal to the annuity of the production of 1 ton of MP, corresponding to $270 per year (i.e. ~15 – 20% of the total cost (except for hydrogen based production through water electrolysis, i.e. 9%, Table S9), a conservative estimate. Overhead costs include labor, maintenance, quality testing, packaging, depreciation and miscellaneous other minor expenses. Note that the labor costs depend on the world's area where MP is produced as well as on single countries governments (e.g. to support a particular industry with taxation, assistance in the recruitment of highly skilled labor, etc.). Assessing the value of these differences was beyond the scope of this paper and therefore a fixed (rather conservative value) was used.

Figures and Tables



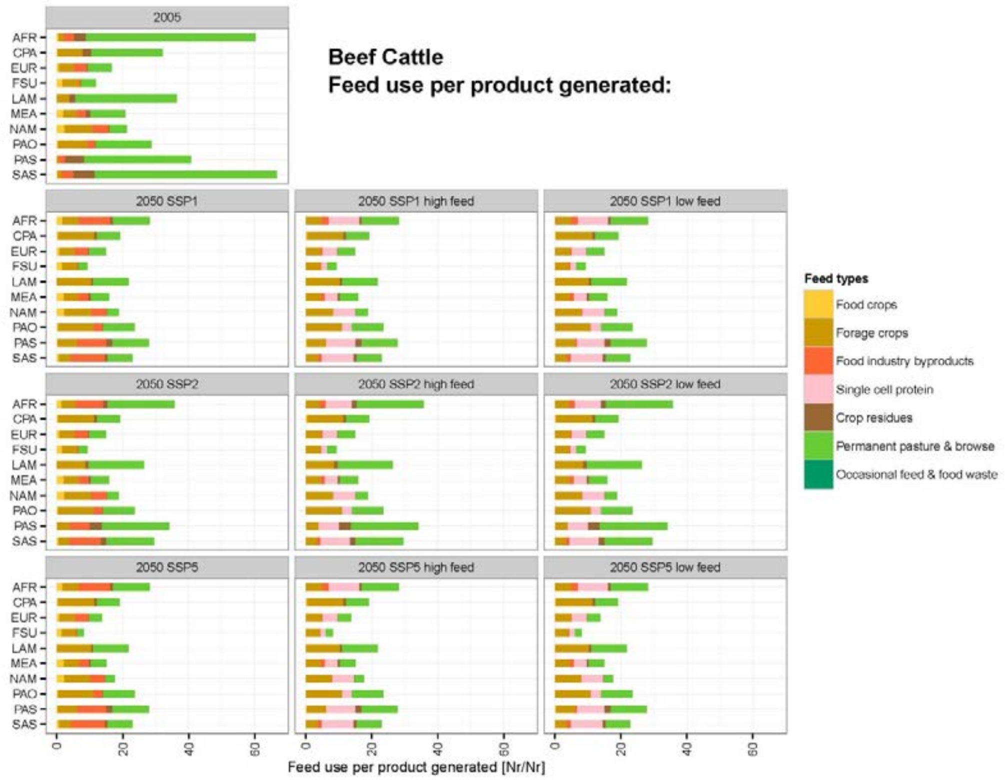
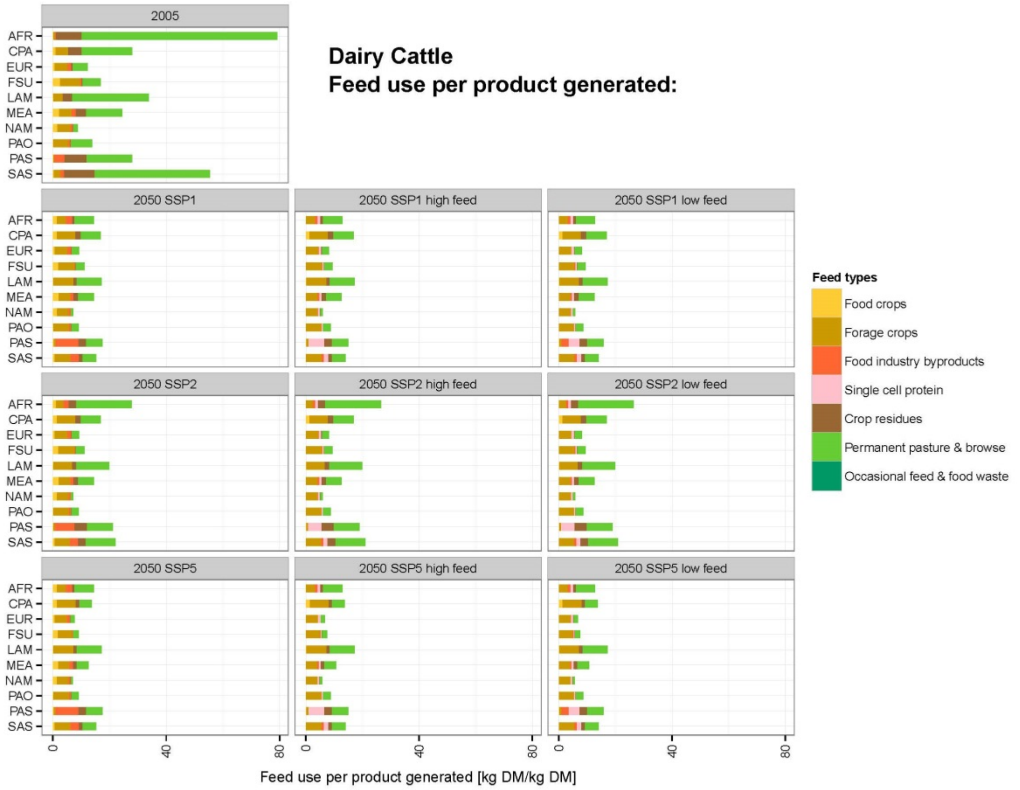


Figure S11. Feed basket for Beef Cattle. Top: Feed use per product generated expressed in kg DM / kgDM feed



