gradient. Of course, genetic variation other than proteorhodopsin tuning is likely to influence the fitness of population variants along the depth gradient. Other genetic loci may also co-vary with tuned absorption maxima, indicating a depth-optimized adaptation for these other traits as well. Genomic approaches should provide a means to test such hypotheses.

Proteorhodopsin-mediated phototrophic energy generation may have a significant impact on carbon and energy flux in the ocean. Although it is not known whether proteorhodopsin-bearing bacteria fix CO_2, their ability to generate energy from light should reduce overall respiratory energy requirements, as has been suggested for aerobic, bacteriochlorophyll-containing marine bacteria[19]. Bioenergetic calculations based on our data suggest that proteorhodopsin-based phototrophy could provide a large fraction of energy requirements for cell maintenance and reproduction. The widespread distribution and high abundance of SAR86 bacteria[5,10,20–22], combined with the biophysical and genetic data that we report, indicate that proteorhodopsin phototrophy is a biologically significant, globally distributed marine microbial process. □

Methods

Cell collection and membrane preparation

Antarctic coastal waters were collected off Palmer Station (64.4° S, 64.0° W), Anvers Island, Antarctic Peninsula, in August 1996 (ref. 23). Hawaiian HOT station (22.4° N, 158.0° W) waters were collected on 17 March 1998. Cell membranes were prepared from picoplankton cells concentrated from surface seawater (700 l) collected in early winter 2000, roughly 26 miles offshore from Moss Landing, California (36.7° N, 122.4° W). Water samples were prefiltered through a GF/A glass-fibre filter (approximate particle size $< 0.6\ \mu m$) to remove the larger eukaryotic phytoplankton cells. We concentrated the filtrate by tangential flow filtration[10] and prepared the membranes[24] as described. Aliquots of the cell preparation were fixed in 3.7% formalin for subsequent fluorescence *in situ* hybridization and taxon-specific cell quantification.

Fluorescence *in situ* hybridization

BAC clones 31A08 and 27G05 (ref. 10) were used as templates to generate SAR86-specific polyribonucleotide probes targeted to large-subunit ribosomal RNA. We prepared fluorescently labelled polyribonucleotide probes that targeted a variable region of large subunit rRNA by *in vitro* transcription (M. Leclerc *et al.*, manuscript in preparation), and carried out subsequent whole-cell hybridization assays as described[25]. The percentage of SAR86-type cells relative to total 4′,6-diamidino-2-phenylindole dihydrochloride (DAPI)-stained cells was calculated as described[25].

Spectroscopy

One-half of the cell-membrane suspension (corresponding to 350 l of seawater) was suspended in 2 ml of 100 mM sodium phosphate buffer, pH 7.0. Absorption spectra were measured on an Aminco DW2000 UV-visible absorption spectrophotometer (SLM; Urbana, IL). Flash-induced absorption transients in the millisecond to seconds time domain were acquired on a digital oscilloscope (Nicolet, Integra20) after a Nd-YAG laser flash (532 nm, 6-ns pulse duration, 40 mJ; Continuum, Surelight I) in a laboratory-constructed flash-photolysis system as described[6]. Each transient was obtained by averaging 64 acquisition sweeps collected at 30-s intervals at various wavelengths between 360 and 680 nm. We performed kinetic analyses with the program IGOR Pro, v3.1 (WaveMetrics, Lake Oswego, OR).

