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Nitrogen deposition makes a minor contribution to carbon sequestration in temperate forests

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Humans have altered global nitrogen cycling such that more atmospheric N_2 is being converted ('fixed') into biologically reactive forms by anthropogenic activities than by all natural processes combined[1]. In particular, nitrogen oxides emitted during fuel combustion and ammonia volatilized as a result of intensive agriculture have increased atmospheric nitrogen inputs (mostly NO_3 and NH_4) to temperate forests in the Northern Hemisphere[2–4]. Because tree growth in northern temperate regions is typically nitrogen-limited[5], increased nitrogen deposition could have the effect of attenuating rising atmospheric CO_2 by stimulating the accumulation of forest biomass. Forest inventories indicate that the carbon contents of northern forests have increased concurrently with nitrogen deposition since the 1950s[6–8]. In addition, variations in atmospheric CO_2 indicate a globally significant carbon sink in northern mid-latitude forest regions[9–12]. It is unclear, however, whether elevated nitrogen deposition or other factors are the primary cause of carbon sequestration in northern forests. Here we use evidence from ^{15}N-tracer studies in nine forests to show that elevated nitrogen deposition is unlikely to be a major contributor to the putative CO_2 sink in forested northern temperate regions.

Although northern temperate forests might now function as significant CO_2 sinks, predicting their future role in the global carbon budget requires that causative factors for carbon sequestration be identified[4,11–15]. Estimates of the effects of nitrogen deposition on forest carbon sequestration[3,4,16,17] vary from 0.1 to 2.3 Pg carbon yr^{-1}. By comparison, the total terrestrial sink is currently 1.5–1.9 Pg yr^{-1}, with most CO_2 uptake occurring in northern mid-latitudes[9,10]. If the higher estimates of the effects of nitrogen deposition on forest carbon uptake are accurate, then the terrestrial sink could persist well into the coming century as nitrogen deposition increases. Alternatively, if factors such as reforestation, changes in forest use and management, CO_2 fertilization, or nutrient transfers from soils to trees as a result of climate warming are mainly responsible for the terrestrial carbon sink, then rates of CO_2 uptake by temperate forests might follow different trajectories over the coming decades.

The role that nitrogen deposition plays in determining sink strengths of forests for CO_2 depends on where nitrogen inputs to forests ultimately reside[12,18]. If the primary recipients are trees with woody tissues, high carbon-to-nitrogen (C:N) mass ratios (of between 200 and >500) and long turnover times, then the effects of nitrogen deposition on forest carbon uptake are relatively large. However, if nitrogen inputs are sequestered mainly in soils (C:N = 10–30) are exported as nitrate to drainage water or as nitrogen gases to the atmosphere, then the resultant carbon storage is minor.

Global analyses[3,4] underscore the importance of determining the fates of nitrogen deposition in forests. The Townsend–Holland groups predicted global patterns of NO_y and NH_x deposition by combining estimates of NO_x and NH_x emissions with three-

Table 1 ^{15}N-labelled nitrogen inputs to North American and European NITREX forests

Site	Location	Tree species	Ambient throughfall nitrogen (kg ha^{-1} yr^{-1})	^{15}N-forms added	^{15}N-labelled inputs* (kg ha^{-1} yr^{-1})
Bear Brooks (Maine, USA)	44° 52′ N, 68° 06′ W	Beech–maple–spruce (*Fagus grandifolia, Acer* sp., *Picea rubens*)	4	$^{15}NO_3$, plots $^{15}NH_4$, watershed	32 and 60 on plots 29 on watershed
Harvard Forest (Massachusetts, USA)	42° 30′ N, 72° 10′ W	Oak (*Quercus velutina, Q. rubra*)	8	$^{15}NH_4NO_3$ & $NH_4{}^{15}NO_3$	8 (ambient) 58 (fertilized)
Harvard Forest (Massachusetts, USA)	42° 30′ N, 72° 10′ E	Red pine (*Pinus resinosa*)	8	$^{15}NH_4NO_3$ & $NH_4{}^{15}NO_3$	8 (ambient) 58 (fertilized)
Gårdsjön (Sweden)	58° 04′ N, 12° 01′ E	Norway spruce (*Picea abies*)	13	$^{15}NH_4{}^{15}NO_3$	49 (fertilized)
Aber (Wales, UK)	53° 12′ N, 4° 00′ W	Sitka spruce (*Picea sitchensis*)	15	$^{15}NH_4{}^{15}NO_3$ also $Na^{15}NO_3$	35 (fertilized), 75 (fertilized, $Na^{15}NO_3$ only)
Alptal (Switzerland)	47° 03′ N, 8° 24′ E	Norway spruce (*Picea abies*)	17	$^{15}NH_4{}^{15}NO_3$	47 (fertilized)
Klosterhede (Denmark)	56° 29′ N, 8° 24′ E	Norway spruce (*Picea abies*)	20	$^{15}NH_4{}^{15}NO_3$	20 (ambient) 55 (fertilized)
Speuld (Netherlands)	52° 13′ N, 5° 39′ E	Douglas fir (*Pseudotsuga menzieseii*)	50	$^{15}NH_4$ (as $(^{15}NH_4)_2SO_4$)	50 (ambient) 4 (roof exclosures)
Ysselsteyn (Netherlands)	51° 30′ N, 5° 55′ E	Scots pine (*Pinus sylvestris*)	58	$^{15}NH_4$ (as $(^{15}NH_4)_2SO_4$)	58 (ambient) 6 (roof exclosures)