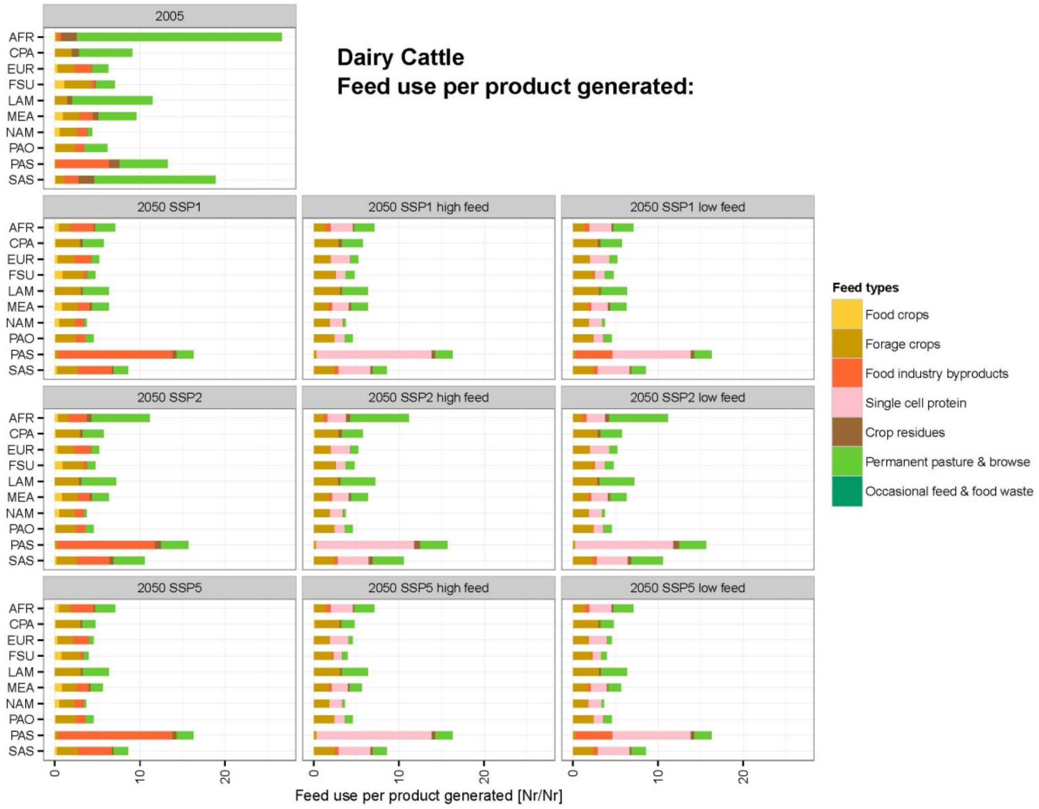
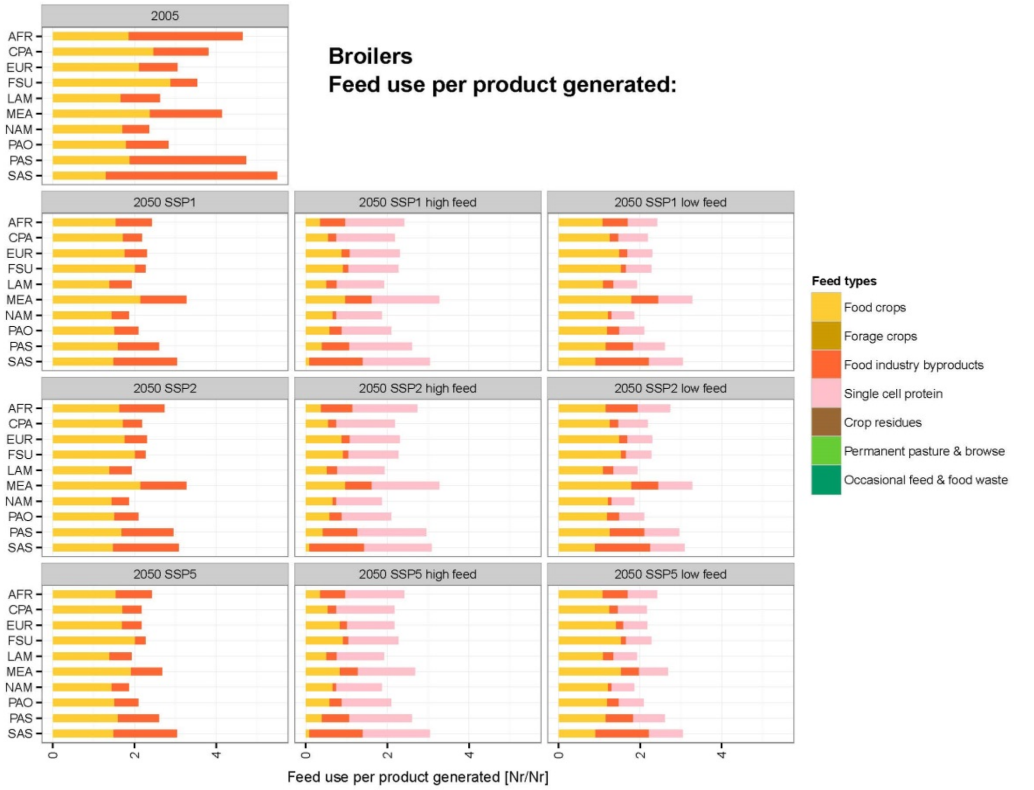


Figure S12. Feed basket for Dairy Cattle. Top: Feed use per product generated expressed in kg DM / kg DM feed