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Large-scale forest girdling shows that current photosynthesis drives soil respiration

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The respiratory activities of plant roots, of their mycorrhizal fungi and of the free-living microbial heterotrophs (decomposers) in soils are significant components of the global carbon balance, but their relative contributions remain uncertain[1,2]. To separate mycorrhizal root respiration from heterotrophic respiration in a

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boreal pine forest, we conducted a large-scale tree-girdling experiment, comprising 9 plots each containing about 120 trees. Tree-girdling involves stripping the stem bark to the depth of the current xylem at breast height terminating the supply of current photosynthates to roots and their mycorrhizal fungi without physically disturbing the delicate root–microbe–soil system. Here we report that girdling reduced soil respiration within 1–2 months by about 54% relative to respiration on ungirdled control plots, and that decreases of up to 37% were detected within 5 days. These values clearly show that the flux of current assimilates to roots is a key driver of soil respiration; they are conservative estimates of root respiration, however, because girdling increased the use of starch reserves in the roots. Our results indicate that models of soil respiration should incorporate measures of photosynthesis and of seasonal patterns of photosynthate allocation to roots.

Flux measurements suggest that boreal forests can be either sources or sinks for atmospheric carbon dioxide (CO_2; refs 3, 4), and that there is considerable interannual variability in this respect[5,6]. The forest carbon (C) balance is the net result of CO_2 fixation by photosynthesis occurring above ground and the release of C as CO_2, notably from the below-ground compartment through the respiratory activities of plant roots, their symbiotic mycorrhizal fungi and the free-living microbial and faunal populations of the soil. Of the two compartments, the below-ground system is the most difficult to evaluate[2].

Almost all approaches to its study have used methods that physically disturb the intimately linked processes in which C is allocated to fine roots, their mycorrhizal symbionts, and through these to the wider soil community. Estimates of the contribution of root respiration to total soil respiration vary from 10 to 90% (ref. 2). Although some of this variability reflects differences among types of ecosystems, a considerable proportion is probably caused by problems of experimental design or methodology. As a result, our knowledge of the absolute and relative contributions of different components of the root–heterotroph–soil continuum to the forest C balance is limited[2].

The soil remains a 'black box' despite the need to obtain accurate models of this vital ecosystem compartment. This gap in our understanding becomes particularly serious in the context of boreal forests—one of the two most extensive terrestrial biomes on earth, which accounts, together with adjacent ecosystems of high latitudes, for the largest global capital of soil organic C (ref. 7). The identification of factors that control C source–sink relations is a prerequisite for understanding the boreal ecosystem C balance and the potential effects of raised temperatures, atmospheric CO_2 concentrations and deposition of nitrogen.

To determine the main drivers of soil respiration and to evaluate more precisely the relative contributions to CO_2 efflux made by the different biota, we conducted a large-scale stem-girdling experiment in a boreal Scots pine (*Pinus sylvestris* L.) forest in northern Sweden. Girdling has the instantaneous effect of terminating the flux of photosynthates from the tree canopy through the phloem to the roots, while enabling water transport in the reverse direction through the xylem. Thus, girdling has no immediate impact on the principal physical soil parameters, moisture and temperature, nor does it physically displace roots and soil organisms or sever roots and fungal hyphae.

Retention of the integrity of the root–fungus pathway is especially important in ecosystems of this type, in which the surfaces of virtually all roots are ensheathed by a thick mantle of fungal hyphae to form ectomycorrhizas[8]. The extensive but inherently delicate mycelial system extending from these symbiotic structures into the soil not only scavenges for mineral nutrients required by the trees, but also provides the conduits through which the C passes to enable the fungal sexual structures—the sporocarps—to develop.

To indicate the potential of influx of C from ungirdled trees into plots with girdled trees, we examined the distribution of ectomycorrhizal sporocarps. To avoid edge effects, which are inevitably associated with small-scale girdling experiments, our plots covered large (900 m^2) areas. Soil respiration was measured within a metre of the centre of each plot. All trees in six such large plots, with about 120 trees in each plot, were girdled either in early June (early girdled) or in August (late girdled). This duplication was designed

Table 1 Sporocarps of ectomycorrhizal fungi and starch in fine roots

(a) Ectomycorrhizal fungi*

Treatment	No. of species	No. of sporocarps	Biomass (g dry wt)
Control	11 ± 3	252 ± 116	84.4 ± 24.4
Early girdling	1 ± 1 (72)	4 ± 4 (72)	0.5 ± 0.5 (72)
Late girdling	10 ± 1 (2–3)	108 ± 48 (2–3)	40.9 ± 8.5 (2–3)

