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Supplement of

Long-term nutrient fertilization and the carbon balance of permanent grassland: any evidence for sustainable intensification?

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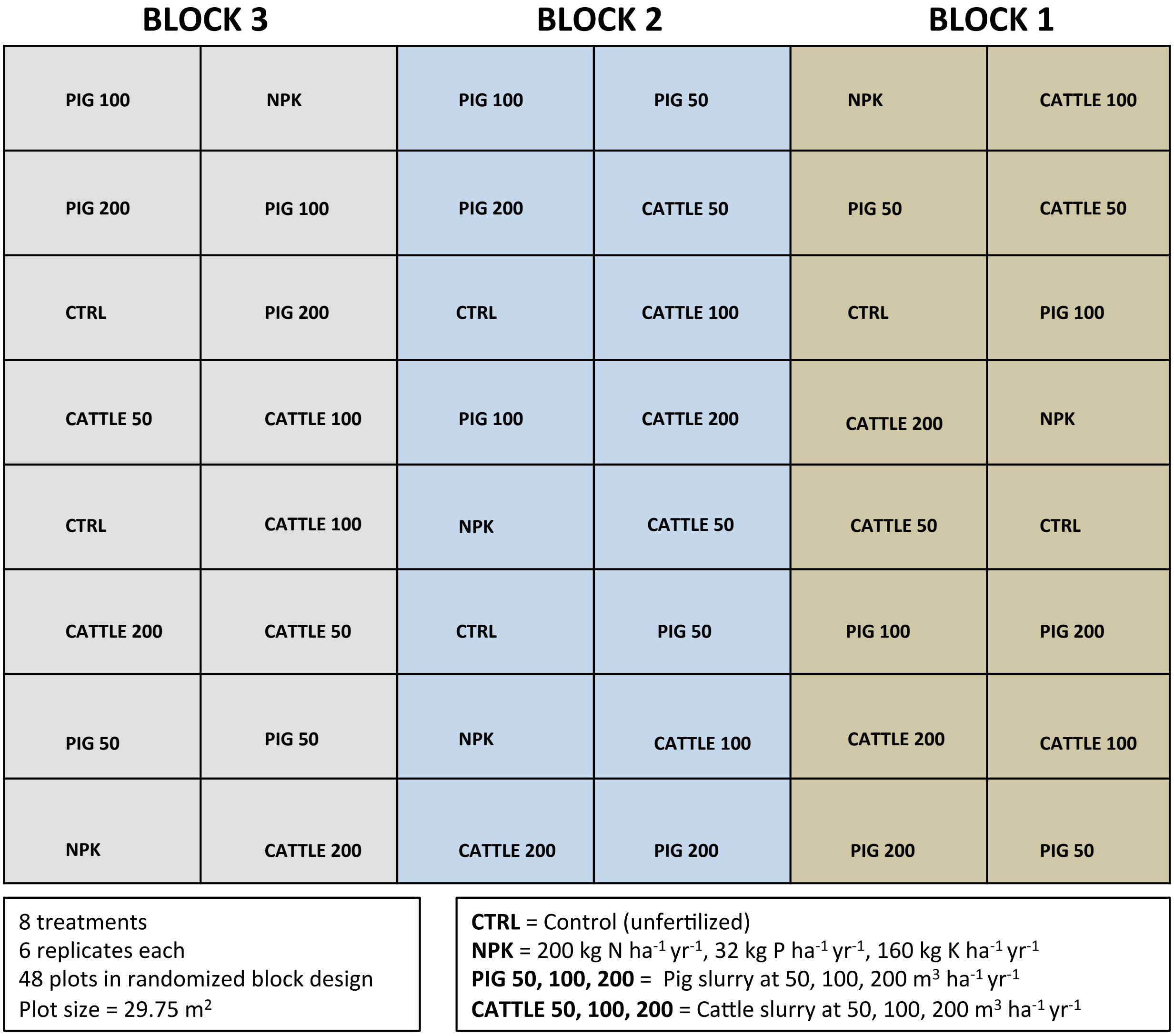
Supplementary Material

Fig. 1. Design of the Long-Term Slurry (LTS) experiment established at Hillsborough (UK) in 1970. There are six replicates for each of eight different nutrient applications giving 48 experimental plots arranged in a randomized block design.



