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Supplementary information

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Functionally rich crop rotations increase calorie and macronutrient outputs across Europe

In the format provided by the authors and unedited

Description of the Long-Term Experiments

Table S1 Climatic conditions are summarized by annual mean temperature (T) and total precipitation (P), averaged over the years for which yield data are available, based on data from EObs[1] at 0.1° resolution v23.1e released in March 2021. Years of data indicate the period covered and the length of the record. Rotation length is the number of years needed to complete the rotation cycle. In crop rotation, crops followed by (f) are used as forage. Leys are specified as mixture of grasses only (grass), mixture of grasses and red clover (grass+rc), mixtures of grasses and white clover (grass+wc), and pure alfalfa (alfalfa). Cereals undersown with ley are denoted by *. Functional types in the rotations are cereals (C), broadleaves (BL), legumes (Leg) and ley (Ley). Fertiliser treatment indicates whether mineral (min), organic (org – manure or slurry) or a combination of both fertilisations (both) were used. In most sites, different fertiliser input levels were considered. Application rates of fertilisers and crop protection chemicals were either the same across diversity treatments or variable based on need. Tillage regimes (con: conventional; red: reduced) were always the same across diversity levels. Long-term experiment Tulloch was sheep grazed.

Site (code), Country	Site location: Latitude, longitude	Climatic conditions T (°C), P (mm)	Years of data (length)	Rotation ID	Crop rotation	Crop species	Rotation length	FR	Functional types	Fertiliser Treatments (levels considered)	Tillage treatment	Crop protection	Study design	Number of replicates	Reference
Ås (As), Sweden	63.25 °; 14.57 °	T=3; P=553	1966: 2009 (44)	As1	spring barley*, ley-grass+rc (f), ley-grass+rc (f), forage rape (f), potato, rye	5	6	3	C, BL, Ley	both, min (3)	conv	variable	split plot	1	[2]
				As6	spring barley	1	1	1M	C						
				As7	spring barley, spring barley, oat	2	3	1C	C						
				As8	spring barley, spring barley, potato	2	3	2	C, BL						
				As9	spring barley*, ley-grass+rc (f), ley-grass+rc (f),	2	3	2	C, Ley						
Bologna (Bo), Italy	44.55 °; 11.35 °	T=14; P=611	1984: 2020 (36)	Bo1	maize, winter wheat, maize, winter wheat, maize, winter wheat, ley–alfalfa (f), ley-alfalfa (f), ley-alfalfa (f)	3	9	2	C, Ley	org, both, min (8)	conv	variable	split plot	2	[3]
				Bo2	maize, winter wheat	2	2	1C	C						
				Bo4	maize	1	1	1M	C						
				Bo5	winter wheat	1	1	1M	C						
Broadbalk (Bb), UK	51.82 °; -0.35 °	T=11; P=650	2000: 2017 (18)	Bb1	winter wheat	1	1	1M	C	org, min, both (17)	conv	same	split-split plot	1 for Bb1, 5 for Bb2	[4]
				Bb2	oats, maize(f), winter wheat, winter wheat, winter wheat	3	5	1C	C						
Brody (Bd), Poland	52.43 °; 16.3 °	T=9; P=548	1986: 1999 (14)	Bd3	spring barley	1	1	1M	C	min, both (1)	conv	same	split plot	4	[5,6]
				Bd2	winter rye	1	1	1M	C						
				Bd1	potato, spring barley, ley-alfalfa (f), ley-alfalfa (f), winter oilseed	5	7	3	C, BL,						

