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Contrasting Soil pH Effects on Fungal and Bacterial Growth Suggest Functional Redundancy in Carbon Mineralization▽†

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The influence of pH on the relative importance of the two principal decomposer groups in soil, fungi and bacteria, was investigated along a continuous soil pH gradient at Hoosfield acid strip at Rothamsted Research in the United Kingdom. This experimental location provides a uniform pH gradient, ranging from pH 8.3 to 4.0, within 180 m in a silty loam soil on which barley has been continuously grown for more than 100 years. We estimated the importance of fungi and bacteria directly by measuring acetate incorporation into ergosterol to measure fungal growth and leucine and thymidine incorporation to measure bacterial growth. The growth-based measurements revealed a fivefold decrease in bacterial growth and a fivefold increase in fungal growth with lower pH. This resulted in an approximately 30-fold increase in fungal importance, as indicated by the fungal growth/bacterial growth ratio, from pH 8.3 to pH 4.5. In contrast, corresponding effects on biomass markers for fungi (ergosterol and phospholipid fatty acid [PLFA] 18:2ω6,9) and bacteria (bacterial PLFAs) showed only a two- to threefold difference in fungal importance in the same pH interval. The shift in fungal and bacterial importance along the pH gradient decreased the total carbon mineralization, measured as basal respiration, by only about one-third, possibly suggesting functional redundancy. Below pH 4.5 there was universal inhibition of all microbial variables, probably derived from increased inhibitory effects due to release of free aluminum or decreasing plant productivity. To investigate decomposer group importance, growth measurements provided significantly increased sensitivity compared with biomass-based measurements.

The soil microbial community is responsible for most nutrient transformations in soil, regenerating minerals that limit plant productivity. Fungi and bacteria are the two groups that dominate the microbial decomposer community, and, crudely defined, they share the function of decomposing organic matter in soil, indicating that there is a strong potential for interaction. There are potentially important differences between their properties, however, such as biomass elemental composition (21), nutrient demand (22, 53, 63, 65), turnover rate (54), metal tolerance (52), temperature dependence (51), and food web linkage (33, 37, 46). Consequently, anthropogenic impacts, such as changes in nutrient input, climate change, and soil management, have the potential to directly or indirectly affect the bacterial and fungal composition, with consequent impacts on soil function.

Efforts to distinguish between these two components of the microbial community have almost exclusively used biomass-based techniques, e.g., microscopy and biochemical markers, including phospholipids fatty acids (PLFAs) (28, 66) and glucose amines (5), as well as DNA-based molecular techniques (18, 44). However, biomass-based measurements are only indirectly related to process contribution, and estimating process contribution from biomass requires knowledge of its status (ranging from dormant to highly active), which often is difficult to differentiate. Attempts have been made to relate gross activity (respiration) to the biomass composition of fungi and bacteria (16, 17, 36). This causal connection is weak due to decoupling by variable growth efficiency (60, 61), i.e., variation in the proportion of substrate C used for biomass production compared with the amount expended for energy in respiration, which may result in a lack of correlation of basal respiration with direct estimates of fungal and bacterial growth (53, 55).

One of the most influential factors affecting the microbial community in soil is pH. pH strongly influences abiotic factors, such as carbon availability (4, 42), nutrient availability (1, 41, 42), and the solubility of metals (24, 25). In addition, soil pH may control biotic factors, such as the biomass composition of fungi and bacteria (23), in both forest (12, 17, 28) and agricultural (6, 15) soils. An inherent problem in studying soil pH effects is its varied influence on multiple parameters. Experimentally manipulating the pH of a soil may result in changes in several factors that are hard to separate. Conversely, comparing pHs of different natural soils introduces confounding factors, frequently unidentifiable, derived from differences in soil type and management regimen that also vary between soils.

Our aim was to assess how the relative importance of fungal and bacterial decomposers was affected by soil pH. Instead of inferring process contributions only from biomass estimates, we estimated them directly using measurements of fungal and bacterial growth. To do this, we studied the effects of soil pH on fungal and bacterial growth and biomass, as well as basal respiration, in soil samples obtained along the Hoosfield acid strip (1, 2), which has a pH gradient from pH 4.0 to 8.3, a uniform history of management regimen, and the same soil type.

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MATERIALS AND METHODS

Soil, sampling, and preparation. The soil in the Hoosfield acid strip at Rothamsted Research, United Kingdom, is classified as Typic Paleudalf (62) or Chromic Luvisol (26). Such soils were originally acidic and well drained to moderately well drained and developed in a relatively silty (loess-containing) superficial deposit overlaying and mixed with clay with flints. The topsoil is a flinty, silty clay loam (18 to 27% clay).