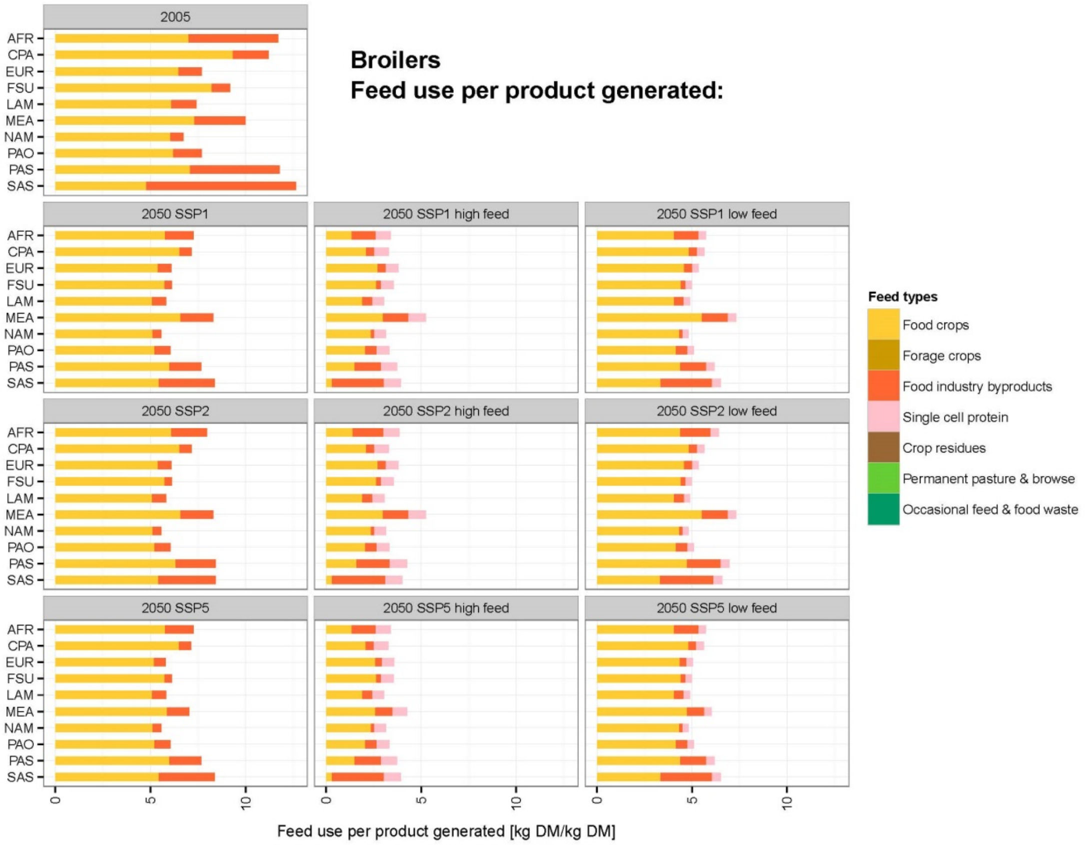
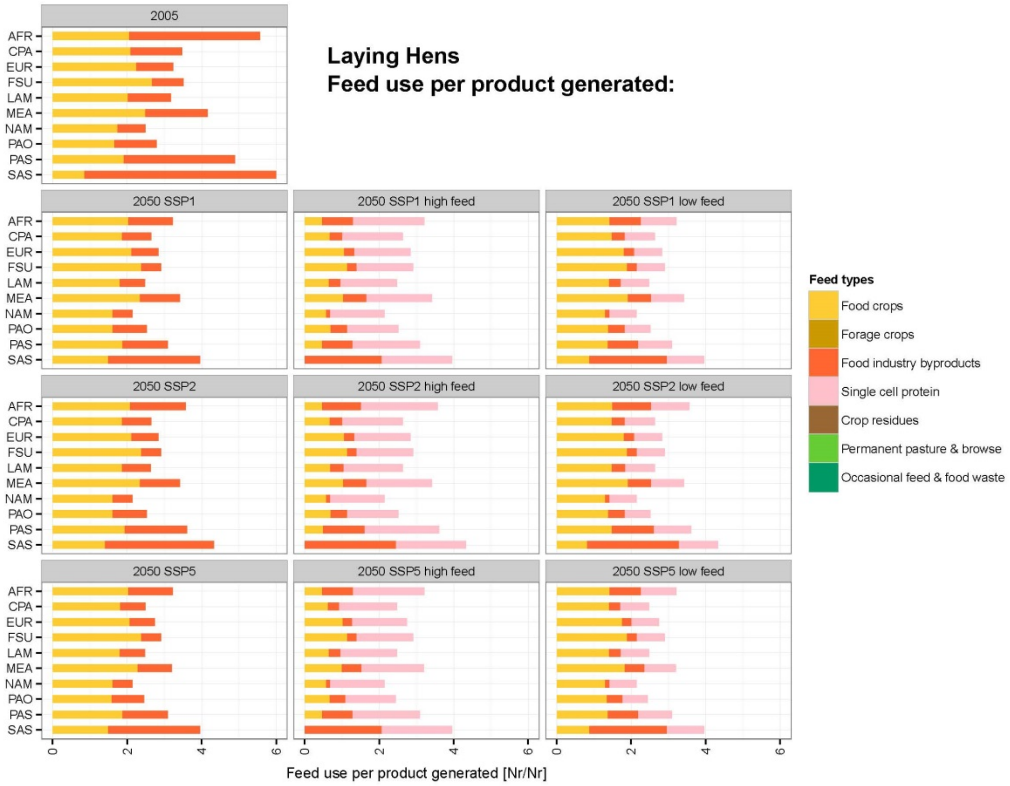


Figure S13. Feed basket for Broilers. Top: Feed use per product generated expressed in kg DM / kg DM feed