BLOCK 3		BLOCK 2		BLOCK 1	
PIG 100	NPK	PIG 100	PIG 50	NPK	CATTLE 100
PIG 200	PIG 100	PIG 200	CATTLE 50	PIG 50	CATTLE 50
CTRL	PIG 200	CTRL	CATTLE 100	CTRL	PIG 100
CATTLE 50	CATTLE 100	PIG 100	CATTLE 200	CATTLE 200	NPK
CTRL	CATTLE 100	NPK	CATTLE 50	CATTLE 50	CTRL
CATTLE 200	CATTLE 50	CTRL	PIG 50	PIG 100	PIG 200
PIG 50	PIG 50	NPK	CATTLE 100	CATTLE 200	CATTLE 100
NPK	CATTLE 200	CATTLE 200	PIG 200	PIG 200	PIG 50

8 treatments
6 replicates each
48 plots in randomized block design
Plot size = 29.75 m^2

CTRL = Control (unfertilized)
NPK = 200 kg N ha^{-1} yr^{-1}, 32 kg P ha^{-1} yr^{-1}, 160 kg K ha^{-1} yr^{-1}
PIG 50, 100, 200 = Pig slurry at 50, 100, 200 m^3 ha^{-1} yr^{-1}
CATTLE 50, 100, 200 = Cattle slurry at 50, 100, 200 m^3 ha^{-1} yr^{-1}

Fig. 2. Changes in soil N stocks from 1970 to 2013 (0-15 cm soil depth) under either organic or inorganic nutrient additions or no-nutrients (i.e. control). Nutrient application rates: NPK = 200 kg N, 32 kg P, 160 kg K ha^{-1} yr^{-1}; Pig (L), Pig (M) and Pig (H) = Pig slurry applications at 50 (Low), 100 (Medium) and 200 (High) m^{3} ha^{-1} yr^{-1}, respectively; Cattle (L), Cattle (M), Cattle (H) = Cattle slurry applications at 50 (Low), 100 (Medium) and 200 (High) m^{3} ha^{-1} yr^{-1} respectively. Best fit lines (dashed lines): Cattle (H) ($y = 0.084x - 161$; $R^2 = 0.98$); Cattle (M) ($y = 0.053x - 100$; $R^2 = 0.87$); Cattle (L) ($y = 0.032x - 58.418$; $R^2 = 0.88$); Pig (H) ($y = 70.1*\ln(x) - 526$; $R^2 = 0.66$); Pig (M) ($y = 0.023x - 39.5$; $R^2 = 0.57$); Pig (L) ($y = 0.025x - 44.23$; $R^2 = 0.81$); NPK ($y = 0.022x - 37.6$; $R^2 = 0.74$); Control ($y = 0.014x - 21.8$; $R^2 = 0.60$).