* Labelled nitrogen inputs include throughfall nitrogen + fertilizer nitrogen, or, in the case of exclosures, filtered throughfall.

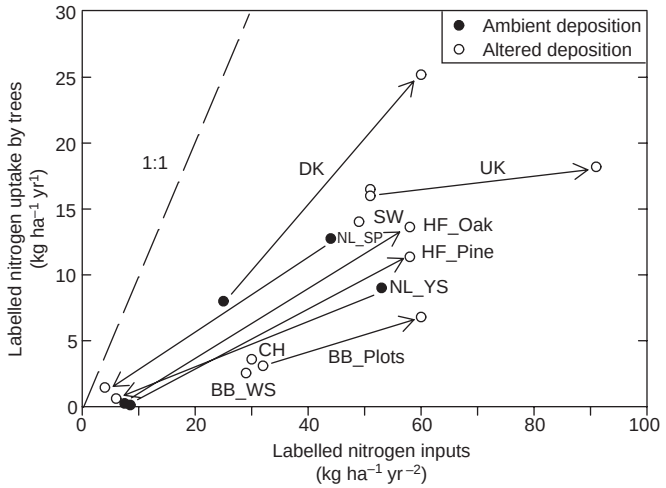

Figure 1 Uptake of ^{15}N-labelled throughfall by tree biomass after 1 to 3 years of tracer additions to non-fertilized (ambient deposition), fertilized and 'reverse fertilized' (throughfall nitrogen removed by means of roofs) plots in North America and Europe. Arrows show shifts in relationships at individual sites between ambient nitrogen deposition and either fertilized or reverse-fertilized conditions. Data for the Danish (DK), Welsh (UK), Dutch (NL_SP, Speuld; NL_YS, Ysselsteyn) sites are from ref. 19. Data from the Swiss (CH) and Swedish (SW) sites are from ref. 20 and O. J. Kjønaas (unpublished data), respectively. Data for Harvard Forest (HF_Pine, HF_Oak) and Bear Brooks (BB_WS, watershed study; BB_Plots, plot study) are from refs 21–23. Where $^{15}NH_4$ and $^{15}NO_3$ were applied separately, averages are shown. The masses of labelled nitrogen uptake by trees (*y*-axis) and labelled nitrogen inputs (*x*-axis) are linearly correlated. The least-squares regression is: $y = -0.87 + 0.25x$ ($r^2 = 0.68$, $P < 0.0001$).

dimensional atmospheric transport models ($NO_y = HNO_3 + NO_3^- + HNO_2 + HO_2NO_2 + N_2O_5 + NO_x + PAN$ (peroxyacetyl nitrate); $NO_x = NO + NO_2$; $NH_x = NH_4^+ + NH_3$). They then used spatially explicit estimates of nitrogen inputs and climate data as drivers for a process-based biogeochemical model (NDEP) to simulate ecosystem carbon dynamics globally at a $1° \times 1°$ scale. Their simulations predicted that CO_2-carbon uptake due to NO_y deposition on the Earth's land surfaces currently ranges from 0.3 to 1.4 Pg yr^{-1}, and up to 2.0 Pg yr^{-1} with NH_x deposition included. Their simulations also indicate that most nitrogen deposition and carbon uptake resulting from this deposition in 1990 occurred between about 25° N and 55° N, mainly in temperate forests of eastern North America and Europe[3,4]. The Townsend–Holland analyses allow for variations in the degree of ecosystem nitrogen retention (permitting system with high nitrogen deposition to retain smaller proportions of nitrogen inputs) and are highly sensitive to the proportion of nitrogen-induced plant growth that is allocated to woody tissues. Low estimates resulted when nitrogen loss:input ratios increased with nitrogen deposition and when carbon allocation by plants to woody tissue was low (compared with foliage and roots). Their highest estimates resulted when trees took up 80% of nitrogen inputs and when proportional allocation of the resultant carbon fixation to woody tissues (compared with leaves and fine roots) was high.