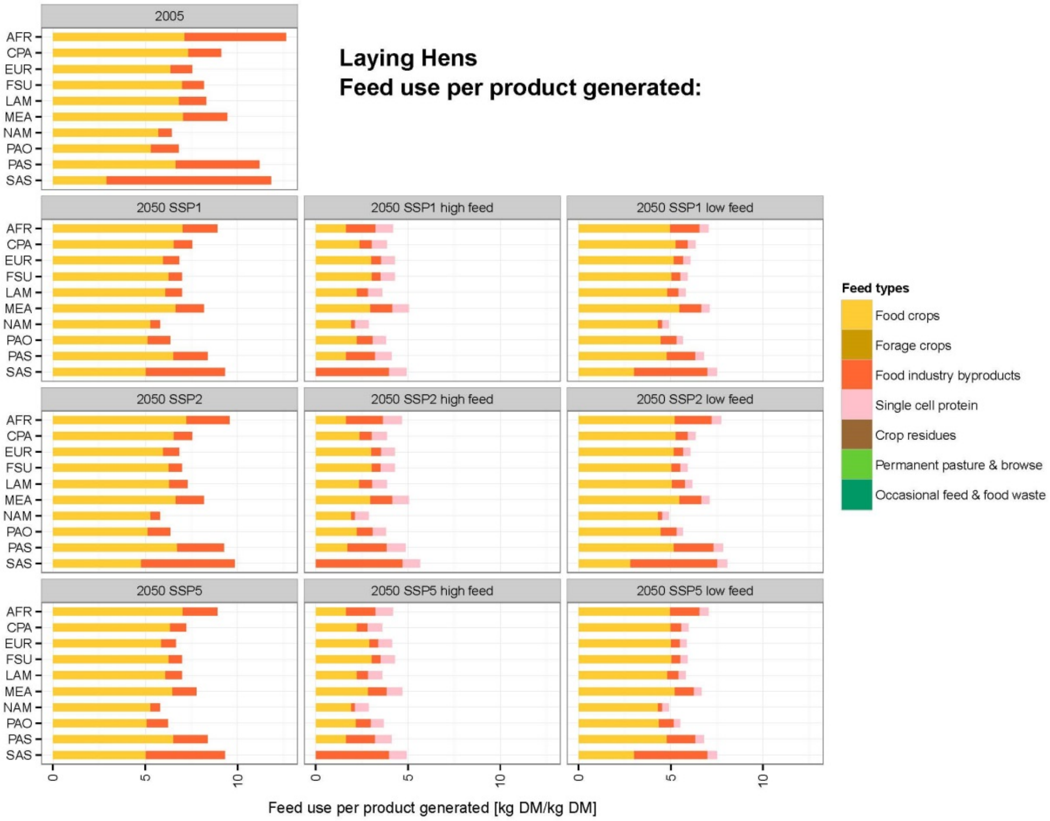
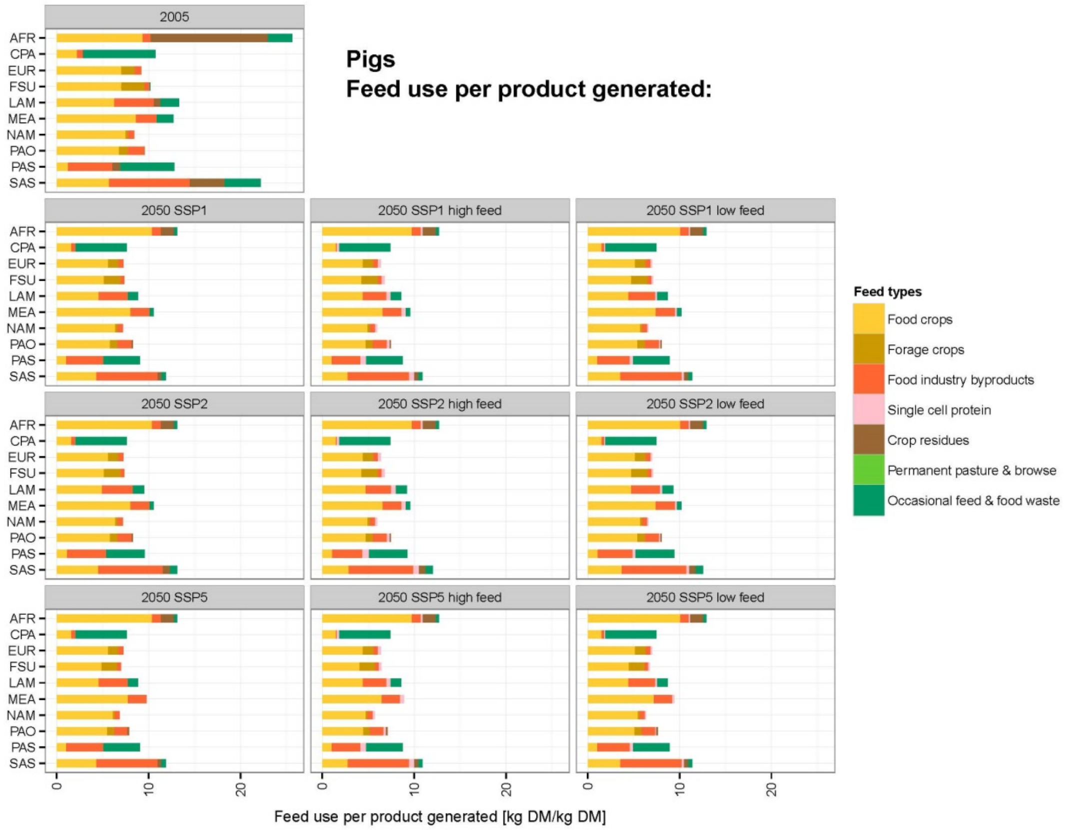


Figure S14. Feed basket for laying hens. Top: Feed use per product generated expressed in kg DM / kg DM feed



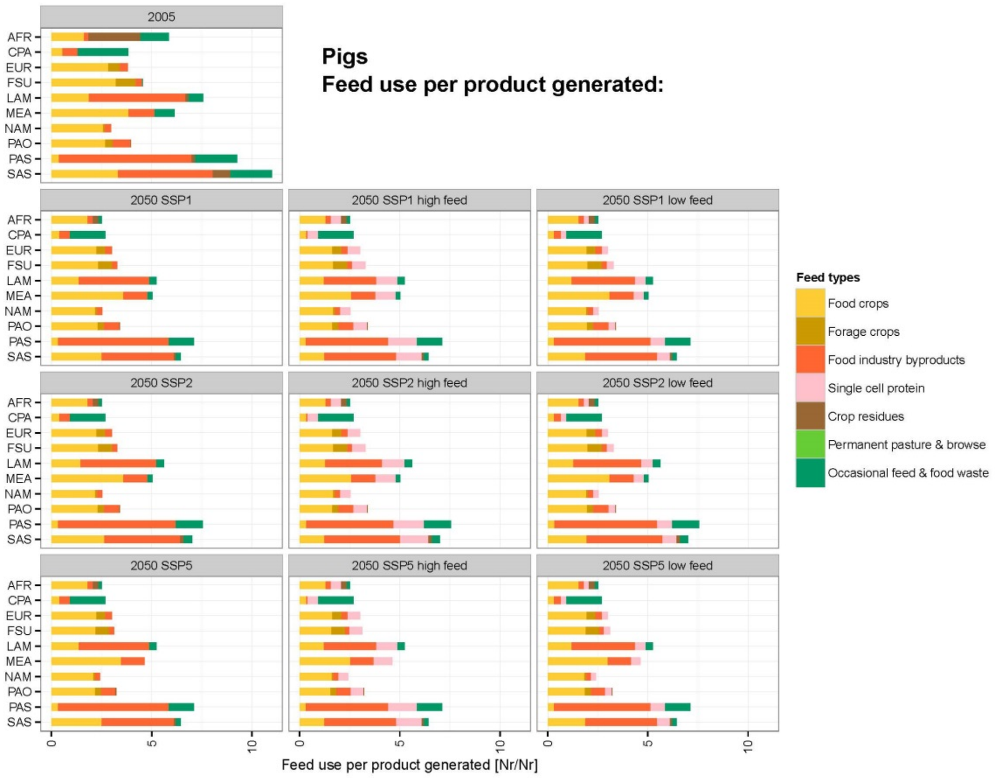


Figure S15. Feed basket for Pigs. Top: Feed use per product generated expressed in kg DM / kgDM feed