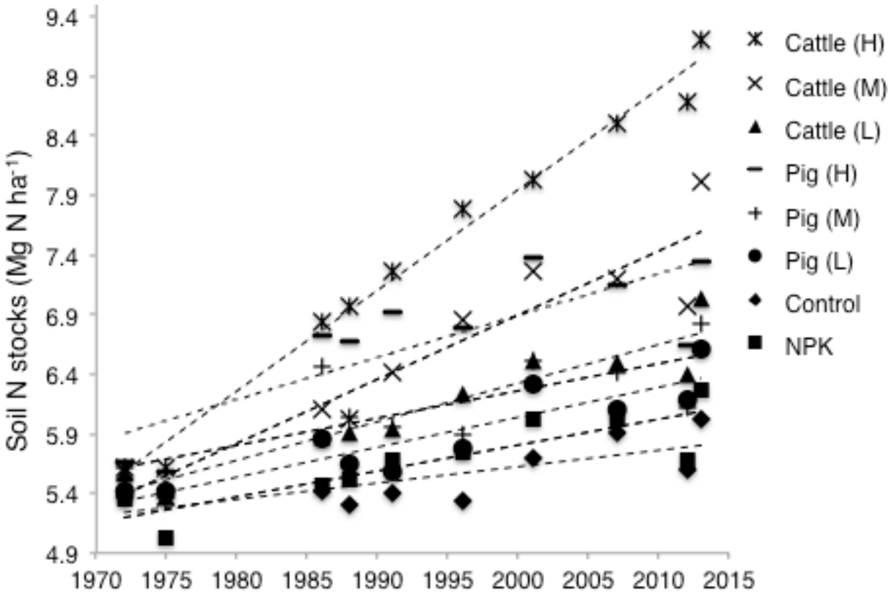


Fig. 3. Relationship between net soil N sequestration rates (Mg N ha^{-1} yr^{-1}; 0-15 cm depth) and nutrient application treatments. Nutrient application rates as for Supplementary Fig. 2. Standard errors indicate variation among six replicate values for each treatment.

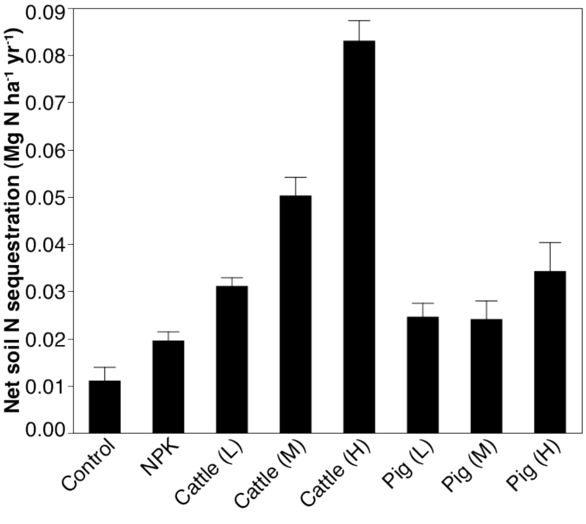


Fig. 4. Relationship between mean soil N (%) content at three soil depths (A=0-5 cm; B=5-10 cm; C=10-15 cm) and different nutrient treatments. Nutrient application rates as for Supplementary Fig. 2. Standard errors indicate variation among six replicate values for each treatment.