Hoosfield has been under arable management since before the 19th century. It probably received only a single heavy (150 to 250 tons ha^{-1}) dressing of chalk in the latter half of the 19th century. Chalk dressing was dug from infield "bell pits" or from "dell holes" on bordering slopes. The soil from the site which we studied (the Hoosfield acid strip) has not received any other amendment, including chemical or organic fertilizer, since then (A. E. Johnston and P. R. Poulton, personal communication). By the 1950s, the reserves of $CaCO_3$ remaining from the earlier application had become exhausted by leaching at distances furthest from the original chalk pits. At these locations the soil had become acidic (7).

We sampled along the first 180 m of the strip by taking 5-cm-diameter, 0- to 23-cm depth cores at each sampling position along the gradient. The gradient was sampled every 10 m between 0 and 40 m, then every 5 m between 40 and 120 m, and then every 10 m between 120 and 180 m of the gradient. The greater number of samples between 40 and 120 m was based on the faster pH changes found previously in that interval (1). The 27 resulting soil samples were sieved (<2.8 mm) in the laboratory, and water contents were determined (105°C, 24 h). The moisture contents of the soil samples were about 40% of the water-holding capacity, so further moisture adjustment was not required. The soil samples were incubated at 22°C for 1 week before the microbial analyses were performed. Three replicates from each soil sample were used for all microbial analyses except respiration ($n = 2$). Subsamples of the sieved soil were frozen until the PLFA analysis was performed 3 weeks later. Other sieved samples were air dried and ground (<180 μm) for chemical analyses. All measurements given below are based on soil oven dry weights (105°C, 24 h).

Between about pH 4.5 and 8.3 no differences in crop yield could be discerned; however, there was virtually no crop yield below pH 4.5 (J. C. Aciego-Pietri, unpublished Ph.D. thesis, University of Nottingham, United Kingdom, 2001). To reflect the difference, the data are presented separately as (i) data for pHs below pH 4.5 and (ii) data for pH 4.5 to 8.3 (see Discussion).

Bacterial growth. The bacterial growth rate was estimated using leucine (Leu) (43) and thymidine (TdR) (29) incorporation into bacteria extracted from soil using the homogenization and centrifugation techniques described by Bååth (8, 9) and Bååth et al. (14). Soil samples (1 g, fresh weight) were mixed with 20 ml distilled water using a multivortex shaker at maximum intensity for 3 min. This was followed by low-speed centrifugation at 1,000 × *g* for 10 min to create a bacterial suspension in the supernatant. Aliquots (1.5 ml) of this suspension were transferred to 2-ml microcentrifugation tubes, and 2 μl [^{3}H]Leu (37 MBq ml^{-1} and 5.74 TBq $mmol^{-1}$; Amersham) was added with nonlabeled Leu to each tube, resulting in 275 nM Leu in the bacterial suspensions. After 2 h of incubation, growth was terminated with 75 μl 100% trichloroacetic acid. Washing and subsequent measurement of radioactivity were performed as described by Bååth et al. (14). To ascertain that the measured bacterial growth response was not derived from altered communities with different abilities to incorporate the precursor Leu into bacterial protein along the gradient, we also used the independent precursor TdR for bacterial DNA. The same procedure was thus used with dual labels. Five microliters of [^{14}C]Leu (1.85 MBq ml^{-1} and 11.3 GBq $mmol^{-1}$; Amersham) and 5 μl of [^{3}H]TdR (37 MBq ml^{-1} and 925 GBq $mmol^{-1}$; Amersham), were added to each tube, resulting in 130 nM [^{3}H]TdR and 550 nM [^{14}C]Leu in the bacterial suspensions. The amounts of Leu and TdR incorporated into extracted bacteria per hour per gram of soil were used as measures of bacterial growth.

Fungal growth and biomass. Fungal growth was assessed using the acetate-into-ergosterol-incorporation method (47) adapted for soil (11), with modifications. Briefly, 1 g (fresh weight) of soil was transferred to test tubes to which 20 μl [1-^{14}C]acetic acid (sodium salt; 7.4 MBq ml^{-1} and 2.04 GBq $mmol^{-1}$; Amersham), 480 μl 1 mM unlabeled sodium acetate, and 1.5 ml distilled water were added, resulting in a final acetate concentration of 220 μM. The resulting soil slurry was incubated at room temperature (22°C) without light for 4 h, after which 1 ml 5% formalin was added to terminate growth. Ergosterol was then extracted, separated, and quantified using high-performance liquid chromatography and a UV detector (282 nm) (52). Fungal biomass was estimated by assuming that 5 mg of ergosterol corresponded to 1 g of fungal biomass (38, 57). The ergosterol peak was collected, and the amount of incorporated radioactivity was determined. The amount of acetate incorporated into fungal ergosterol per hour per gram of soil was used as a measure of fungal growth.