We used results from ^{15}N-tracer studies conducted in six European and three North American forests (Table 1) to evaluate the potential of elevated nitrogen deposition to alter the sink strengths of temperate forests for atmospheric CO_2. This set of experiments is unique in that tracers were applied to large plots (10^2–10^5 m^2) across one- to three-year intervals, either directly (without fertilizer, simulating ambient nitrogen inputs, and with chronic fertilizer applications), or in forest-canopy throughfall from which most NH_4^+ and NO_3^- ions were removed ('reverse-fertilization', using sub-canopy roofs and filtration). Tracing the movements of the ^{15}N additions through ecosystem components enabled us to estimate fluxes of N deposition (ambient, enhanced or diminished) into tree biomass and soil pools in a variety of forest types located along wide, natural and experimentally induced nitrogen-deposition gradients in the northern temperate region.

Our results indicate that trees are not the primary sinks for nitrogen deposition in these forests (Fig. 1). Across the entire nitrogen-deposition gradient, under both ambient and manipulated nitrogen inputs, the average tracer recovery was only 20% of one- to three-year ^{15}N additions. Within tree biomass, most tracers were recovered in leaves, fine roots, bark and twigs. The mean ^{15}N recovery in wood was 3% of inputs. The remaining fractions of applied tracers were recovered in litter, forest floor and mineral soil[19–23]. Solution losses of labelled inputs in drainage were <10% of inputs at most sites. However, larger fractions of tracers were exported through drainage water at some European sites with high rates of nitrogen inputs[19,24]. The two sites with the highest ambient rates of nitrogen deposition, Speuld and Ysselsteyn, lost 33% and 17% of ^{15}N tracers as dissolved inorganic nitrogen, respectively. Inorganic nitrogen losses at these sites were ⩽10% of inputs under reverse-fertilization. At Aber, in north Wales, 25–50% of applied tracers were lost as nitrate at the end of the labelling period, with the greatest losses occurring at inputs of 75 kg nitrogen ha^{-1} yr^{-1} (with $NaNO_3$ as the added nitrate form; 1 ha = 10^4 m^2).

At the one- to three-year timescale of these tracer studies, most labelled nitrogen inputs to the forests entered the forest floor and soil pools, or, under high rates of nitrogen input, were exported as inorganic nitrogen. Thus, our results indicate that soil rather than tree biomass is the primary sink for NO_y and NH_x inputs to temperate forests. This is consistent with analyses of tree growth, which have not yet shown detectable increases in tree biomass accumulation with nitrogen deposition at our ^{15}N-labelled sites[24–26]. If little accumulation of woody biomass results from chronic addition of atmospheric nitrogen to temperate forests, then elevated nitrogen deposition is not the primary contributor to the northern-

Table 2 Estimated influence of $NO_y + NH_x$ deposition on CO_2-carbon uptake by forests

Forest ecosystem pool (1)	Nitrogen deposition allocated to pool (%) (2)	C:N (3)	Nitrogen deposition in pool (Tg) (= 5.1 Tg nitrogen × (2)) (4)	CO_2-carbon uptake (Pg) (= (3) × (4) × 10^{-3}) (5)
Non-woody biomass	15	25	0.77	0.019
Woody biomass	5	500	0.25	0.125
Soil (forest floor + mineral)	70	30	3.57	0.107
Leaching + gaseous losses	10	0	0.51	0.000
Totals	100		5.10	0.251