Table S1. Feed substitution potential of MP within livestock feed baskets.

Type of livestock	MP replacement in feed baskets	refs	Remarks
	21% (dry matter basis)	30	In this study, feeding trials using 24 pigs (with a mean body weight 44 kg) were used to determine the digestibility of energy, nitrogen and amino acids. MP was added to the basal diet (21% dry matter basis) as such that the overall crude protein content of the feed basket remained constant at 18±0.5% crude protein on a DM basis. The inclusion of MP was compared with the addition of fish meal added to the basal diet at 24% (dry matter basis). Pigs fed with MP were found to have higher net protein utilization rates and apparent and true digestibility of amino acids than the pigs fed with fish meal.
	10% – 20% (dry matter basis)	31	In this study, the impact of 10% and 20% inclusion of MP in diets offered to 27 piglets from 21 – 42 days of age and compared with diets with fish meal. The diets were characterized by a slight increase in overall crude protein content of the feed baskets from 21±1% to 27±1% crude protein content on a dry matter basis when increasing the MP replacement share from 10 to 20%, respectively. No difference in growth rate was observed with the piglets converting equally efficient as piglets offered diets containing fish meal.
	5%, 10.5%, 16% (dry matter basis)	32	In this study, soy bean meal was replaced in basal diet (which comprised of soy, wheat and barley) by MP (methane based production of bacterial protein) at three replacement rates (i.e. 5%, 10.5% and 16% of DM basis) as such that the overall crude protein content of the feed basket remained constant at 22±0.5% crude protein (DM basis). No increase in plasma concentrations was observed, indication the absence of any uricogenic effect.
	4%, 8%, 12% (dry matter basis)	33	In this study, the effects of replacing fish meal, soy bean meal, and meat and bone meal in conventional diets with MP (methane based production of bacterial protein) at a replacement rate of 4%, 8% and 12% on the growth of weanling pigs examined. Similar growth performance to that obtained with a conventional diet was observed. Note that that the overall crude protein content of the feed basket within the different feed baskets remained constant at 23±0.5% crude protein on a DM basis.
	5%, 10%, 15% (dry matter basis)	34	Feeding trials were conducted to investigate the effects of increasing the dietary content of MP (methane based production of bacterial protein) on the protein and energy metabolism of pigs from weaning to a weight of 80 kg. Soya-bean meal was replaced with MP at a replacement rate ranging from 0 – 15% (DM basis) as such that the overall crude protein content of the feed basket remained constant at 21.8±0.7% crude protein content on a DM basis. Both the overall protein and energy metabolism in growing pigs were not affected in all MP replacement rates used.
Pigs	17%, 20%, 35%, 40%, 52%, 60% (digestible N basis)	35	In this study, feeding trials were conducted in which experimental diets contained increasing levels of MP (methane based production of bacterial protein) as replacement of soybean meal as such that the

			overall crude protein content of the feed basket remained constant at 38.3±0.5% crude protein content on a DM basis. It was found that MP was a suitable protein source without any significant differences compared to the soybean meal diet.
	6%, 12%, 15% (dry matter basis)	[36]	In this study, it was found that replacement of soy beans by MP at replacement rates up to 12% (dry weight basis) in diets for pigs from 26 kg live weight until slaughter had no adverse effect on their overall growth performance as well as carcass lean or fat content. However, MP levels of up to 15% reduced growth rates during the piglet period and increased carcass fat content. The latter was found to be caused by marginal dietary lysine levels. Overall, it was concluded that the addition of MP achieved a dose dependent improvement in the utilization of total amino acids and lysine and the quality of back fat determined as fat firmness and fat color. Note that that the overall crude protein content of the feed basket within the different feed baskets remained constant at 20±0.7% crude protein on a DM basis.
	5%, 10%, 15% (dry matter basis)	[37]	In this study, MP replacement rates and dietary compositions were used according to. [34, 36] It was found that the inclusion up to 15% MP on a dry matter basis did not affect the metabolic function, as reflected in the measured blood parameters.
	5%, 10%, 15% (dry matter basis)	[38]	In this study, feeding trials were conducted in which experimental diets contained increasing levels of MP (methane based production of bacterial protein) as replacement of soybean meal. The inclusion of MP was balanced by a reduction in amount of soybean in the diets in order to maintain constant crude protein levels within the different diets. Pigs fed diets containing MP had reduced thiobarbituric acid reactive substances (TBARS) value in backfat and muscle, reduced intensity of odor and rancid odor and taste in pork after short-time storage, and reduced off-odor and off-taste after intermediate-time storage. To conclude, adding MP to diets for pigs changed the fatty acid profile, improved the oxidative stability, and sensory quality of pork.
	23% (dry matter basis)	[39]	Feeding trials were conducted to determine the digestibility of MP grown on methane. MP was added at a replacement rate of 23% to the basal diet. Total tract apparent digestibility and the ileal nitrogen digestibility were found to be 85.4% and 78.1%, respectively.
In the MAgPIE model simulations, we used 4%, 8% and 15% of MP replacements shares to the feed baskets for pigs as low, default and ambitious feeding ratio, respectively. Replacement shares are indicated on dry matter basis of the total feed basket.			
Beef and dairy cattle	0, 10, 20% (dry matter basis)	[40]	During feeding trials over a period of 32 weeks it was found that replacing groundnut oil meal with up to 20% MP as protein source in the feed baskets had no detrimental effects on performance in calves.
	0%, 5%, 7.5% (dry matter basis)	[41]	Feeding trials using veal calves were conducted using MP at replacement rates of 2.5, 5 and 7.5% as a replacement for milk protein as such that the overall crude protein content of the feed basket remained constant at 25.5±0.7% crude protein content on a DM basis. Similar growth rates during the course of the fattening period (i.e.