					rape, winter rye, winter rye				Ley						
La Canaleja (LC), Spain	40.35 °; -3.33 °	T=14; P=396	2010: 2020 (11)	LC1	winter wheat	1	1	1M	C	min (1)	conv, red, none	variable	split plot	4	7–9
				LC2	winter wheat, vetch (f), winter barley, fallow	4	4	2	C, Leg						
Lanna_ley (LL), Sweden	58.33 °; 13.12 °	T=7; P=612	1981: 2018 (38)	LL1	winter oilseed rape, winter wheat, oats, spring barley*, ley-grass+rc (f), ley-grass+rc (f)	6	6	3	C, BL, Ley	min (4)	conv	variable	split plot	1	10
				LL2	winter oilseed rape, winter wheat, oats, spring barley*, ley-grass (f), ley-grass (f),	6	6	3	C, BL, Ley						
				LL3	winter oilseed rape, winter wheat, oats, spring barley, spring wheat, fallow	6	6	2	C, BL						
Öjebyn (Oj), Sweden	65.35 °; 21.38 °	T=2; P=547	1966: 2009 (44)	Oj1	spring barley*, ley-grass+rc (f), ley-grass+rc (f), forage rape (f), potato, rye	5	6	3	C, BL, Ley	both, min (3)	conv	variable	split plot	1	2
				Oj6	spring barley	1	1	1M	C						
				Oj7	spring barley, spring barley, oats	2	3	1C	C						
				Oj8	spring barley, spring barley, potato	2	3	2	C, BL						
				Oj9	spring barley*, ley-grass+rc (f), ley-grass+rc (f),	2	3	2	C, Ley						
Osiny (Os), Poland	51.46 °; 22.05 °	T=9; P=555	1996: 2019 (24)	Os1	winter wheat	1	1	1M	C	min (1)	conv	variable	block design	1	11
				Os2	winter oilseed rape, winter wheat, spring barley (1996-2003)/spring wheat (2004-2019)	3	3	2	C, BL						
Padova (Pa), Italy	45.35 °; 11.97 °	T=14; P=719	1990: 2019 (30)	Pa1	winter wheat	1	1	1M	C	both, min (2)	conv	variable	split plot	3	12
				Pa2	maize	1	1	1M	C						
				Pa3	winter wheat, maize	2	2	1C	C						
				Pa4	maize, sugar beet, maize, winter wheat, ley-alfalfa (f), ley-alfalfa (f)	4	6	3	C, BL, Ley						

				Pe1	winter wheat	1	1	1M	C						
				Pe2	winter wheat, maize	2	2	1C	C						
				Pe3	winter wheat, winter wheat, maize	2	3	1C	C						
				Pe4	winter wheat, winter wheat, winter wheat, maize	2	4	1C	C						
Perugia (Pe), Italy	42.96 °; 12.38 °	T=14; P=762	1974: 2013 (40)	Pe5	winter wheat, winter wheat, winter wheat, winter wheat, maize	2	5	1C	C						
				Pe6	winter wheat, winter wheat, winter wheat, winter wheat, winter wheat, maize	2	6	1C	C						
				Pe8	winter wheat, sunflower	2	2	2	C, BL						
				Pe9	winter wheat, sorghum	2	2	1C	C						13
				Pe10	winter wheat, fava bean	2	2	2	C, Leg	min (1)	conv	variable	split plot	3	
				Rb1	spring barley*, ley-grass+rc (f), ley-grass+rc (f), forage rape (f), potato, rye	5	6	3	C, BL, Ley						
				Rb6	spring barley	1	1	1M	C						
Röbäcksdalen (Rb), Sweden	63.82 °; 20.28 °	T=3; P=636	1966: 2009 (44)	Rb7	spring barley, spring barley, oats	2	3	1C	C	both, min (3)	conv	variable	split plot	1	2
				Rb8	spring barley, spring barley, potato	2	3	2	C, BL						
				Rb9	spring barley*, ley-grass+rc (f), ley-grass+rc (f),	2	3	2	C, Ley						
				SL1	winter oilseed rape, winter wheat, oats, spring barley*, ley-grass+rc (f), ley-grass+rc (f)	6	6	3	C, BL, Ley						
Säby_ley (SL), Sweden	59.82 °; 17.70 °	T=6; P=620	1981: 2016 (36)	SL2	winter oilseed rape, winter wheat, oats, spring barley*, ley-grass (f), ley-grass (f)	6	6	3	C, BL, Ley	min (4)	conv	variable	split plot	1	10
				SL3	winter oilseed rape, winter wheat, oats, spring barley, spring wheat, fallow	6	6	2	C, BL						