Percentages of nitrogen deposition allocated to ecosystem pools are estimated from US and NITREX ^{15}N-tracer studies as follows. Tracer-based estimates of nitrogen deposition taken up into tree biomass (leaves, fine roots, twigs, buds and wood + bark) averaged 20% across 9 sites and 18 treatments. The mean flux into woody biomass was 3% of labelled nitrogen inputs, but was conservatively estimated at 5%. C:N values were estimated as follows. Non-woody biomass (leaves, fine roots, twigs and buds) were assumed to contain 2% nitrogen and 48% carbon. Woody biomass was assumed to be 0.13% nitrogen (the mean concentration of bolewood from all 9 sites) and 48% carbon. Soil C:N was estimated to be 30, the high end of values reported for temperate forest floors for North America[27] and Europe[28]. This is an upper limit as C:N ratios in forest floors subject to chronically high nitrogen deposition are as low as 20 and mineral soil values can be as low as 12. Losses of nitrogen (mostly as dissolved NO_3^- and possibly N_2O, NO and N_2 gases) can vary from <5 to 100% of inputs[28]. We assumed relatively small losses. The 5.1-Tg estimate[4] of nitrogen deposition on forests includes 3.1 Tg NO_y-nitrogen and 2.0 Tg NH_x-nitrogen deposition on forests, most of which currently falls on temperate forests in eastern North America, Europe and eastern Asia[3,4].

latitude CO_2 sink as suggested by modelling scenarios which assume that tree biomass accumulates 80% of atmospheric nitrogen inputs[3,4]. To illustrate this point, we used our tracer results to construct a simple budget to estimate the effects of nitrogen deposition on carbon sequestration by temperate forests (Table 2). Our budget uses annual inputs of 5.1 Tg nitrogen ($NO_y + NH_x$) to the Earth's forests and the mean percentage assimilation of labelled nitrogen into non-woody biomass, wood and soils as measured in the 18 treatments at our 9 forest sites. We assumed that, on average, 10% of nitrogen inputs were exported as either dissolved NO_3^- or nitrogen gasses. Woody biomass constitutes the smallest nitrogen sink, but is the largest carbon sink owing to high C:N ratios. In contrast, although soil assimilates almost 15 times as much nitrogen deposition as wood, carbon sequestration in soil is slightly lower owing to low C:N ratios. Assuming that our budget is representative of patterns of nitrogen assimilation in temperate forests, we estimate that nitrogen deposition currently accounts for an uptake of 0.25 Pg carbon yr^{-1} by forest. This value is between 6 and 8 times lower than the recent estimate[4] of 1.5–2.0 Pg yr^{-1} that is possibly attributable to nitrogen deposition on forests located predominantly in northern temperate regions.

Our budget (Table 2) is subject to some uncertainties. First, it applies results from temperate forest studies to forests in general. Patterns of assimilation of nitrogen inputs in tropical and boreal forests could differ from those in temperate forests. In particular, long-term fertilization studies in some boreal and sub-boreal forests suggest chronic, low-level nitrogen fertilization can lead to increased tree biomass[29]. However, because temperate regions of eastern North America, Europe and eastern Asia currently receive nearly all of the 5.1 Tg yr^{-1} of anthropogenic nitrogen inputs to the Earth's land surfaces, we feel that generalizing from the results of temperate forests is acceptable in the current climate. Too few tracer studies of nitrogen assimilation by boreal and tropical forests exist to determine how nitrogen deposition will be distributed between tree biomass and soils in these regions. Second, it is possible that the ^{15}N-labelled inputs assimilated into tree biomass at our sites substituted to some extent for mineralized nitrogen that might otherwise have been taken up by trees. Thus, uptake of labelled nitrogen inputs does not necessarily lead to increased tree growth as assumed in our budget. Third, our budget assumes that tree and soil C:N ratios are fixed even though studies along nitrogen-deposition gradients indicate that tree tissue and upper soil horizon C:N ratios decrease under conditions of chronically elevated nitrogen deposition[27,28]. If uptake of atmospherically deposited nitrogen substitutes for nitrogen mineralized in soil that would otherwise be taken up by trees, or if biomass or soil C:N ratios decrease with elevated nitrogen inputs, the effect of nitrogen deposition on carbon sequestration as estimated in Table 2 would be diminished. Therefore, our estimate of 0.25 Pg CO_2-carbon uptake yr^{-1} resulting from nitrogen deposition is likely to be an upper limit.