			increase in weight of the veal calves from 60 – 150 kg) at all MP replacement shares.
	0, 5, 10, 15% (dry matter basis)	[42]	Feeding trials were carried out to determine the impact of yeast, grown on methanol, as a protein source using 4 – 5 months old fattening lambs. Similar performance was observed amongst the range of yeast inclusion tested.
	0%, 10.2% and 22.9% (dry matter basis)	[43]	In this study, the impact of inclusion of yeast derived MP from sugar cane, as a replacement of soy bean, on dry matter intake and digestibility, milk production and quality was examined in dairy goats. During the 90 day feeding trial, it was found that MP can be used as a protein supplement without any differences in milk production and quality observed. The different diets were characterized by a constant crude protein content of 23.8±0.9% on a dry matter basis.
	Not reported	[44]	The replacement of groundnut-cake protein with MP significantly ($P<0.05$) increased the milk yield in lactating dairy goats without observing any changes in the milk composition.
	8% (dry matter basis)	[45]	In 1995 the European Union approved the use of bacterial protein grown on natural gas as protein source in veal calves up to a replacement share of 8% (Council Directive No. 82/471/EEC).
In the MAgPIE model simulations, we used 4%, 8% and 15% of MP replacements shares to the feed baskets for beef cattle and dairy cattle as low, default and ambitious feeding ratio, respectively. Replacement shares are indicated on dry matter basis of the total feed basket.			
Broiler chicken and laying hens	2%, 4%, 6%, 8%, 10% (dry matter basis)	[46]	Overall, substitution of soybean meal protein with increasing levels of MP significantly lowered feed-to-gain ratio during the last part of the feeding period. Sensory analysis of thigh meat after 2 month of frozen storage showed that meat from 35-d-old chickens fed with inclusion of 6% and 10% MP in their diets had less odor intensity and less rancid flavor than meat from the control group. Other sensory attributes were not affected by treatment. Note that that the overall crude protein content of the different diets remained constant at 22.7±0.4% crude protein on a DM basis.
	6% (dry matter basis)	[47]	It was concluded that 6% of MP (methane based production of bacterial protein) can replace soybean meal in diets for broiler chickens without impairing growth performance. The overall crude protein content of the different diets remained constant at 26.3±1% crude protein on a DM basis.
	2%, 4%, 6% (dry matter basis)	[48]	The effects of replacing soybean meal or fish meal with 2, 4 or 6% MP on growth performance, digestibility of amino acids and sensory quality of meat, were examined using 630 broiler chickens. It was concluded that MP can replace soybean meal or fish meal protein in broiler chicken diets within the inclusion rates tested (i.e. up to 6%). Diets were characterized by a constant crude protein content of 23±1% on a dry matter basis.
	9.6%, 10%, 19.2%, 20%, 29% (dry matter basis)	[49]	It was found that the inclusion of 10% – 20% of MP as a replacement of soybean meal to 5 day-old male broiler chicks reduced growth rate and efficiency of food conversion over a 14 days period. In the same study, it was found that MP fed to chicks (with an age of 21 days) at a

			rate of 9.6% marginally improved growth rates, efficiencies of food utilization and nitrogen retention. Further increasing the MP inclusion rate up to 19.2 and 29% caused adverse effects. The different diets tested were characterized by constant crude protein contents on a dry matter basis.
	2%, 4%, 6% (dry matter basis)	50	In this study, the effect of the inclusion of MP to basal diet with increasing concentrations (0%, 4% and 6 %) on the energy metabolism and carcass composition was investigated. It was concluded that the overall protein and energy metabolism as well as carcass composition were not influenced by a dietary content of up to 6% MP. The latter corresponds to 20% of dietary nitrogen. The different diets tested were characterized by a constant crude protein content of 24.4±1% on a dry matter basis.
	4%, 8%, 12% (dry matter basis)	51	In this study, the effects of the inclusion of MP (methane based production of bacterial protein) to basal diet as a replacement of soybean meal with increasing concentrations (4%, 8% and 12%) on growth performance and carcass quality in broiler chickens were examined. reduced feed intake and improved gain, but did not affect weight gain compared to the soybean meal based control diet. The different diets were characterized by a constant crude protein content of 25.5±0.5% on a dry matter basis.
	4%, 8%, 12% (dry matter basis)	52	In this study, the effects of the inclusion of MP to basal diet with increasing concentrations (4%, 8% and 12% on fatty acid composition, the profile of volatiles by dynamic headspace gas chromatography-mass spectrometry, and sensory quality of frozen-stored broiler chicken thigh meat was examined. In the basal diet, containing e.g. wheat and maize), soy bean meal was used as protein source. Replacing soy bean by MP resulted in a reduction in lipid oxidation products in frozen-stored meat. The latter is important, as it is associated with quality deterioration and reduced consumer acceptance. The different diets tested were characterized by a constant crude protein content of 26.9±0.8% (DM basis).
	8% (dry matter basis)	53	In this study, the effects of bacterial protein meal at an 8% replacement rate (as a replacement of soybean meal) to broiler chickens (1–35 days of age) on the fatty acid composition, lipid oxidation and sensory quality on frozen thigh meat stored frozen for 6 month was examined. It was found that the inclusion of MP did not affect the sensory quality parameters, but had a positive effect in terms of reduced volatiles in frozen-stored broiler meat. The latter was hypothesized to be related to antioxidant properties of the bacterial autolysate. The different diets tested were characterized by a constant crude protein content of 26.5±0.85% (DM basis).
In the MAgPIE model simulations, we used 3%, 6% and 12% of MP replacements shares to the feed baskets for broiler chicken and laying hen as low, default and ambitious feeding ratio, respectively. Replacement shares are indicated on dry matter basis of the total feed basket.			

Table S2. Environmental costs of reactive nitrogen losses to the biosphere.