Säby_LTE (SLTE), Sweden	59.82 °; 17.70 °	T=6; P=614	1974: 2010 (36)	SLTE4	fallow, winter oilseed rape, winter wheat, oats, barley, spring wheat	6	6	2	C, BL	min (4)	conv	variable	split plot	2	[10]
				SLTE5	spring oats	1	1	1M	C						
				SLTE6	spring barley	1	1	1M	C						
				SLTE7	spring wheat	1	1	1M	C						
Stenstugu_ley (St), Sweden	57.60 °; 18.43 °	T=7; P=571	1968: 2020 (53)	St1	winter oilseed rape, winter wheat, oats, spring barley*, ley-grass+rc (f), ley-grass+rc (f)	6	6	3	C, BL, Ley	min (4)	conv	variable	split plot	1	[10]
				St2	winter oilseed rape, winter wheat, oats, spring barley*, ley-grass (f), ley-grass (f)	6	6	3	C, BL, Ley						
				St3	winter oilseed rape, winter wheat, oats, spring barley, spring wheat, fallow	6	6	2	C, BL						
Tulloch (Tu), UK	57.18 °; -2.25 °	T=9; P=796	1991: 2006 (16)	TuE1	ley-grass+wc (f), ley-grass+wc (f), ley-grass+wc (f), spring oats, swedes, oats*	3	6	3	C, BL, Ley	org (1)	conv	none	ran-dom-ised block	2	[14,15]
				TuE2	ley-grass+wc (f), ley-grass+wc (f), ley-grass+wc (f), ley-grass+wc (f), spring oats, oats*	2	6	2	C, Ley						
Woodside (Ws), UK	57.63 °; -3.40 °	T=9; P=706	1991: 2012 (12)	Ws1	ley-grass+wc (f), ley-grass+wc (f), ley-grass+wc (f), spring oats, potatoes, oats*	3	6	3	C, BL, Ley	org (1)	conv	none	ran-dom-ised block	2	[14,15]
				Ws2	ley-grass+wc (f), ley-grass+wc (f), spring oats, potatoes, oats*, ley-grass+wc (f), swedes, oats*	4	8	3	C, BL, Ley						

Supplementary Results

To assess the effect of the selected long-term experiments and the robustness of our conclusions, we repeated the analyses by leaving out one long-term experiment at a time and checking differences in the sign of statistically significant (at $p<0.005$) coefficients. Removing data relative to the experiments in Bologna, Broadbalk or Padova influenced some of the contrasts, but not our main conclusions, as discussed next.

Removing long-term experiment Bologna resulted in larger reductions in carbohydrate outputs at FR 1C and smaller at FR 2, compared with the entire dataset, although both remained lower than FR 1M irrespective of time (Figure S1). Fat outputs at FR 1C were not different from those at FR 1M. Time had no longer an effect on calories at FR 1C till after 10 years since implementation and on fats at FR 1M 20 yrs after implementation.

Removing long-term experiment Broadbalk resulted in a benefit of FR 1C over 1M for carbohydrates (Figure S2), instead of a loss when considering the entire dataset. The fat benefits of rotating different cereals emerged already 5 years after implementation. Proteins at FR 1C were the same of FR 1M. Calories and carbohydrates remained stable over time at FR 1C, whereas a slight decline in fats emerged 10 years after the implementation.

After removing data from long-term experiment Padova, carbohydrates from FR 3 were lower than from FR 1M. Moreover, calories, carbohydrates and fats at FR 1M, as well as carbohydrates at FR 3, were no longer affected by time elapsed after rotation implementation, whereas a positive trend in proteins from FR 1C emerged. (Figure S3).

Long-term experiments Bologna and Broadbalk contrasted winter wheat (and maize) monocultures with a two- or five-year cereal only rotation, including maize and winter wheat (Table S1) and have high or intermediate potential productivity (Extended Data Figure 1).

This explains the positive changes in carbohydrates at FR 1C. Long-term experiment Padova comprised winter wheat and maize monocultures and a six-year FR 3 rotation, including two years of maize and one of sugar beet. This starch-rich rotation in a highly productive site (Extended Data Figure 1) likely affected the carbohydrate benefits at FR 3.