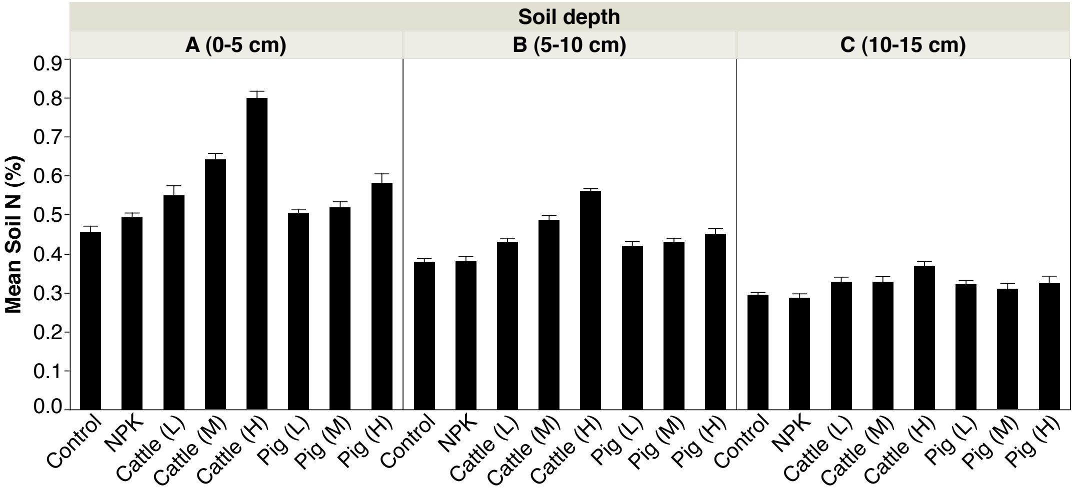
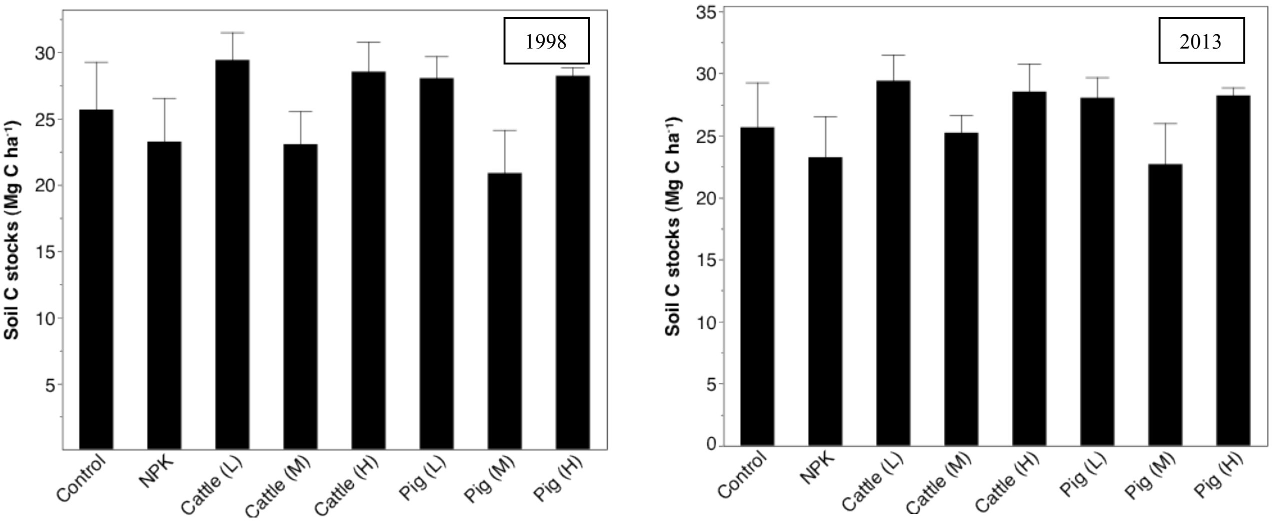


Fig. 5. Relationship between soil C stocks (Mg C ha^{-1}) in the 15-30 cm depth layer and nutrient application treatments. Soil stocks between 15-30 cm depth were measured in 1998 and 2013. Note that in 1998 the control treatment plots were not sampled. Nutrient application rates as for Supplementary Fig. 2. Standard errors indicate variation among six replicate values for each treatment.

a) **b)**



Global Warming Potential (GWP) calculations

To estimate the long-term net C balance of our permanent grassland we calculated the Global Warming Potential (GWP) associated with each experimental nutrient treatment (Robertson et al., 2000; Fornara et al., 2011). Net changes in soil CO_2 sequestration were calculated between 1970 and 2013 in the 0-15 cm soil depth layer. We are aware that *IPCC Guidelines* define soil carbon stocks as organic carbon incorporated into mineral soil horizons to a depth of 30 cm. In our study, we have measured soil C stocks between 15 and 30 cm depth in 1998 and 2013 (see Supplementary Fig. 5); we did not find, however, any significant difference in C stocks between nutrient treatments. We observed significant changes in soil C content and stocks only in the 0-15 cm soil depth and we thus used these rates of soil C sequestration in our GWP calculations. All GHG emissions associated with the management of our permanent grassland were calculated using information from IPCC reports (Dong et al., 2006; De Klein et al., 2006; Myhre et al., 2013) and from multiple peer-reviewed scientific papers. GHG emissions were converted to CO_2 equivalents (CO_2-eq) assuming a 100 year time horizon (Myhre et al., 2013). To simulate grassland management intensification we assumed a cattle-stocking rate of 2 animals (i.e. livestock units) per hectare.