Respiration. Three grams (fresh weight) of soil was weighed into 20-ml glass vials, and the vials were sealed. After 30 min, any surplus CO_2 in the atmosphere in the respiration vials was purged with pressurized air, setting the initial CO_2 level to the ambient level, after which the vials were sealed with crimp caps and incubated in the dark at 22°C for 22 to 24 h. The CO_2 content was determined using gas chromatography.

Biomass composition of the community. The PLFA pattern was determined using 2 g of frozen soil as described by Frostegård et al. (28). The PLFAs chosen to indicate bacterial biomass were i15:0, a15:0, i16:0, 16:1ω9, 16:1ω7c, 10Me16:0, cy17:0, i17:0, a17:0, 18:1ω7, and cy19:0, while PLFA 18:2ω6,9 was used to indicate fungi (27). The fatty acid concentrations were estimated using 19:0 as an internal standard.

Soil chemical analyses. Soil pH was measured at a soil/water ratio of 1:2.5 (wt/wt). Air-dried soil (10 g, <2.8 mm) and 25 ml distilled water were shaken together for 2 min and left to settle for 30 min, this procedure was repeated once, and then the pH was determined with a pH electrode. Total N and organic C were measured by dry combustion using a Leco CNS-2000 autoanalyzer.

RESULTS

Soil chemical analyses. Starting at about pH 8.0 at the gradient origin, the pH reached a maximum of 8.3 at 10 m along the gradient. After 50 to 60 m along the gradient (pH 8.0) the pH declined almost linearly to about 4.1 at 115 m (Fig. 1A). After 115 m the pH remained between 4.0 and 4.1 until 180 m, the last sampling position (pH 4.1).

The soil organic C content increased rapidly from pH 4 to about pH 4.5, from about 0.75 to 0.90%, and then apparently plateaued at around this concentration, although there was considerable scatter in the data (Fig. 1B). The total soil N content showed a pattern similar to that of the soil organic C content, increasing from about 0.09 to 0.11% from pH 4 to 4.5 (Fig. 1C). Consequently, soil C/N values were quite stable, varying only between 8.4 and 9.2 over the entire gradient. These data are consistent with those previously reported for the same site (2).

Bacterial growth. Bacterial growth as indicated by [^{3}H]Leu incorporation was fastest at pHs above pH 7, after which it declined steadily with decreasing pH by 80%, from about 25 pmol Leu g^{-1} h^{-1} at pHs above pH 7.0 to about 5 pmol Leu g^{-1} h^{-1} at pH 4.5 (Fig. 2A) ($R^2 = 0.45$, $P = 0.005$). The rate of Leu incorporation continued to decrease to the lowest rate, about 1 pmol Leu g^{-1} h^{-1} at pH 4, equivalent to a total decrease in bacterial growth over the whole pH gradient of more than 90%. The results of the independent run measuring TdR and Leu incorporation with the dual labels [^{3}H]TdR and [^{14}C]Leu corroborated this result with identical representations of the bacterial growth response to pH (see Fig. S1 in the supplemental material). This resulted in linear relationships between [^{3}H]Leu and [^{3}H]TdR ($R^2 = 0.95$, $P < 0.0001$), as well as between [^{3}H]Leu and [^{14}C]Leu ($R^2 = 0.97$, $P < 0.0001$). The results for the dual precursors [^{14}C]Leu and [^{3}H]TdR also correlated well with each other in the same run ($R^2 = 0.97$, $P < 0.0001$).

Fungal growth. Fungal growth as indicated by acetate incorporation into ergosterol was related to pH in a very different way than bacterial growth (Fig. 2B). Between pH 8.3 and 4.5, fungal growth increased fivefold as an exponential function ($R^2 = 0.97$, $P < 0.0001$), from about 5 to about 25 pmol acetate g^{-1} h^{-1}, with decreasing pH. Below pH 4.5 the relationship with pH was reversed, and between pH 4.5 and 4.0 fungal growth decreased sharply from about 25 to 5