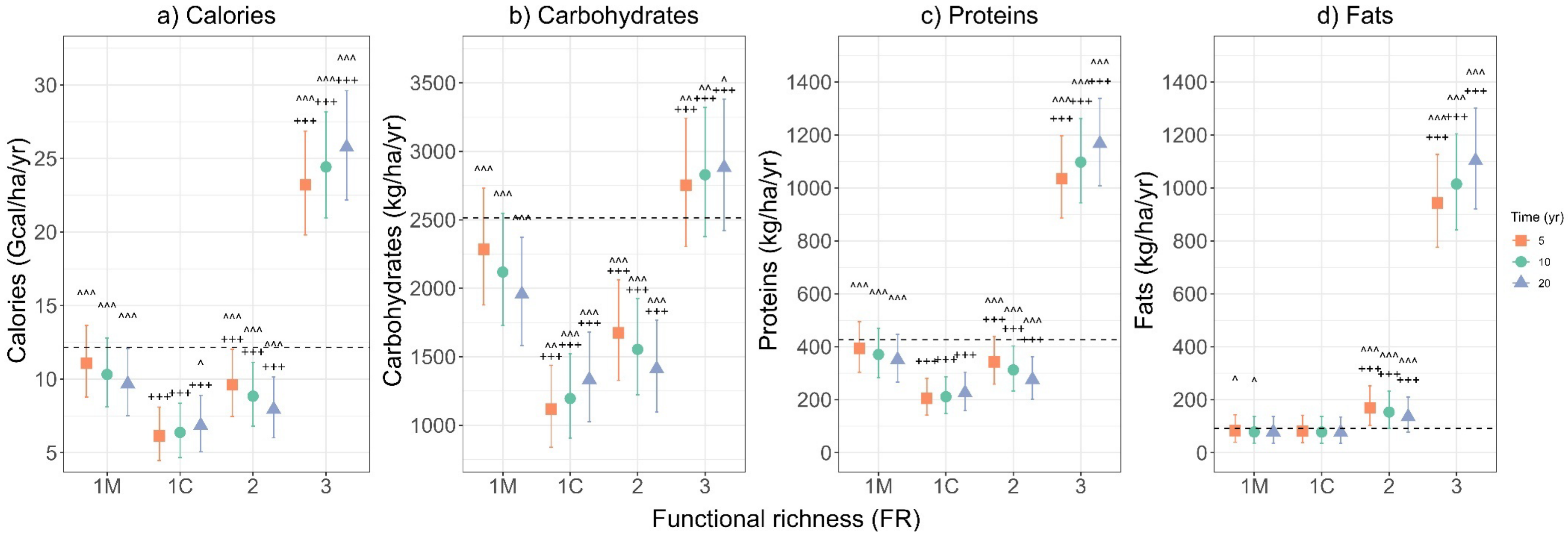

Figure S1 Same as Figure 1 in the main text but excluding long-term experiment Bologna from the analyses. The statistical models are based on fitting the 10137 whole rotation outputs relative to the remaining 15 long-term experiments (see Table S1 for details). Forage crops were assumed to be used for milk production. Closed symbols are model predictions relative to five (orange squares), ten (green diamonds), and twenty (blue triangles) years following the implementation of the rotation. Whiskers extend over the 5 to 95% confidence intervals. Symbols indicating significances refer to contrasts across levels of FR within each time, using FR 1M at the same time as baseline (+ indicates p<0.05; ++ p<0.01; +++ p<0.001) and across time within each FR level with time 0 at the same FR as baseline (^ indicates p<0.05; ^^ p<0.01; ^^^ p<0.001). *Post-hoc* tests were two-sided and adjusted for multiplicity using multivariate t-distribution. Note the difference in the y axis scale among plots.