(b) Concentrations of starch in fine roots†

	Starch (% of dry wt)		
Treatment	July 6	August 17	October 16
Control	10.3 ± 1.3	4.6 ± 0.5	5.1 ± 0.6
Early girdling	3.5 ± 1.4 (28)	1.0 ± 0.3 (68)	0.6 ± 0.1 (130)
Late girdling	ND	5.0 ± 0.5	1.5 ± 0.4 (59)

Data are means ± s.e.m. (n = 3) per plot. Figures in parentheses give the number of days since the girdling treatment.
* The number of species, the number of sporocarps and the biomass of ectomycorrhizal fungi on the central 100 m^2 of the experimental plots in the different treatments in the tree girdling experiment at Åheden.
† Concentrations of starch in fine (< 2 mm in diameter) roots of *P. sylvestris*.

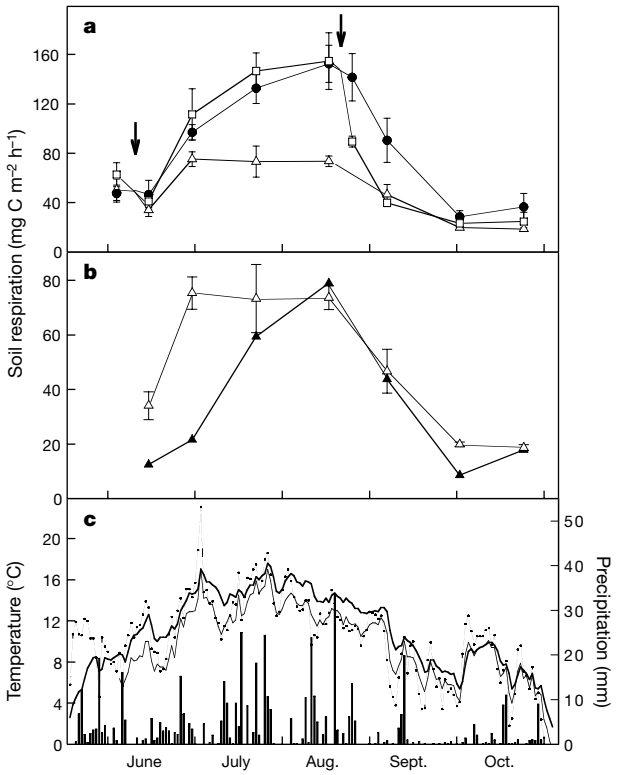

Figure 1 Soil respiration in the different tree-girdling treatments in Scots pine forest at Åheden in relation to meteorological data. **a**, Respiratory soil CO_2 efflux from ungirdled control (filled circles), early girdled (open triangles) and late girdled (open squares) plots. **b**, Calculated root respiration (filled triangles, respiration on control plots minus respiration on early girdled plots) and heterotrophic respiration (open triangles, respiration on early girdled plots). **c**, Open-field meteorological station data for precipitation (bars), daily averages of air temperature and soil temperature at 5-cm soil depth in the mineral soil (broken and thick solid lines, respectively), and average soil temperatures at 4-cm depth below the mor layer but above the mineral soil (thin solid line), in the studied forest. Arrows indicate times of early and late girdling.

to allow the detection of any phenological effects on respiratory activity. Three plots of equivalent size were established in adjacent areas but left ungirdled as controls. The respiratory activity remaining on girdled plots was ascribed to heterotrophic activity, whereas the difference in respiration between control and girdled plots was ascribed to respiration by mycorrhizal roots. Concentrations of starch were measured to determine whether girdling resulted in increased use of stored carbohydrates in roots.