1) Liming contribution to GWP through soil CO_2 emissions.

Nutrient treatments	$CaCO_3$ applications ($Mg\ ha^{-1}\ yr^{-1}$)	Total CO_2 emissions ($Mg\ ha^{-1}$) *	Total CO_2 emissions ($g\ m^{-2}\ y^{-1}$)
Control	0.07	0.031	3.1
NPK	0.09	0.04	4
Cattle (L)	0.04	0.018	1.8
Cattle (M)	0.006	0.003	0.3
Cattle (H)	0.0001	0.0003	0.03

* All C in $CaCO_3$ is emitted in the atmosphere as CO_2 thus keeping in mind atomic weights of C (12), O (16) and Ca (40) this means that 1 tonne of $CaCO_3$ applied releases 0.44 tonnes of CO_2.

2) Liming contribution to GWP due to the production and transport of lime.

Nutrient treatments	Total $CaCO_3$ applications ($Mg\ ha^{-1}$)	Total CO_2 emissions ($Mg\ ha^{-1}$) due to lime production & transport	Total CO_2 emissions ($g\ m^{-2}\ y^{-1}$) due to lime production & transport
Control	0.07	0.0025	0.25
NPK	0.09	0.0032	0.3
Cattle (L)	0.04	0.0014	0.1
Cattle (M)	0.006	0.0002	0.02
Cattle (H)	0.0001	0.00002	0.002

Previous studies (West and Marland, 2002; West and McBride, 2005) estimate that production of crushed limestone and transport to site of application (on an average distance of 160 km) is responsible for emissions of about 0.036 tonnes of CO_2 per tonne of limestone.

3) CO_2-eq emissions from the enteric fermentation of ruminant livestock

CO_2 emissions from enteric fermentation were estimated using emission factors given for Western Europe (Dong et al. 2006). These emission factors are comparable to those determined from local studies under Irish grassland conditions (Casey and Holden, 2005; Yan et al., 2010). We used 117 for dairy cattle and 57 for beef (meat) cattle.

TIER 1 ENTERIC FERMENTATION EMISSION FACTORS FOR CATTLE

Regional characteristics	**Cattle category**	**Emission factor (kg CH_4 $head^{-1}$ yr^{-1})**	**Comments**
North America: Highly productive commercialized dairy sector feeding high quality forage and grain. Separate beef cow herd, primarily grazing with feed supplements seasonally. Fast-growing beef steers/heifers finished in feedlots on grain. Dairy cows are a small part of the population	Dairy	128	Average milk production of 8,400 kg $head^{-1}$ yr^{-1}.
	Other Cattle	53	Includes beef cows, bulls, calves, growing steers/heifers, and feedlot cattle.
Western Europe: Highly productive commercialized dairy sector feeding high quality forage and grain. Dairy cows also used for beef calf production. Very small dedicated beef cow herd. Minor amount of feedlot feeding with grains.	Dairy	117	Average milk production of 6,000 kg $head^{-1}$ yr^{-1}.
	Other Cattle	57	Includes bulls, calves and growing steers/heifers.
Eastern Europe: Commercialised dairy sector feeding mostly forages. Separate beef cow herd, primarily grazing. Minor amount of feedlot feeding with grains.	Dairy	99	Average milk production of 2,550 kg $head^{-1}$ yr^{-1}.
	Other Cattle	58	Includes beef cows, bulls and young