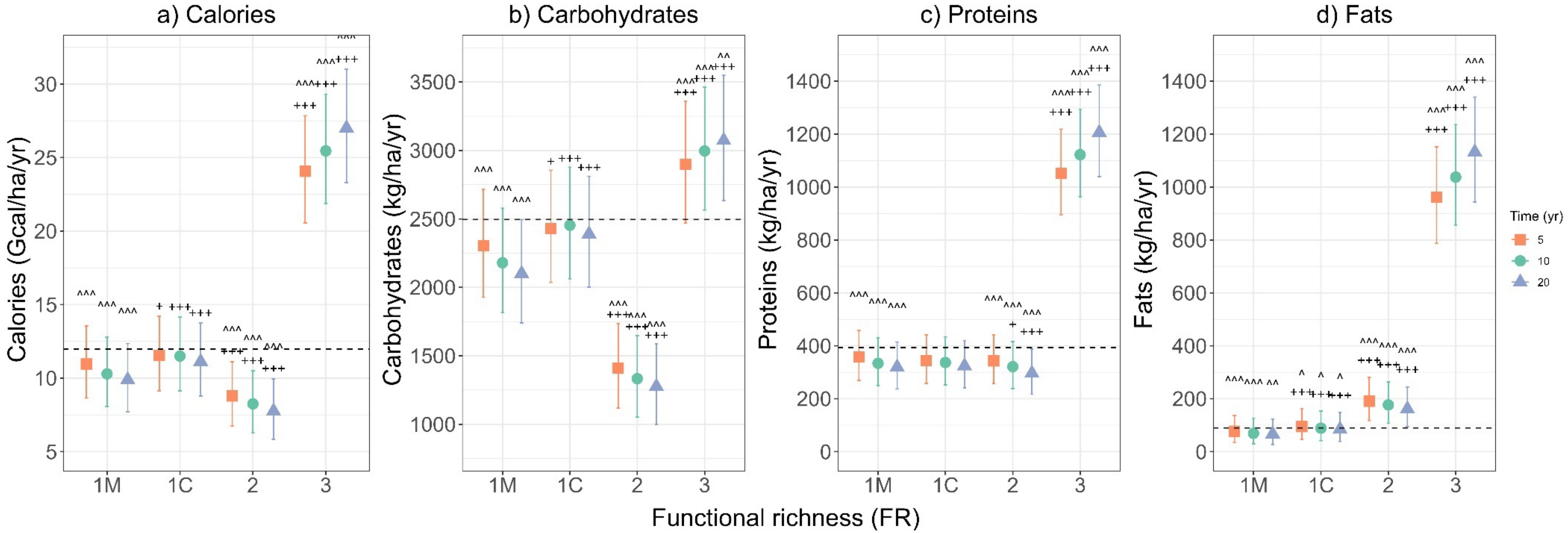

Figure S2 Same as Figure 1 in the main text but excluding long-term experiment Broadbalk from the analyses. The statistical models are based on fitting the 11051 whole rotation outputs relative to the remaining 15 long-term experiments (see Table S1 for details). The meaning of bars and symbols are detailed in the caption to Figure S1.

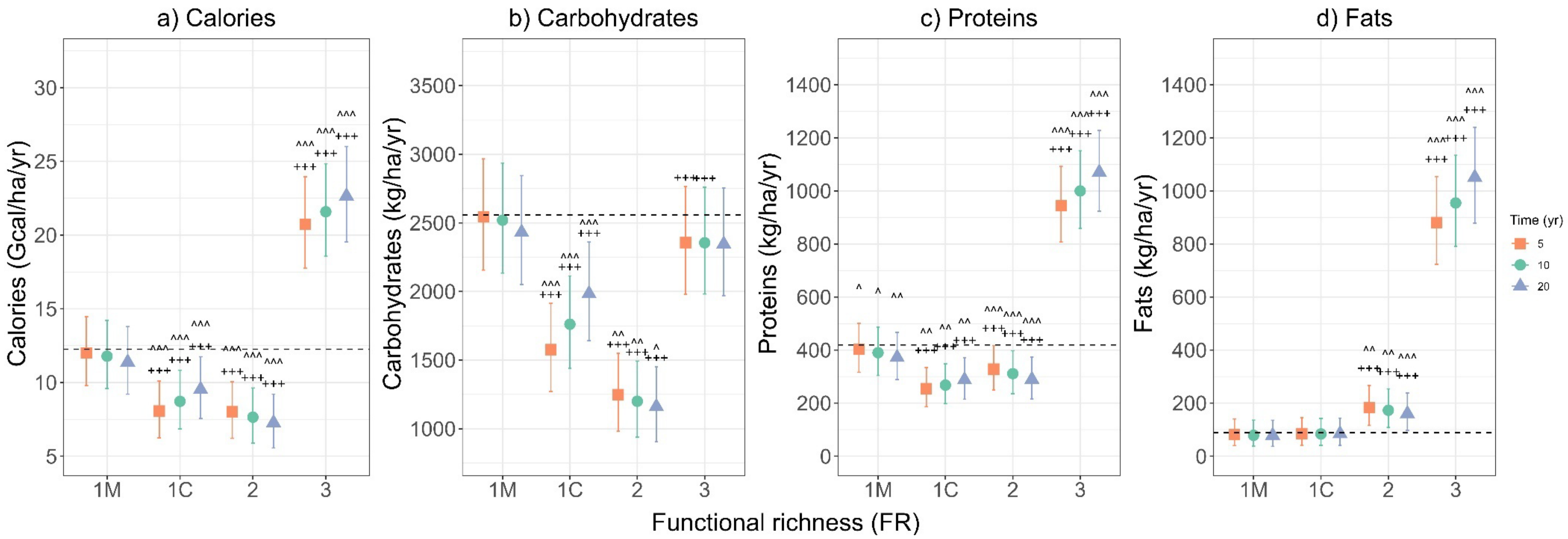

Figure S3 Same as Figure 1 in the main text but excluding long-term experiment Padova from the analyses. The statistical models are based on fitting the 11865 whole rotation outputs relative to the remaining 15 long-term experiments (see Table S1 for details). The meaning of symbols is detailed in the caption to Figure S1.