Two months after the initiation of the early girdling, comparisons of the numbers and biomass of ectomycorrhizal fungal fruit bodies in the central 100 m^2 of the early girdled and control plots showed that these structures had been virtually eliminated by the girdling procedure (Table 1a). An increasing abundance of sporocarps closer to the edge of treated plots (data not shown) suggested an influence of untreated trees outside treated plots. These observations supported the use of 900-m^2 plots, and especially the use of the centre of the plots for measurements of treatment effects on soil respiration. The observations also support the view, based mainly on experiments carried out in laboratory microcosms (for example, see ref. 9), that the ectomycorrhizal mycelial system is strongly dependent on current assimilates. In contrast, there was no reduction in the numbers or biomass of saprotrophic fungi in the girdled relative to the control plots (data not shown).

Soil respiration on the control plots followed a seasonal pattern with rates of 20–60 mg CO_2–C m^{-2} h^{-1} early and late in the growing season, and with a maximum of 150 mg CO_2–C m^{-2} h^{-1} in mid-August (Fig. 1a). In the early girdled plots, soil respiration showed within 5 days a decrease of 27% relative to that measured in control plots. This relative difference between treatments was retained for a further 2 weeks, although soil respiration increased in both treatments. By this time, at the end of June, respiration in the early girdling treatment had reached its maximum. Respiration on control plots continued to increase for a further 7 weeks until mid-August, indicating that the increase was due to root rather than heterotrophic activity. This resulted in a 52% lower respiration on early girdled plots compared with on control plots. The late girdling produced even faster effects; soil respiration declined by 37% relative to control plots within 5 days of treatment (Fig. 1a). This precipitous drop in activity continued such that by 2 weeks the CO_2 efflux was 56% lower than in control plots. As a result, the respiratory activity of the late girdled plots rapidly reached a level almost identical to that seen in early girdled plots (Fig. 1a).

Two factors will have contributed to the more rapid response of soil respiration in the late girdling treatment. First, temperate forest conifers preferentially allocate C to support shoot, needle and bud expansion in the early part of their growing season[10]. Second, at the same time roots can contain twice the concentration of starch seen later in the summer[11], as confirmed by our data from control plots (Table 1b). As a consequence, root respiration is less dependent on C import in the early growing season than in the late growing season.

On the basis of root biomass data from this forest stand[12], and the depletion of starch observed after the early girdling (Table 1b), we calculated an average loss of about 10 mg CO_2–C m^{-2} h^{-1}, which we assumed was due to use of starch during the first month after the early girdling. This corresponds to 10–20% of the respiratory flux from the soil during this period of June–July (Fig. 1a). The use of starch reserves after July in the early girdling treatment and by late girdled trees was calculated to be of less significance for the soil CO_2 efflux. Thus late, as opposed to early, girdling curtails C supply to the roots at a time when both flow of, and demand for, photosynthates are at a maximum.

With the respiration from early girdled plots used as a proxy for heterotrophic respiration, we calculated the levels of respiration by roots and their mycorrhizas by subtracting the values of respiratory activity on the early girdled plots from those on the control plots (Fig. 1b). Early in the summer, calculated root respiration increased later than heterotrophic respiration, whereas during the autumnal decline (Fig. 1c) heterotrophic and root respiration were similar (Fig. 1b). However, the apparently greater heterotrophic respiration early in the summer is probably an overestimate, owing to the 10 mg CO_2–C m^{-2} h^{-1} use of starch by the roots (see above) in the period immediately after girdling. When this amount is subtracted from the heterotrophic component, and, as is appropriate, added to the values obtained for root respiration, the curves for CO_2 production by the two compartments run closer together.