In summary, despite some uncertainties, our results indicate that nitrogen deposition currently accounts for $<$20% of the annual 1.5–1.9 Pg CO_2-carbon uptake attributed to forest growth. Although recent budgetary and modelling analyses indicate that nitrogen deposition could account for an annual uptake of 0.1–2.3 Pg CO_2-carbon from the atmosphere[1,3,4], our analysis suggests that the effect of elevated nitrogen deposition on carbon uptake by forests is at the low end of this range of estimates. Other factors, such as long-term redistribution of nutrients from soils to trees, resulting from reforestation or climate warming, are more likely to be responsible for the putative carbon sink in northern mid-latitude forests. Finally, because rates of nitrogen deposition in boreal, subtropical and tropical regions are projected to increase in upcoming decades[2], it is important to determine whether the relationship we have observed between nitrogen deposition and carbon sequestration in temperate forests applies to forests in higher and low latitudes. □

Methods

Sites. The research was conducted in nine closed-canopy forests (dominant trees $>$35 years old at the start of treatments) in Europe and the northeastern United States (Table 1). The soils vary in texture, depth of forest floors and depth of solum, but all can be considered podzolic or gleyic. Ambient nitrogen deposition (throughfall ammonium plus nitrate) at the sites ranges from 4 to 58 kg ha^{-1} yr^{-1}. Detailed descriptions are available elsewhere[19–23].

^{15}N additions. In all cases, ^{15}N tracers were added systematically to forest floors of large plots for at least an entire growing season. Two tracer experiments were conducted at Bear Brooks (Maine, USA). In the first, $^{15}NO_3$ was mixed with nitric acid applications (added at either 28 or 56 kg nitrogen ha^{-1} yr^{-1}) to replicate 15 m $\times$ 15 m plots ($n = 3$) across three growing seasons (August 1988 through October 1991). In the second experiment, $^{15}NH_4$ was mixed with ammonium sulphate fertilizer additions (25 kg ha^{-1} yr^{-1}) to a 10-ha watershed for two seasons (April 1991 to December 1992). Tracer experiments at the Harvard Forest (Massachusetts, USA) were conducted in a red pine forest and in an oak-dominated deciduous forest. In both forests, tracers were applied to single fertilized (50 kg nitrogen ha^{-1} yr^{-1} as NH_4NO_3 solutions) and non-fertilized plots (30 m $\times$ 30 m) in each forest type for two growing seasons (1991 and 1992). Each Harvard Forest plot was split with respect to ^{15}N-ion additions, with one half of a lot (15 m $\times$ 30 m) receiving $^{15}NH_4$ and the other half receiving $^{15}NO_3$. The NITREX plot treatments included nitrogen fertilization (NH_4NO_3 at Aber, Alptal, Gårdsjön, Klosterhede and also $NaNO_3$ at two fertilization levels at Aber) and nitrogen removal from throughfall using sub-canopy roofs (Speuld, Ysselsteyn). ^{15}N tracers were added to plots subject to ambient nitrogen deposition at Klosterhede, Speuld and Ysselsteyn. Tracers were added as $^{15}NH_4$ at Speuld and Ysselsteyn, both to ambient plots and beneath roofs. Both $^{15}NH_4$ and $^{15}NO_3$ were added together at the other NITREX plots, except for the $NaNO_3$-fertilized plots at Aber, which received only $^{15}NO_3$. NITREX plot sizes ranged from 100 to 1500 m^2 and tracers were added across a single calendar year (April or May 1992 to April or May 1993 at five sites; April 1995 to April 1966 at Alptal).

Tracer Recoveries. Analyses for ^{15}N were conducted on samples collected before and at the end of tracer additions using isotope-ratio mass spectrometry[30]. Although samples were collected shortly after the final tracer additions at most sites, samples collected 9 months after labelling were used for assessing tracer movements at three of the sites (Speuld, Ysselsteyn and Alptal). Recoveries of ^{15}N tracers were estimated by mass balance[30].

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The *mahogany* protein is a receptor involved in suppression of obesity

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Genetic studies have shown that mutations within the *mahogany* locus[1] suppress the pleiotropic phenotypes, including obesity, of the *agouti-lethal-yellow* mutant[2,3]. Here we identify the *mahogany* gene and its product; this study, to our knowledge, represents the first positional cloning of a suppressor gene in the mouse. Expression of the *mahogany* gene is broad; however, *in situ* hybridization analysis emphasizes the importance of its expression in the ventromedial hypothalamic nucleus, a region that is intimately involved in the regulation of body weight and feeding. We present new genetic studies that indicate that the *mahogany* locus does not suppress the obese phenotype of the melanocortin-4-receptor null allele[4] or those of the monogenic obese models (*Lep^{db}*, *tub* and *Cpe^{fat}*). However, *mahogany* can suppress diet-induced obesity, the mechanism of which is likely to have implications for therapeutic intervention in common human obesity. The amino-acid sequence of the *mahogany* protein suggests that it is a large, single-transmembrane-domain receptor-like molecule, with a short cytoplasmic tail containing a site that is conserved between *Caenorhabditis elegans* and mammals. We propose two potential, alternative modes of action for mahogany: one draws parallels with the mechanism of action of low-affinity proteoglycan receptors such as fibroblast growth factor and transforming growth factor-β, and the other suggests that mahogany itself is a signalling receptor.