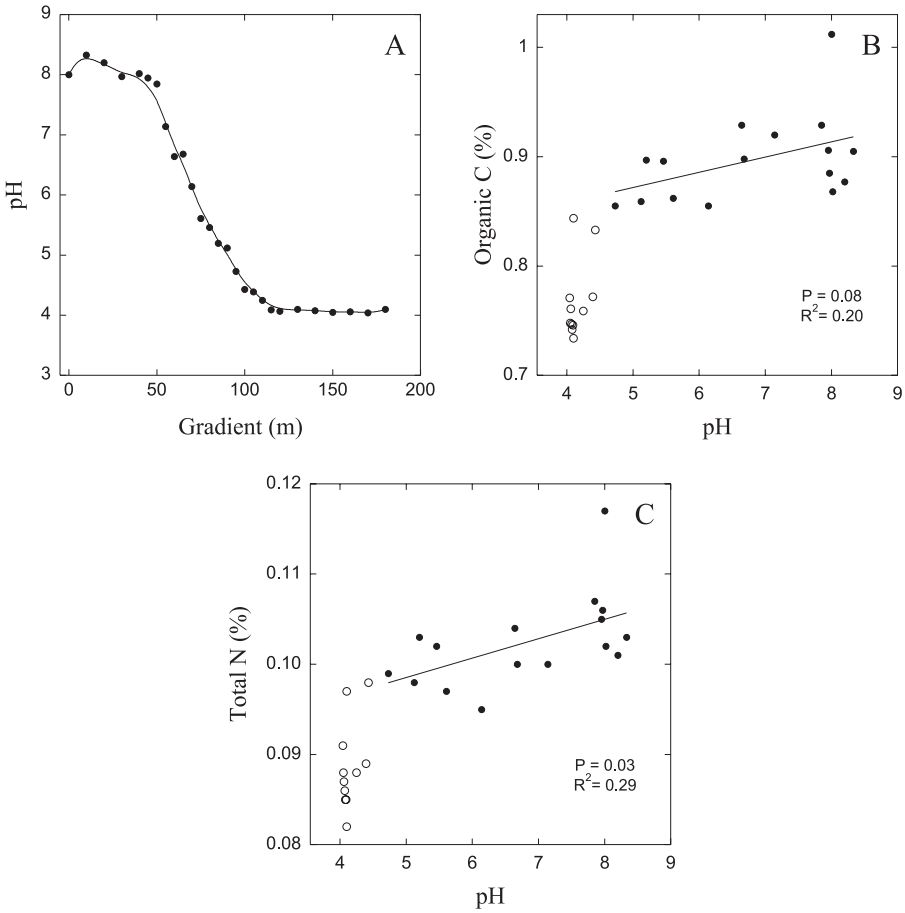

FIG. 1. Soil pH along the Hoosfield acid strip (A) and the effect of pH on organic C (B) and total N (C). Data for pHs less than pH 4.5 (open circles) were not used in the regression analyses (see Discussion).

pmol acetate g^{-1} h^{-1}. Thus, the maximum fungal growth occurred at about pH 4.5.

Fungal growth and bacterial growth were negatively correlated between pH 4.5 and 8.3 (Fig. 3A) ($R^2 = 0.66$, $P < 0.0001$). The fungal growth/bacterial growth ratio increased at lower pHs by a maximum of about 30-fold, from about 0.15 to about 5 between pH 8.3 and 4.5 (Fig. 3B) ($R^2 = 0.91$, $P < 0.0001$). Below pH 4.5 the growth ratio did not show a clear trend, and there was a broad range of ratios at pHs around pH 4.

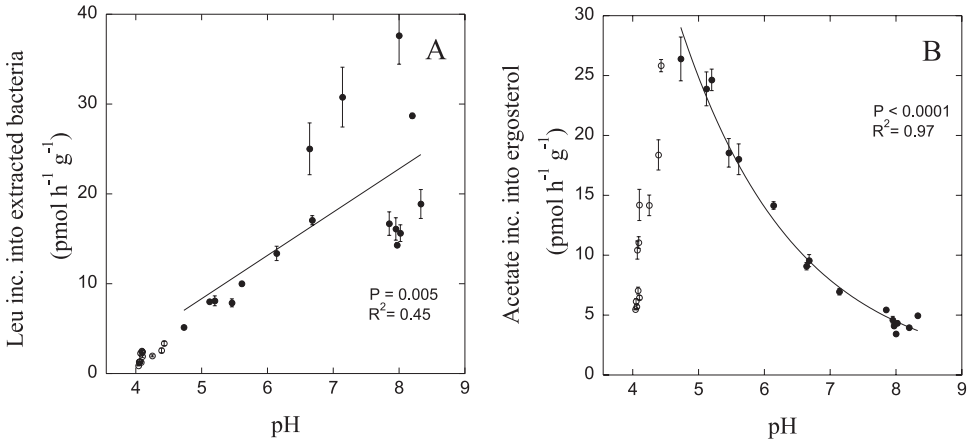

FIG. 2. Effect of pH on bacterial growth as measured by leucine incorporation (A) and on fungal growth as measured by acetate incorporation into ergosterol (B). Data for pHs less than pH 4.5 (open circles) were not used in the regression analyses (see Discussion). The error bars indicate ±1 standard error ($n = 3$). inc., incorporation.