Region	Annual costs	Costs (per kg N)	ref	Remarks
China	US$9.5−31 billion	$2.1-3.8 kg NO_x-N $0.4-3.3 kg NH_3-N $0.3 kg N_2O-N	[54]	This study estimates that agriculture accounted for 95% of the NH_3 and 51% of the N_2O in china in the year 2008. In the same study it was also estimated that the total atmospheric emissions of reactive nitrogen causing related health damage ranged US$19−62 billion per year. Of this number, agricultural induced emissions accounted for more than 50% of the costs.
EU	€35−230 billion	€10-30kg NO_x-N €2-20kg NH_3-N €5-15kg N_2O-N	[55]	This study revealed that the costs of agricultural induced reactive nitrogen losses exceed the economic benefit due to increase primary crop production by a factor 4. Overall, the annual costs associated with agricultural reactive nitrogen losses was estimated to range between €35−230 billion per year.
USA	US$81–$441 billion	No information given on costs per kg N	[56]	This study is the first assessing the cost associated with reactive nitrogen losses to the biosphere from human activities in the United States. The study revealed that the total potential environmental and health economic impact of reactive nitrogen losses from anthropogenic nitrogen summed up to an average of US$210 ($81–$441) billion per year in the beginning of the 21th century. Of this, ~75% of the estimated costs were associated with agricultural induces losses.
World	US$200-2000 billion	No information given on costs per kg N	[57]	In this report, conducted by the European Nitrogen Assessment, a costing procedure based on the European situation was implemented aiming at calculating the global cost of nitrogen pollution. Taking into account that the global costs would be approximately a factor threefold of the European situation, resulting in an overall estimated costs associated with reactive nitrogen losses ranging between 200 to 2000 billion US dollars annually.
USA		$900 ($100 – $59,400) ton NH_3 250 ($20-$1780) ton NO_x	[58]	In this study, the Air Pollution Emission Experiments and Policy (APEEP) model (an integrated assessment model) was used to determine the economic impact of air pollution by means of air quality modeling, exposure, dose-response and valuation for a large range of point sources, based on data of more than 10,000 sources measured by the US EPA.

USA		\$3.03 (\$1.25 – \$4.80) kg NH_3 14.6 (\$2.0-\$27.27) kg NO_x	[59]	This study aimed to determine the environmental and health externalities associated with the production of different agricultural crops such as corn and switch grass for the production of ethanol. While the purposed and crops used are different, the externalities are directly assessed based on the emissions of NH_3 and NO_x.
USA and EU		€3.1 – €30 kg NH_3-N (to air) €13 – €43 kg NO_x-N (to air) €5 – €54 kg Nr (to water) €2-18kg N_2O-N (to air)	[60]	In this study, the findings of several previous studies [61-63] on the externalities of reactive nitrogen emissions in terms of health, ecosystems/coastal systems, crop decline and climate change were summarized.

Table S3. Overview of the substrate requirements of the different technological pathways for production of Microbial Protein.

MP Substrate		**ton substrate / ton MP-N**	**ton substrate / ton MP (@ 70% protein)**	**Microbial route**
High C/N crops	Maize (dry weight)	49.6	5.6	Methane oxidation (via biogas)
	Miscanthus (dry weight)	50.2	5.5	Hydrogen oxidation (via biogas)
	Sugar cane (dry weight)	34.9	4.3	Organic carbon oxidation (fermentation)
Natural gas	CH_4 gas	9.2	1.01	Methane oxidation (direct assimilation)
Hydrogen gas	H_2 gas	4.0	0.46	Hydrogen oxidation (direct assimilation)

Table S4. General assumptions for the economic assessment of the different technological pathways for MP production

Parameter	Value	Unit	Refs.	Remarks
Electricity price	0.10	$/kWh		Price may have regional fluctuations and may be as low as $0.05/kWh. Note that levelized costs for wind energy are already as low as $0.06/kWh [64].
Natural gas price	0.17-0.33	$\$/Nm^3$	[65]	The prices corresponding to 5 and 10 $/mmbtu, respectively. 10 $/mmbtu is the current and predicted future price in Europe, whereas the current price and forecasted price in the US are lower than 5 $/mmbtu.
Sugar price	250	$/ton	[66]	2005 prices. The same report, prepared by the US department of Agriculture, it is forecasted that raw sugar price will increase to around $440 by 2025.
Soy price	400-600	$/ton	[67]	Note that soy has a protein content of around 32 – 40%, compared to 70-75% for MP. Prices substantially differ between regions.
Fishmeal price	1750	$/ton	[68]	The price steadily rising since 2000 and expected to double by 2030.
Market value MP	1750	$/ton		The market value of MP is estimated to be the same as fishmeal as it has a similar protein and amino acid composition, however the protein MP protein content is higher than fishmeal (up to 75% versus 65% for MP and fishmeal respectively).

Table S5. Levelized costs for hydrogen production including CAPEX and OPEX and subsequent impact on the operational expenditure.

Hydrogen production route	**Costs ($/kg H_2)**	**Costs ($/ton MP)[d)]**	**Costs ($/ton MP-protein)[d,e)]**	**Remarks**
Biomass gasification	$1.61	902	$1288	The cost for biomass gasification are based on study of the US department of Energy. [69] The same study reports on future prediction of a further reduction in costs to $1.47/kg H_2 using the H2A production model. [70] This model includes capital, operating, maintenance, feedstock, utility, transport and replacement costs.
Water electrolysis (current technology)	$5.00	2800	$4000[c)]	The costs for hydrogen production by means of PEM electrolysis are based on an electricity price of $0.05/kWh. [71]
Water electrolysis (future predictions)	$3.00	1680	2100	Future predicted levelized costs for hydrogen production using PEM electrolysis are predicted at $3/kg H_2 produced. [71] Note that this also includes compression, storage and dispensing, which are not needed for the production of MP.
Water electrolysis (off-peak electricity)	$0.70	392	$560 [c)]	The predicted future levelized cost are $3/kg H_2 produced. [71] Electricity price of $0.05/kWh or $2.3/kg, corresponding to $0.7/kg H_2, including compression, storage and dispensing.

a) Costs for delivery, compression, storage, and dispensing not required producing MP.
b) Calculated costs per ton MP based on stoichiometric requirements of 4 kg H_2/kg N and conversion of N to protein of 6.25. [72]
c) Additional economic benefits may derive from using CO_2 from industrial point source (e.g. reduction in carbon tax).
d) We assumed 80% hydrogen uptake efficiencies for MP production.
e) Final cost expressed per ton of protein (@70% protein content for the final MP product).