Signalling from α-melanocyte-stimulating factor (α-MSH) through the melanocortin receptors Mc1r and Mc4r regulates pigmentation and body weight, respectively. Overexpression of agouti, an antagonist of Mc1r and Mc4r (refs 5, 6), results in yellow, obese mice. The murine *mahogany* gene (*mg*) acts in a dosage-dependent manner within the agouti pathway to compensate for agouti overexpression and for lack of signalling from an *Mc1r* null allele[2,3,7]. Mice homozygous for both *mg* and a null allele of *Mc1r* (recessive yellow, *Mc1^{e}*) are yellow, as are *Mc1r^{e}*/*Mc1r^{e}* mice, indicating that *mg* does not act downstream of *Mc1r* (ref. 2). We performed a similar experiment with obese *Mc4r* (ref. 4) knockout mice (Fig. 1). For both sexes, all the animals homozygous for the *Mc4r* null allele were roughly equally obese and were heavier than wild-type mice, independently of the *mg* genotype. These data strengthen and confirm the data obtained with *Mc1r* knockouts, strongly indicating that *mg* acts at or upstream of both melanocortin receptors.

It has been assumed that *mg* acts specifically within the agouti pathway. We tested this by asking whether *mg* can suppress the obesity of other monogenic obese mouse mutants and whether it can suppress diet-induced obesity. We set up appropriate genetic crosses to produce mice that segregated *mg* and one of the mouse obesity mutations, *Cpe^{fat}*, *tub*, or *Lepr^{db}*. No suppression of obesity by *mg* was seen for any of the monogenic obese mutants (Fig. 2a–c), lending credence to the assumed specificity of action of *mg* within the agouti pathway. To determine whether *mg* can suppress diet-induced obesity, we weaned C3HeB/FeJ-*mg^{3J}* and C3H/HeJ mice onto either normal chow (physiological fuel value (PFV) 3.63 kcal g^{-1} with 9% fat) or a high-fat diet (PFV 4.53 kcal g^{-1} with 42.2% fat). Converting the grams of food consumed to calories indicated that male C3H/HeJ mice fed on normal chow or the high-fat diet consumed ~97 kcal per week and 96 kcal per week, respectively; male C3HeB/FeJ-*mg^{3J}* mice fed on normal chow or the high-fat diet consumed ~83 kcal per week and ~81 kcal per week, respectively. Despite the equal calorie intake, the C3H/HeJ mice on the high-fat diet gained more weight than the C3H/HeJ mice fed on normal chow (P=0.0004). In contrast, the C3HeB/FeJ-*mg^{3J}* mice on either diet showed no statistically significant difference in weight (Fig. 2d). Female data (not shown) showed the same trends, although there

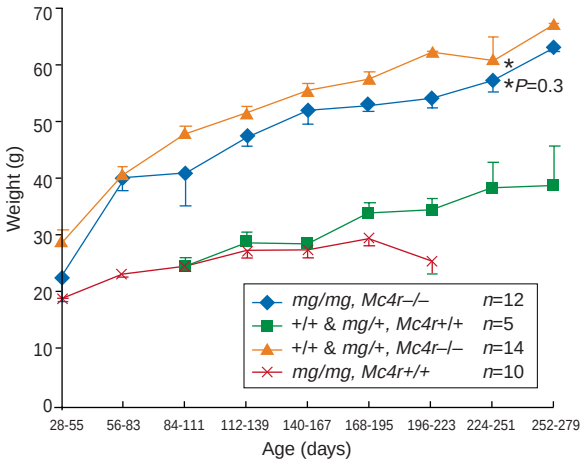

Figure 1 Effect of *mg* on *Mc4r*$^{-/-}$-induced weight gain in females. Values shown are the means ± s.d. within a designated time interval. We performed a *t*-test on the weights of *mg*/*mg*, *Mc4r*$^{-/-}$ animals and +/+, *Mc4r*$^{-/-}$ or *mg*/+, *Mc4r*$^{-/-}$ animals at days 224–251 and found no statistically significant differences in either the female or the male (data not shown) data.