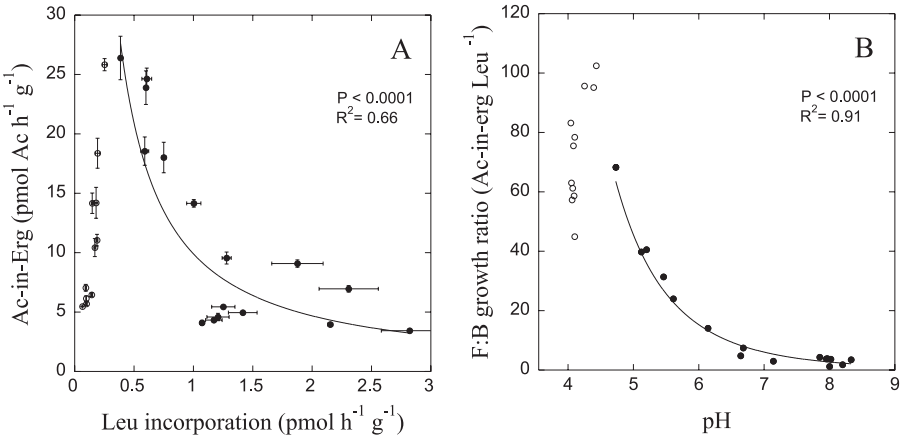

FIG. 3. Relationship between fungal growth (incorporation of acetate into ergosterol [Ac-in-Erg]) and bacterial growth (leucine incorporation) (A) and the effect of pH on the fungal growth/bacterial growth (F:B) ratio (pmol acetate $pmol^{-1}$ Leu) (B). Data for pHs less than pH 4.5 (open circles) were not used in the regression analyses (see Discussion). The error bars indicate ±1 standard error ($n = 3$).

Fungal and bacterial biomass. The bacterial PLFA concentration was highest at the highest soil pH, just over 20 nmol bacterial PLFA g^{-1}, and remained virtually unchanged until the pH was about 4.5 (Fig. 4A), and then it declined to the minimum level, about 10 to 14 nmol bacterial PLFA g^{-1}, at pH 4.

The initial fungal PLFA 18:2ω6,9 concentration was about 0.60 nmol g^{-1} at the highest pH, and then the concentration

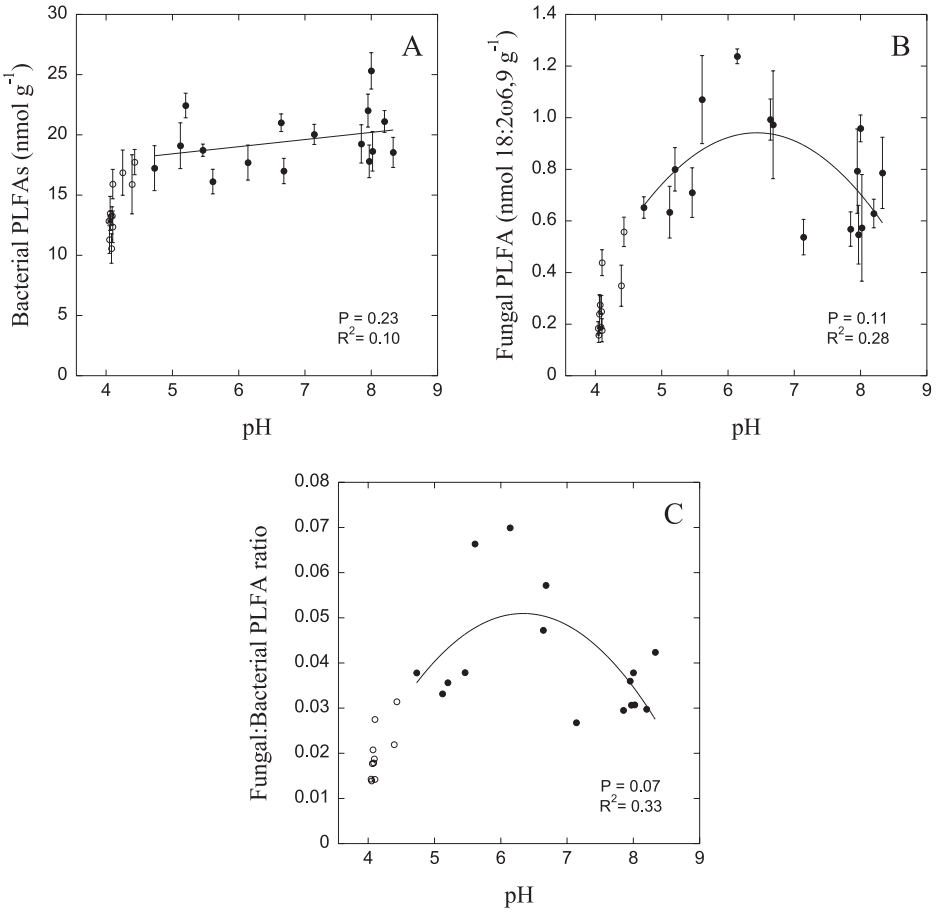

FIG. 4. Effect of pH on bacterial PLFAs (A), fungal PLFA (B), and the fungal PLFA/bacterial PLFA ratio (C). Data for pHs less than pH 4.5 (open circles) were not used in the regression analyses (see Discussion). The error bars indicate ±1 standard error ($n = 3$).