Supplementary Methods

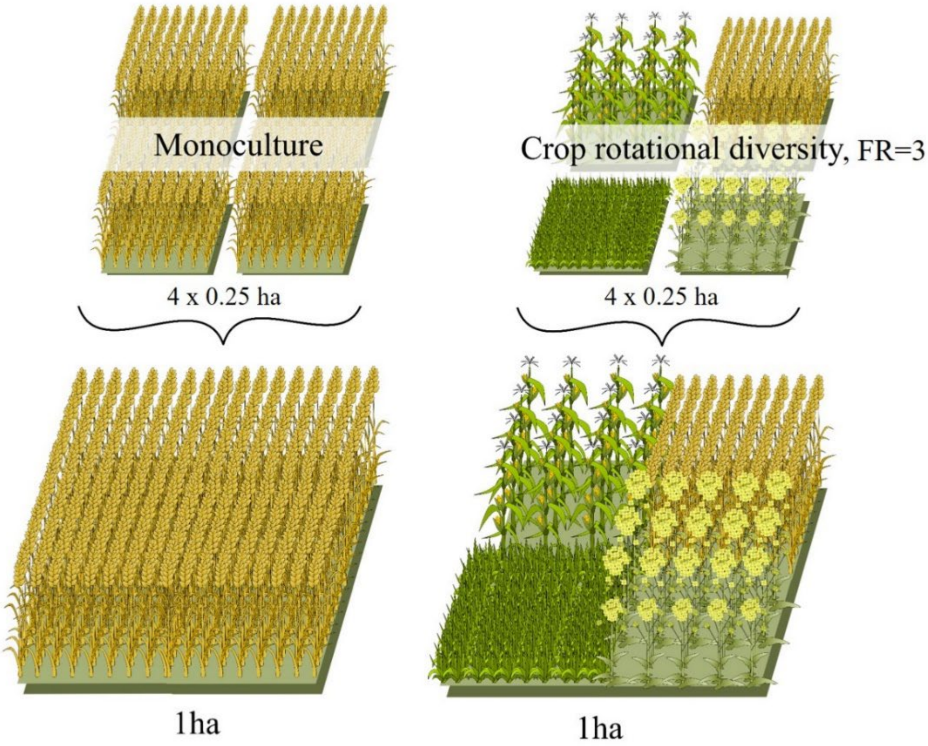

Figure S4 Transformation of the amounts of calories or macronutrient produced by all crops in a year (per unit area) to total calorie or macronutrient output of the rotation during that year, assuming the rotation is implemented on a set area. The example considers a set area of 1 ha, either cultivated as cereal monoculture (i.e., the cereal is cultivated over the entire area; left) or as a rotation with FR 3, encompassing two cereals, one ley, and one oil crop (right). Since all crops are cultivated every year, each occupies a fraction of the set area proportional to the inverse of the rotation length, i.e., 1 ha for the cereal monoculture (left) and 0.25 ha for the four course rotation (right). Figure created by J Heinen.

Table S2 Selected retail product, water, calorie and macronutrient contents, raw to retail conversion, and refuse factor relative to the crops included in the long-term experiments. Retail product is the abbreviated description of food items as per the USDA database (Shrt_Descr in https://www.ars.usda.gov/ARSUserFiles/80400525/Data/SR-Legacy/SR-Legacy_Doc.pdf; last accessed October 14th, 2025). Functional type refers to the classification of the crop. Crop to retail conversion factors for each crop *c* and retail product, α_c, were obtained from https://www.fao.org/fileadmin/templates/ess/documents/methodology/tcf.pdf. Columns denoted as calories, carbohydrates, proteins, and fats report the nutrient conversion rates, $\gamma_{n,c}$, for each nutritional output *n* and crop *c* (Eq. 1 in the Methods in the main text). $f_{H2O,c}$, $f_{ref,c}$, as well as calorie and macronutrient conversion rates $\gamma_{n,c}$ were sourced from https://www.ars.usda.gov/northeast-area/beltsville-md-bhnrc/beltsville-human-nutrition-research-center/food-surveys-research-group/docs/fndds-download-databases/, FNDDS 2019-2020, FNDDS Nutrient Values.xls and SR28 Dataset – table FOOD_DES (last accessed October 14th, 2025). The crop to retail conversion and water fraction for sugar beet was obtained from ref. [16], whereas the nutritional value of white sugar was obtained from the USDA as all the other products. The conversion factors for forage to milk and beef are based on results from farms across the Netherlands[17] and from USDA[18] respectively. These values are aligned with those emerging from other assessments throughout Europe (for milk, ref. [19–21]; for beef, ref. [20–22]), although other estimates are higher (for milk, ref. [22–24]; for beef, ref. [25,26]).