Several factors suggest that our estimates of a 52–56% contribution of root-mycorrhizal respiration to overall soil CO_2 efflux are conservative. Girdling can be expected not only to lead to a transient increase in the use of starch reserves of the roots, as observed by us (Table 1b), but also potentially to enhanced decomposition of starved roots and their symbionts by heterotrophs. Both of these processes should contribute to an increase, rather than a decrease, in soil respiration. In addition, all our plots supported small populations of the dwarf shrubs *Vaccinium vitis-idaea* L. and *Calluna vulgaris* L., whose root respiration will have contributed to the values attributed to non-root respiration from girdled plots. But even our conservative estimates of the contribution from root respiration are still considerably greater than those reported from the boreal zone, which were based on less direct and more intrusive methods.

In a study of *Picea mariana* in Canada, tree root respiration was calculated, on the basis of plant below-ground C allocation and estimates of root growth, to constitute only 24% of total soil respiration[3]. In a young Swedish Scots pine forest located 500 km to the south of the one used in our study, root respiration, also calculated on this basis, accounted for only 10 of the 63% of assimilates allocated below ground[13]. Our results contrast markedly with those of the only reported girdling experiment[14], which was carried out in temperate deciduous forest but found no effect of the treatment on soil respiration over the next 2 years. However, this experiment, which was unreplicated, used plots so small as to make edge effects likely.

Our experiment shows clearly that the flux of photosynthates has a big impact on soil respiratory losses—these being more influenced by seasonality than by variations in soil temperature. Thus, for example, the highest levels of root and total respiration were found in mid-August, well after both the maximum solar irradiance at the end of June and a long period of high air and soil temperatures in July (Fig. 1c). Previous studies have focused on the role of soil temperature as determinant of soil respiration (such as ref. 15). Our results indicate that the seasonal pattern of below-ground C allocation may be more important than soil temperature in determining root respiration. Improved models of forest C balances will be necessary to mitigate the process of global climate change, and we have shown that such models, when applied to the largest terrestrial ecosystem of the northern hemisphere, must take into account the fact that roots and their fungal symbionts contribute considerably more to soil respiratory C loss than acknowledged previously. Because these losses are driven directly by current assimilate allocated from the canopy, the coupling of photosynthetic activity to soil C efflux emerges as a key determinant of the ecosystem C budget, as has been suggested by a study of a temperate grassland[16]. Clearly, seasonal variations in these C allocation fluxes need to be considered in addition to the conventional physical parameters, if realism is to be achieved in assessing global C budgets. □

Methods

Girdling experiment

Nine square plots of 900 m^2 and 120 ± 12 trees each were established in a naturally regenerated 45–55-year-old Scots pine (*Pinus sylvestris* L.) forest at Åheden, northern Sweden (64° 14′ N, 19° 46′ E, 175 m above sea level). The soil is a weakly podzolized sediment of sandy silt. There is a sparse understorey of the dwarf shrubs *Vaccinium vitis-*

idaea L. and *Calluna vulgaris* L. In early June 2000, the trees on three plots were girdled at 1.5-m above ground, by complete removal of the bark over 0.3-m long sections around the circumferences of the stems (early girdled plots). In mid-August 2000, we girdled the trees on three other plots (late girdled plots). On both occasions, it took 2 people 4 days to girdle all (~360) the trees. Three plots of ungirdled trees were used as controls throughout the experiment. Air temperature, soil temperature at 5-cm soil depth in the mineral soil, and precipitation were measured continuously at a standard meteorological station in an open field 150 m from the centre of the experiment (Fig. 1c). On one early girdled, one late girdled and one control plot, sensors below the organic mor layer (4-cm deep) recorded soil temperatures continuously (Fig. 1c). This, and more detailed measurements of surface soil temperatures and gravimetric soil moisture contents revealed no changes in response to the girdling treatments during the period of study.