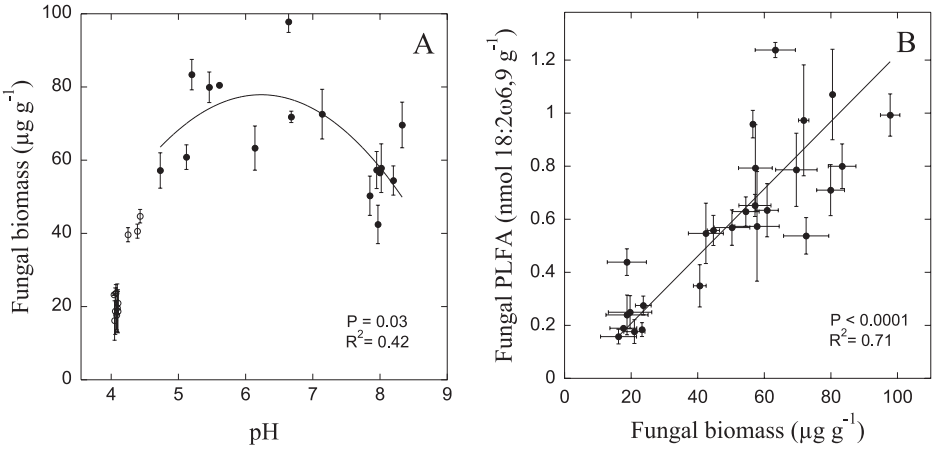

FIG. 5. Effect of pH on the fungal biomass estimated using ergosterol (A) and the relationship between fungal PLFA and ergosterol (B) analyzed using type II major axis linear regression analysis. The error bars indicate ±1 standard error (n = 3).

appeared to increase slightly to about 1 nmol g^{-1} at pH 6 and declined to about 0.65 nmol g^{-1} at pH 4.5 (Fig. 4B). The fungal PLFA concentration was lowest at pH 4, 0.20 nmol 18:2ω6,9 g^{-1}. The comparatively large influence of pH on the fungal PLFA compared to the marginal responses to pH of the bacterial PLFAs resulted in a fungal PLFA/bacterial PLFA ratio that closely mirrored the PLFA 18:2ω6,9 concentration (Fig. 4C), except for a somewhat slower decline below pH 4.5, when the bacterial PLFA concentration also declined.

The fungal biomass, estimated from the soil ergosterol concentration, was influenced by pH similar to the fungal PLFA marker; it started at about 50 μg g^{-1} at pH 8, increased to a maximum of about 80 μg g^{-1} at pH 6, and then decreased at lower pHs to about 60 μg g^{-1} at pH 4.5 (Fig. 5A) and to a minimum of about 20 μg g^{-1} at pH 4. The concentration of the fungal PLFA marker 18:2ω6,9 consequently correlated linearly with the fungal biomass estimated from ergosterol (R^2 = 0.71, $P < 0.0001$) (Fig. 5B), and 1 nmol 18:2ω6,9 corresponded to about 0.40 μg ergosterol, equivalent to 80 μg fungal biomass.

Respiration. Respiration decreased by about one-third toward lower pHs, from 0.55 μg CO_2 g^{-1} h^{-1} at pH 8 to 0.35 μg CO_2 g^{-1} h^{-1} at pH 4.5. Below pH 4.5 it decreased rapidly to a minimum of about 0.10 μg CO_2 g^{-1} h^{-1} at pH 4 (Fig. 5).