CH_4 emissions were converted to CO_2 equivalents using the following equation:

$$Y \text{ g CO}\cdot \text{ m}\cdot \text{ y} = \text{y g CH - Cha} \cdot \text{d}\times 16 \text{ g CH28 g CH - C}\times 365 \text{ d1 y}\times 1 \text{ ha10 m}\times 28 \text{ g CO1g CH}$$

Nutrient treatments	Enteric fermentation emissions ($g\ CH_4\ m^{-2}\ y^{-1}$)	Enteric fermentation emissions ($g\ CO_2\text{-eq}\ m^{-2}\ y^{-1}$)
Control	0	0
NPK	0	0
Meat		
Cattle (L)	68400	109.4
Cattle (M)	68400	109.4
Cattle (H)	68400	109.4
Dairy		
Cattle (L)	140400	224.6
Cattle (M)	140400	224.6
Cattle (H)	140400	224.6

4) CO_2-eq emissions from manure management (CH_4)

Emissions for CH_4 from organic manure management were calculated from (Dong et al., 2006) emission factors for Western Europe, with average annual temperatures of ≤10°C (see Table below). We used 21 for dairy cattle and 6 for beef (i.e. meat) cattle.

Manure management methane emission factors by temperature for cattle, swine and buffalo (kg CH_4 head^{-1} yr^{-1})

Regional Characteristics	Livestock species	CH4 emission factors by average annual temperature (°C)		
		Cool		
		≤ 10	**11**	**12**
North America: Liquid-based systems are commonly used for dairy cows and swine manure. Other cattle manure is usually managed as a solid and deposited on pastures or ranges	Dairy Cows	48	50	53
	Other Cattle	1	1	1
	Market Swine	10	11	11
	Breeding Swine	19	20	21
Western Europe: Liquid/slurry and pit storage systems are commonly used for cattle and swine manure. Limited cropland is available for spreading manure.	Dairy Cows	21	23	25
	Other Cattle	6	7	7
	Market Swine	6	6	7
	Breeding Swine	9	10	10

5) CO_2-eq emissions from manure management (N_2O-N)

N_2O-N emissions from organic manure management were calculated from experimental data collected at the AFBI Hillsborough Farm (AgriSearch, 2009) as well as default values (i.e. IPCC). N_2O emissions were estimated for livestock and manure management including Direct and Indirect Emissions. We assumed cattle stocking rates of 2 (i.e. 2 animal units per hectare), thus the total below is multiplied by 2 in our final GWP calculations

	Nitrous Oxide Emissions ($kg\ N_2O\ m^{-2}\ y^{-1}$)	CO_2 equivalent ($g\ CO_2$-eq $m^{-2}\ y^{-1}$)
Livestock and manure management	0.09	7.5
Direct emissions		
Dung and urine deposited by cattle	0.95	79.12
Slurry spreading	0.47	39.14
Indirect emissions		
Volatilization from slurry spreading, dung and urine by grazing animals	0.19	15.82
Leaching losses	0.28	23.32
Total	1.98	164.9

N_2O emissions were converted to CO_2 equivalents using the following equation:

EQ5: *Y* g CO· m· y = x g NO - Nha · d×44 g NO28 g NO - N×365 d1 y×1 ha10 m×265 g CO1g NO

6) CO_2-eq emissions from managed soils (N_2O-N)

To estimate N_2O emissions from our managed grassland soils we used the default value of 1% set by (De Klein et al., 2006) and applied this conversion factor to the amount of N that is applied to our experimental plots every year either through organic (cattle slurry) or inorganic (NPK) nutrient applications (see Table below).

Nutrient treatments	Total N applied ($Kg\ ha^{-1}\ yr^{-1}$)	N_2O emissions ($g\ N_2O\ ha^{-1}\ yr^{-1}$)	Total CO_2-eq emissions ($g\ CO^2\ m^{-2}\ yr^{-1}$)
Control	0	0	0
NPK	200	2000	83.3
Cattle (L)	162	1620	67.5
Cattle (M)	324	3240	134.9
Cattle (H)	648	640	269.8

7) CO_2-eq emissions from managed soils (CH_4)

CH_4 emissions from cattle excrement in the field were estimated to be 1.7g CH_4 day^{-1} for dairy cows and 1.6 g CH_4 day^{-1} for non-dairy cattle (Jarvis et al., 1995).