Analysis of soil respiration

Soil respiration was measured as described previously[17] on nine occasions during June to October. Within 1 m of the centre of each plot, three 0.0464-m^2 plastic cylinders were placed on the ground before measurements, the superficial layer of lichens, mosses and litter was temporarily removed, and a lid was placed on each cylinder to form a 6-l headspace. Starting 2 min after the lid was put on, five 12-ml gas samples were removed by a syringe through a rubber membrane in the lid at 2-min intervals. At the two last sampling times, when soil respiration rates were low, the intervals between samples was 4 min. Gas samples were transferred to pre-evacuated vials, which were analysed for their contents of CO_2 on a gas purification module coupled on-line to an isotope-ratio mass spectrometer[17]. The rate of CO_2 evolution from the soil was calculated by linear regression. In mid-August, 2–3 days after the last girdling and during the optimal period of fruiting, sporocarps of fungi were collected from the ground in the central 100-m^2 area of each plot. These were identified by species, and classified into two functional categories, ectomycorrhizal or saprotrophic. The number of species, the number of sporocarps, and their total dry biomass was determined. We sampled roots for starch analysis on three occasions (Table 1b). Fine (< 2 mm diameter) roots of *P. sylvestris* were extracted from auger samples taken just outside the central 100-m^2 plots, dried (70 °C, 48 h) and analysed for starch[18]. For comparisons among treatments, we used plot mean values. In calculations of the contribution of root respiration to total soil respiration, we used treatment mean values.

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Proximity signal and shade avoidance differences between early and late successional trees

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Competitive interactions between plants determine the success of individuals and species. In developing forests, competition for light is the predominant factor. Shade tolerators acclimate photosynthetically to low light[1–3] and are capable of long-term survival under the shade cast by others, whereas shade avoiders rapidly dominate gaps but are overtaken in due course by shade-tolerant, later successional species. Shade avoidance[4–6] results from the phytochrome-mediated perception of far-red radiation (700–800 nm) scattered from the leaves of neighbours, provides early warning of shading[7], and induces developmental responses that, when successful, result in the overgrowth of those neighbours[8]. Shade tolerators cast a deep shade, whereas less-tolerant species cast light shade[9], and saplings tend to have high survivorship in shade cast by conspecific adults, but high rates of mortality when shaded by more-tolerant species[9]. Here we report a parallel relationship in which the shade-avoidance responses of three tree species are inversely proportional to proximity signals generated by those species. On this basis, early successional species generate small proximity signals but react strongly to them, whereas late successional species react weakly but generate strong signals.

Shade-avoiding plants respond to the relative amounts of red (R; 600–700 nm) and far-red (FR; 700–800 nm) photons in incident radiation by establishing different concentrations of the active 'Pfr' form of the phytochromes[10]. For many plants, the elongation growth rate of the stems bears an inverse linear relationship to the established concentration of Pfr, which is usually expressed as the proportion of total phytochrome (P) that is present as Pfr (that is, the photoequilibrium or Pfr/P)[11,12]. The slope of this relationship may be regarded as the 'shade avoidance response sensitivity'. This varies between species, with strong shade avoiders presenting a steep slope and weak shade avoiders presenting a shallow slope[13]. In addition, different species have different intrinsic elongation growth rates. These elements—intrinsic elongation rate and response sensitivity—combine to describe the overall shade avoidance response of plants in the natural environment. To explain the dynamics of tree growth in developing canopies, we sought to identify and quantify both intrinsic growth rates (R_i) and response sensitivities (S) of tree species with contrasting growth dynamics.

We grew stands of tree species at different spacings, and measured height growth, leaf area, leaf distribution and within-canopy radiation environment throughout several growing seasons. Three species of contrasting growth habits and leaf morphology were studied—*Acer pseudoplatanus* (a late successional species known as sycamore in the UK and sycamore-maple in North America), *Betula pendula* (silver birch, an early successional species) and *Populus deltoides* × *trichocarpa* cv. Beaupré (a hybrid poplar bred for rapid elongation growth). These species were grown in both pure stands (that is, single species) or in mixed stands that included another species, *Salix viminalis* (osier willow), chosen because it is a shrubby species with dense leaf coverage of stems and thereby