393 **Table S6.** Nitrogen and phosphorus requirements for MP production and associated OPEX.

Parameter	**Value**	**Unit**	**Remarks**
Haber-Bosch nitrogen requirements	0.112	ton NH_3-N/ton MP	Based on a conversion of nitrogen-N to protein of 6.25 [72, 73], at an average protein content of MP of 70% on a dry weight basis.
Price of Haber-Bosch nitrogen	800	$/ton N	Food-grade ammonia nitrogen is used.
Costs NH_3-N per ton MP produced	89.6	$/ton MP	N/A
Phosphate required	32	kg PO_4/ton MP	N/A
Price phosphoric acid	1000	$/ton	Food grade phosphoric acid used.
Costs phosphate per ton MP produced	32	$/ton MP	N/A
Subtotal N and P costs	121.6	$/ton MP	N/A

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Table S7. Oxygen requirements and generation of industrial grade oxygen for MP production and associated OPEX

Production platform	Oxygen Required		Oxygen Cost		Remarks
	Value	Unit	Value	Unit	
Hydrogen	2.05	kg O_2/kg MP	90	$/ton MP	Based on equation 1, Table S10.
Methane	2.50	kg O_2/kg MP	109	$/ton MP	Based on equation 2, Table S10.
Heterotrophic	0.85	kg O_2/kg MP	37	$/ton MP	Based on equation 3, Table S10.
Industry grade oxygen generation	0.05 (0.04-0.06) 35 (28-42)	\$/m^3 O_2 $/ton O_2	N/A	N/A	Based on [74] and electricity price of $0.10/kWh
Oxygen utilization efficiency	80	%	N/A	N/A	N/A

Table S8. Energy requirements dewatering, sterilization and drying and associated OPEX.

Operation	Parameter	Value	Unit
Centrifugation	Energy requirement for centrifugation	700	kWh/ton MP
	Electricity price	0.10	$/kWh
	Dry solids content MP after centrifuge	25	wt %
	Energy costs per ton dry MP	**70**	**$/ton MP**
Spray-drying	Energy requirements spray-drying [a)]	3500[29]	MJ/ton H_2O
	Energy content natural gas	38.7	MJ/m^3
	Natural gas required	90	m^3/ton H_2O
	Price natural gas	0.33 (0.17-0.33) [65]	$\$/m^3$
	Dry solids content final product	100%	wt%
	Energy requirements per ton dry MP	8167	MJ/ton MP
		211	m^3 CH_4/ton MP
	Energy costs	**$90**	**$/ton MP**

a) Referred to spray drying integrated with a fluidized bed, a common method in the food industry. [29]

Table S9. Summary of operational expenditure (OPEX) for the MP production pathways. Table reports the production strategies (*Production technology*), direct expenses for production (Production *cost* also referred to as OPEX) and the total costs (*Total costs*) for producing MP from 3 different technologies (*production pathways*) using the reported carbon and energy sources (*Production pathway* and *type of substrate*); the cost section reports expenses for substrate (*Substrate*) and other demands such as secondary substrates (*Nitrogen & Phosphate*), gassing (O_2 and CO_2), running costs for fermenting (*Mixing & pumping*) and final processing (*Dewatering* and *Drying/sterilization*). Nitrogen and Phosphate and overhead costs were assumed to be the same for all conditions.

Production technology		**Production costs, OPEX**									**Total costs**	
Production pathway	**Type of Substrate**	**Annuity of capital expenditure**	**Substrate**	**O_2**	**Nitrogen & Phosphate**	**CO_2**	**Mixing & pumping**	**Dewatering**	**Drying / sterilization**	**Overhead**	**(70% protein) [$/ton MP]**	**(100% protein) [$/ton MP]**
High C/N crops	Methane (after energy maize anaerobic digestion)	$203	$831	$109	$149	N/A	$38	$70	$90	$203	$1,692	$2,417
	Hydrogen (cellulose gasification - current price scenario)		$886	$90		N/A	$38	$70	$90		$1,727	$2,466
	Hydrogen (biomass gasification - target price scenario)		$666	$90		N/A	$38	$70	$90		$1,507	$2,153
	Sugar cane (dry weight)		$504	$37		N/A	$38	$70	$90		$1,293	$1,848
Natural gas	CH_4 gas (low price scenario)		$300	$109		N/A	$38	$70	$90		$ 1,162	$1,660
	CH_4 gas (high price scenario)		$583	$109		N/A	$38	$70	$90		$1,444	$2,064
Hydrogen	H_2 gas (water electrolysis - standard price)		$1650	$90		$117	$38	$70	$90		$2,725	$3,894
	H_2 gas (water electrolysis - off peak energy)		$385	$90		$117	$38	$70	$90		$1,460	$2,086

Table S10. Stoichiometry of MP production normalized per mole of biomass. Same biomass composition and nitrogen assimilation efficiency were assumed for MP produced from hydrogen or methane oxidation.

Feedstock	Reaction stoichiometric	Anabolic products (mol)	Catabolic products (mol)	References
Hydrogen oxidation	$21.36H_2 + 6.21O_2 + 4.09CO_2 + 0.76NH_3$	$C_{4.09}H_{7.13}O_{1.89}N_{0.76}$	$18.70\ H_2O$	[75]
Methane oxidation	$7.59\ O_2\ + 6.13\ CH_4\ + 0.76\ NH_3$	$C_{4.09}H_{7.13}O_{1.89}N_{0.76}$ [a]	$2.69\ g\ CO_2$	[76]
Organic carbon oxidation	$0.67\ C_{12}H_{22}O_{11} + 3.00\ O_2 + 1.00\ NH_3$	$C_5H_7O_2N$	$3.00\ CO_2 + 5.33\ H_2O$	[77]

[a)] Assuming same biomass composition for autotrophic hydrogen MP production; an equivalent amount of nitrogen was included in the stoichiometry for biomass formation from methane.

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