Crop	Retail product	Water fraction, $f_{H2O,c}$ (%)	Calories (kcal/100g)	Protein (%)	Fat (%)	Carbohy-drate (%)	Raw crop to retail conver-sion, α_c	Refuse factor, $f_{ref,c}$
Barley	Barley, hulled	9.44	354	12.48	2.3	73.48	0.72	0
Broad beans dry	Broad beans (fava beans), mature seeds, raw	10.98	341	26.12	1.53	58.29	1.0	0
Fallow	Fallow	0	0	0	0	0	0.0	1

Maize	Corn flour, whole grain	10.91	361	6.93	3.86	76.85	0.82	0
Oats	Oat flour, partly debranned	8.55	404	14.66	9.12	65.7	0.53	0
Oil seed rape	Oil, canola	0	884	0	100	0	0.38	0
Potatoes	Potatoes, flesh and skin, raw	79.25	77	2.05	0.09	17.49	1.0	0.25
Rutabaga/Swede	Rutabagas, raw	89.43	37	1.08	0.16	8.62	1.0	0.15
Rye	Rye flour, medium	10.97	349	10.88	1.52	75.43	0.80	0
Sorghum	Sorghum, flour, whole grain	10.26	359	8.43	3.34	76.64	0.90	0
Sugar beet	Sugar, white, granulated or lump	76	401	0	0.32	99.6	0.16	0
Sunflower seeds	Oil, sunflower, high oleic (>70%)	0	884	0	100	0	0.41	0
Wheat	Wheat flour, whole grain	10.74	340	13.21	2.5	71.97	0.79	0
Products obtained from forage crops								
Cow milk, whole	Milk, whole, 3.25% fat, without added vitamin A and vitamin D	88.13	61	3.15	3.27	4.78	1.05	0
Beef, boneless	Beef, grass-fed, ground, raw	67.13	198	19.42	12.73	0	0.047	0
Biofuel	Biofuel	0	0	0	0	0	1.0	0

R statements

We report below the R statements relative to the linear mixed model fitting, model diagnostic and contrasts used to generate the results. In these statements, output *O* is the whole rotation output (calories, carbohydrates, proteins or fats); the dataframe df contains the whole rotation outputs, as well as information on the site, year (time), group, functional richness (FR); back-transformed eff is used to plot the mean and confidence intervals; cntrFR and cntrt are the contrasts, represented in the plot with + and ^ symbols respectively. The code and a sample dataset are available from the Swedish National Data service, Researchdata.se (https://researchdata.se/en), doi: https://doi.org/10.5878/5q25-8572.

```
#linear mixed effect model fitting

FR_model=

lmerTest::lmer(sqrt(O)~poly(time,2)*FR+(1|site:year)+(1|site:group)+(1|site),data=df)

eff=ggeffects::ggeffect(FR_model,terms=c("FR","time[5,10, 20]"))


#model diagnostic

SO_FR= DHARMa::simulateResiduals(FR_model, n=200)

#contrasts

mod.rg <- emmeans::ref_grid(FR_model,at=list(FR=c("1M","1C","2","3"),time=c(0,5,10,20)))

emm <- emmeans::emmeans(regrid(mod.rg), ~ FR * time,
at=list(FR=c("1M","1C","2","3"),time=c(0,5,10,20)),type="response")

##contrasts within time, across FR

cntrFR= emmeans::contrast(emm, "trt.vs.ctrl", by = "FR",adjust="mvt")
```

##contrasts within FRs, across time

```
cntrt= emmeans::contrast(emm, "trt.vs.ctrl", by = "time",adjust="mvt")
```

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