8) CO_2-eq emissions from milk yields

The average C content of milk from a dairy cow was assumed to be 4%, while the average milk yield of the average Irish dairy cow was assumed to be 5000 L (5000 kg) (Jaksic et al., 2006). We assumed a livestock unit per hectare (LU ha^{-1}) of 2. Emissions from milk yields in g CO_2 m^{-2} yr^{-1} were therefore calculated as:

⍰ g CO m yr = LU ha × 500 kg × 0.0410×4412

9) CO_2-eq emissions from beef yields

The average weight of an animal sent for slaughter was estimated to be 550 kg and the C content of the animal was assumed to be 5.1% (Byrne et al., 2006). Emissions were therefore calculated as:

Y g CO m yr=Animals slaughtered as % LU ha × 550 kg × 0.051*10*×4412

10) Emissions from concentrate production and transportation

We estimated the emission value associated with the production and transportation of each 1 kg of dry matter of concentrates as 0.74 kg CO_2e for dairy cows, and 0.6170 kg CO_2e for other cattle (Carbon Trust, 2010; The Scottish Government, 2011).

11) CO_2-eq from microbial oxidation

The GWP associated with microbial oxidation of CH_4 was expressed as CO_2 using the following equation where microbial oxidation was estimated to be 2.5 g CH_4 ha^{-1} day^{-1} (see Fornara et al., 2011):

Y g CO· m· y = y g CH - Cha · d×16 g CH28 g CH - C×365 d1 y×1 ha10 m×28 g CO1g CH

12) CO_2-eq emissions from fertiliser production

Literature data was used to estimate:

a) CO_2 emission equivalents from the production of fertilisers

- Urea: 1326.1 g CO_2 kg^{-1} N (Kongshaug, 1998).
- Triple Superphosphate: 354 g CO_2 kg^{-1} N P_2O_5. CO_2 emissions were calculated per kg of applied P (Kongshaug, 1998).
- Potassium: 111 g CO_2 kg^{-1} K (Williams *et al.*, 2006) (This includes production and transport of fertiliser K).

The total GWP of CO_2 emissions from fertiliser production were calculated as follows, where '*EE'* equates to the corresponding emission equivalent (see above):'*EE'* equates to the corresponding emission equivalent (see above):

Y g CO· m· y = N kg ha yr×Urea *EE*10000+
P kg ha yr×PO *EE*10000+K kg ha yr×K *EE*10000

b) CO_2 emission equivalents from the transportation of fertilisers to the site of application.

Estimations for transportation of N and P fertilisers were estimated using literature data assuming an average distribution distance of 160 km (Davis and Haglund, 1999; West and Marland, 2002).

- N fertilisers: 44 g CO_2 kg^{-1} N
- Triple superphosphate: 12 g CO_2 kg^{-1} N P_2O_5

The total GWP of CO_2 emissions from fertiliser transportation was calculated using the following equations:

Y g CO· m· y = N kg ha yr×44g CO10000

Y g CO· m· y = P kg ha yr×12g CO10000

13) CO_2 emissions from machinery use

CO_2 emissions from machinery (diesel used by farm machinery during fertilisation, liming, silage cutting, baling etc.) were calculated as 24.9 g CO_2 m^{-2} y^{-1} (Downs and Hansen, 1998). This was calculated by multiplying the litres of fuel used per hectare (see Robertson et al., 2000). Estimated diesel consumption was 11.2 L ha^{-1} based on current farming practices.

Yg CO·m· y = x L CHha · y×832 g CH1 L CH×192 g C226 g CH×44 g CO12 g C×1 ha10 m

where x= average annual diesel use (L·ha·yr)

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