DISCUSSION

There appeared to be two separate effects acting on the microbial community along the pH gradient of the Hoosfield acid strip. This was most clearly seen in the fungal growth data, which showed that the peak growth rates were at pHs of about pH 4.5 (Fig. 2A). The narrow pH range between pH 4.0 and 4.5 induced dramatic decreases in the growth rate and biomass, and all of the microbial variables correlated well, suggesting that there is a threshold. Since in forest soils with pHs below 4 the fungal growth rates are higher than those in soils with higher pHs (50) and high biomass concentrations and respiration rates are maintained at pHs even below pH 4 (17), it is likely that it was not the pH per se that was the cause of the decreases in all variables below pH 4.5. Results of previous studies of the same pH gradient suggest two mechanisms for the general decreases in microbial parameters below pH 4.5: (i) below pH 5 a pronounced increase in the available aluminum (an increase from virtually zero above pH 5 to 600 mg Al kg^{-1} soil at pH 4 has been observed [2]), and (ii) crop growth, which decreases to virtually zero below pH 4.5 (Aciego-Pietri, unpublished Ph.D. thesis), decreasing the availability of easily available root-derived C as a substrate input. The lack of plant-derived C is corroborated by the decrease in the organic C level below pH 4.5 (Fig. 1B). Further work is required to rank the relative importance of these factors, but it is possible that the decline in crop yield was related to the high availability of Al and its toxicity, so that plant-derived C and Al toxicity to the microbial community are confounded. However, Aciego Pietri (unpublished Ph.D. thesis) monitored the development of microbial communities in soil samples from the same pH gradient in laboratory incubations following the addition of wheat straw as a substrate. The increase in cumulative respiration and biomass accumulation following substrate addition to soils below pH 4.5 did not differ markedly from the results for soil samples above pH 4.5, indicating that aluminum toxicity was not the limiting factor for the microbial communities in this soil. Irrespective of the mechanism, it is clear that the general inhibitory effects below pH 4.5 in the Hoosfield acid strip are very different from the pH effects above pH 4.5. For this reason, the analyses of the results (Fig. 1 to 6) and the remainder of the discussion concerning the influence of pH on microbial parameters focus exclusively on the pH range above pH 4.5.

The largest effect of pHs above pH 4.5 was on fungal and bacterial growth, and there were opposing pH effects. This resulted in a 30-fold increase in the relative importance of fungi (Fig. 3B), as indicated by the growth ratio; the highest ratio was at about pH 4.5. The influence of pH on bacterial growth has been investigated previously. Bååth and Arnebrant (13) reported that treatment of forest soils with lime and ash, which resulted in pH changes from about pH 4 to 7, increased bacterial growth about fivefold, as measured by TdR incorporation. Similarly, a study that included 19 different soils from areas with various land uses, spanning a pH range from 4 to 8, showed that there was an increase in bacterial growth with

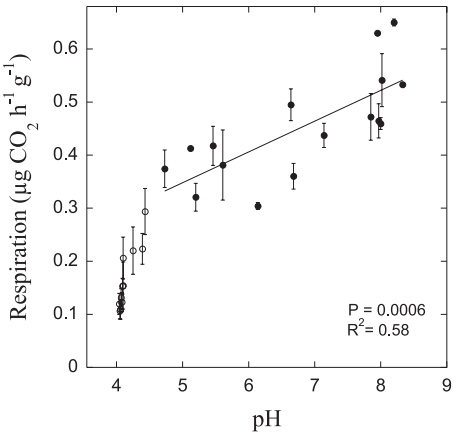

FIG. 6. Effect of pH on respiration. Data for pHs less than pH 4.5 (open circles) were not used in the regression analyses (see Discussion). The error bars indicate ±1 standard error ($n = 2$).

higher pHs as measured by Leu incorporation (10). Bacterial growth increased fourfold between pH 4 and pH 8.

There have been few joint determinations of both fungal and bacterial growth. Previous approaches to investigate the influence of soil pH on fungal and bacterial growth in soils either used soils with a much more restricted pH range (pH 3.6 to 4.1) in forest humus (50) or studied the acute effects of artificially increasing the pH on bacterial and fungal growth (6). However, in both cases increased bacterial growth and decreased fungal growth were found at higher pH. Thus, the general pattern reported previously is clearly corroborated by our results, suggesting increasing fungal dominance of decomposition, as indicated by the growth ratio, at lower soil pHs.

Respiration, a measurement of the total activity of the soil microbial community, was not as strongly affected by pHs between 4.5 and 8.3 as the microbial growth rates were (Fig. 6). A small effect on total activity during such a massive shift within the microbial community has been noted previously during decomposition of added plant material to soil (53) and in an experiment where the bacterial contribution to decomposition was completely inhibited with specific antibiotics (53). The small change in total activity during the shift between the contributions of fungi and bacteria to the process could indicate the complementarity of the two major decomposer groups involved in soil C mineralization, suggesting that they are, in effect, at least partially functionally redundant. It should be noted, however, that the processes of mineralization and microbial growth, despite the intuitive connection, are not directly linked (40, 59), since the partitioning of a substrate into growth and respiration varies, resulting in different growth efficiencies (60, 61).

The close correlation between the decline in bacterial growth and the increase in fungal growth as soil pH declines requires explanation. One potential explanation could be independent physiological limitations by pH of the separate decomposer groups; i.e., low hydrogen ion concentrations limit fungal growth, and high hydrogen ion concentrations limit bacterial growth, with no direct causal connection between the groups of organisms. The negative correlation between fungal growth and bacterial growth is indicative of some dependence between the groups, however. Artificially reducing the bacterial contribution to decomposition using selective inhibitors clearly revealed a negative correlation between bacterial growth and fungal growth, indicating a negative influence of bacteria on fungal growth (55). Selectively manipulating the fungal contribution to decomposition while monitoring the response of bacterial growth to investigate the reciprocal influence of fungi on bacterial growth in soil has not been attempted using growth-related techniques. A possible mechanism for the negative correlation between bacterial growth and fungal growth along the Hoosfield acid strip (congruent with previous findings [55]) is that low pH is physiologically disadvantageous to the bacteria, decreasing bacterial competition and thus favoring fungal growth. Applying selective fungal and bacterial inhibitors (55) may resolve this question and demonstrate, e.g., if an increase in fungal growth can occur if growth of the bacterial population is suppressed.

The biomass-based measurements gave a different picture of the importance of fungi and bacteria along the pH gradient than the growth-based measurements gave (cf. Fig. 3B and 4C). The lack of large effects on the fungal PLFA/bacterial PLFA ratio caused by a change in the soil pH is consistent with earlier results obtained using, e.g., PLFA-based techniques (12, 32) and total soil microbial biomass measurements combined with ergosterol to distinguish fungi (30, 45). This does not support the established concept that fungi are more abundant in acid soils, such as forest soils (39). However, one factor to which studies of pH influence may be particularly susceptible is the influence of mycorrhizae. A natural soil pH gradient is often correlated with a vegetation gradient and thus has different degrees of ectomycorrhizal colonization. Typically, there is a shift toward vegetation with more ectomycorrhizae as the pH declines (48). Consequently, conclusions concerning the effects of pH on the proportions of fungi and bacteria as decomposers may be compromised if biomarkers that also are indicative of ectomycorrhizae, such as ergosterol and PLFA 18:2ω6,9, are used (35, 48), potentially exaggerating the importance of saprotrophic fungi at low pH. The only mycorrhizae potentially present in the Hoosfield acid strip were arbuscular mycorrhizae, which do not contain ergosterol (49), and thus this factor should not have influenced our results.

The selective respiratory inhibition technique (3) has also been used to monitor effects of soil pH on fungi and bacteria. Using this technique, increases in the fungal biomass/bacterial biomass ratio of 4.5-fold between pH 7 and 3 (12) and of two- to sixfold between pH 6 and 3 (17) have been reported. However, the partitioning of potential respiration by using antibiotics to estimate fungal and bacterial biomasses has repeatedly been challenged (56, 64). Still, it is noteworthy that this biomass technique, which inherently relies on active microorganisms, has high responsiveness to pH effects. The results obtained with it thus most closely resemble the results obtained with the growth-based techniques that we used in the present study. However, the 30-fold difference in the fungal growth/bacterial growth ratio resulting from changing soil pH is at least fivefold greater than the fungal biomass/bacterial biomass ratio response (maximum, sixfold) obtained using the selective respiratory inhibition technique.

Why did biomass measurements deviate from growth measurements? It has been suggested that the frequently observed

lack of change in biomass measurements compared to growth measurements can be due to predatory effects (53); i.e., changes in the production of bacterial biomass are not reflected in the biomass since the next trophic level quickly absorbs the increase (20). Increases in bacterial predator biomass have indeed been detected in soil treated to increase bacterial growth (19, 58). With additional assumptions, a similar dynamic could also explain the discrepancy between fungal biomass and growth in the present study.

However, bacterial PLFAs appeared to be particularly stable. This could be due to the active part of the biomass being relatively small compared to the dormant part (53), obscuring significant changes. In addition, bacterial PLFAs may be less sensitive to environmental disturbances than fungal PLFA markers (31, 34, 35), which might indicate different turnover times for different markers (53). However, the microbial community along the Hoosfield acid strip has had numerous decades to adapt its composition to prevalent conditions, rendering this explanation doubtful in the present study.

In conclusion, this study showed that neutral or slightly alkaline conditions favored bacterial growth. Conversely, an acid pH favored fungal growth. This resulted in an increase in the relative importance of fungi by a factor of 30 from pH 8.3 to pH 4.5. The drastic shift in fungal and bacterial growth affected basal respiration in the same pH range to a relatively minor extent, possibly suggesting functional redundancy in C mineralization. It was not possible to reconcile bacterial and fungal biomass measurements with growth measurements, which compromises the reliability of biomass-based methods to properly assess the relative importance of fungi and bacteria in soil. The use of growth-based measurements proved to be a sensitive way to compare the relative importance of the two major decomposer groups in soil, fungi and bacteria.

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