French, D. D.. 1988 Patterns of decomposition assessed by the use of litter bags and cotton strip assay on fertilized and unfertilised heather moor in Scotland. In: Harrison, A. F.; Latter, P. M.; Walton, D. W. H., (eds.) *Cotton strip assay: an index of decomposition in soils.* Grange-over-Sands, NERC/ITE, 100-108. (ITE Symposium, 24).

Patterns of decomposition assessed by the use of litter bags and cotton strip assay on fertilized and unfertilized heather moor in Scotland

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1 *Summary*
Tensile strength losses from cotton strips (CTSL), weight losses from 2 plant litters, and the responses of cotton and litter decay to soil amelioration were compared, using data from treated and untreated plots on a Scottish moor. The rate of decomposition of cotton strips, and the magnitude of responses to nutrients or carbohydrates added to the soil were generally intermediate between decomposition of heather (*Calluna vulgaris*) stems and purple moor-grass (*Molinia caerulea*) leaves, but the detailed patterns (shapes) of their responses to additives were very different.

Some wider comparisons are referred to, and it is generally is concluded that CTSL is a satisfactory index of general decomposer activity in the field, at least in cool temperate and sub-polar sites, if comparisons between sites or plots are made on a fairly gross scale (ie involving appreciable differences in climatic or edaphic parameters) or if only a rank order comparison is required. Cotton strip assay should not be used to estimate absolute decay rates or responses to environmental changes of natural litters, or as a means of comparing very different assemblages of substrates. However, the assay can, in the latter case, help to distinguish environmental from substrate 'quality' effects on decomposition rates.

2 *Introduction*
Much of the justification for the use of the cotton strip assay in ecological studies lies in the 2 related ideas that:

i. cotton strip tensile strength loss (CTSL) is a good index of cellulose decomposition rates in an ecosystem;
ii. cellulose decomposition rates can be used as a general index of the processes of organic matter decomposition and nutrient release.

Howard (1988) is strongly critical of both these assumptions, but direct experimental evidence appears to be scanty.

An experiment to test the effects of added nutrients or carbon sources on decomposition rates on a Scottish moor provided an opportunity, albeit limited, to compare the patterns of decay shown by CTSL with weight losses from plant litters (in bags), using 2 contrasting litters: purple moor-grass leaves and heather stems.

The following questions, related to the 2 assumptions above, were addressed. How does CTSL compare with weight losses from plant litters? Is the response of CTSL to changes in soil conditions comparable with the responses of weight losses from litters? Do the results suggest that CTSL is a good index of overall rates and processes of decomposition and nutrient release under field conditions?

3 *Site description*
The study site was a very uniform heather moor at Glen Dye, Kincardineshire. (Miles (1973) gives a general site description, and some climatic data are given by French (1988a).) The experimental area was mown during the spring of 1979 and the mowings removed. Neither the litter layer nor the underlying soil was disturbed or visibly altered by the mowing. Litters and cotton were inserted in late October of that year, after treatment of the experimental plots. (For details of experimental layout, see French (1988c).)

The plot treatments were:

i. untreated controls

ii. N (ammonium nitrate) at 4, 16 and 40 g m^{-2}

iii. P (potassium dihydrogen orthophosphate) at 6, 24 and 60 g m^{-2}

iv. Ca (calcium carbonate) at 10, 40 and 100 g m^{-2}

v. CHO (carbohydrate–mixture of glucose and potato starch 1:1) at 4, 16 and 40 g m^{-2}

The 3 levels of each additive are hereafter referred to as levels 1, 4 and 10, being those multiples of the lowest levels applied (thus N 1, P 4, etc). All additives were evenly broadcast in dry form, at the above levels, on 3 occasions during September and October 1979, before the cotton and litters were put out. There were then no further additions during the experiment.

4 *Materials and methods*
Shirley Soil Burial Test Fabric (Walton & Allsopp 1977), in 30 cm x 10 cm strips, was wrapped around a turf cut in the upper soil and litter, following the procedure of French and Howson (1982). Of the 5 substrips used in this method, I used data from the top 2 lying horizontally in the litter layer, these being most directly comparable with the decomposing litter in litter bags.

The moor-grass leaves had been gathered immediately after autumnal senescence, so that they were all of almost identical condition. The heather stems had been selected to be between 2 mm and 4 mm in diameter, and cut to 10 cm long. Each litter was dried at 40°C, and 4 g samples were then wrapped in nylon hair nets and placed in the litter layer at the site. Litter bags were retrieved only at 45 weeks. Ingrowing mosses and lichens, extraneous litter fragments, and animals were removed (but not invertebrate faeces), and each sample was dried at 40°C and weighed to determine the weight loss from the sample.

Cotton strips were inserted and retrieved in 3 consecutive batches at 0–19, 19–34 and 34–45 weeks. The test substrips (frayed to 3 cm width) were prepared according to Latter and Howson (1977) and tensile strength (TS) was measured on a Monsanto tensometer. Cloth controls and field controls in all plots and sample periods had identical mean TS values, so all final TS measurements could be related to a single original figure when calculating CTSL.

CTSL was corrected for cementation (French 1984, 1988b), and litter weight losses for ground contact, by linear regression (French 1988c), before analysing the results.

5 *Results*

5.1 Annual weight losses

An estimate of the total decomposition of cotton strips over the 45-week period may be obtained simply by summing CTSL over all 3 batches (Table 1). The total percentage CTSL ranged from 69% in control plots to 85% on plots receiving the higher levels of Ca application. The data in Heal *et al.* (1974) and Latter *et al.* (1988) were used to estimate a relationship between CTSL and weight loss of cotton strips, and the observed CTSL was converted to weight loss. The total CTSL represented weight losses of cotton of about 15–18%. This estimate involves some replacement of fresh material as it decays. An alternative estimate, calculating the expected total weight loss if the first batch of cotton had been left for 45 weeks, assuming a negative exponential decay curve modified only by seasonal effects, gives expected weight loss of about 30–50%. The corresponding range of weight losses over 45 weeks for heather stems was 3–8%, and for moor-grass leaves 30–40%, so the observed CTSL converts to a range of weight losses either intermediate between results for the 2 litters or close to the weight losses from moor-grass leaves.

5.2 Changes in decomposition rates by soil treatments

The overall response of CTSL to the 4 soil additives is again intermediate between the 2 litters (Table 1), with a mean increase over untreated plots of 14%, compared to 9% for moor-grass or 111% for heather. Like the second (exponential) estimate of annual weight loss rates (Section 5.1), the size of changes in CTSL with added nutrients or carbon is more like moor-grass (whose range it overlaps considerably) than heather. The significance of differences from

Table 1. Tensile strength losses (CTSL) from cotton strips, and weight losses (%) from plant litters, after 45 weeks in fertilized and unfertilized plots, with percentage difference from controls

	Heather stems				Cotton strips				Moor-grass leaves			
		Weight loss (%)		% difference from		CTSL (kg)		% difference from		Weight loss (%)		% difference from
Treatment	n	Mean	SE	control	n	Mean	SE	ence	n	Mean	SE	control
Control (untreated)	19	2.4	0.37		20	116	3.5		18	30.4	0.65	
N 1	20	4.5	0.27	88*	20	134	2.4	16*	18	30.7	0.78	1
N 4	20	3.9	0.28	63*	20	129	2.6	11*	18	32.3	0.84	6*
N 10	19	3.7	0.36	54*	20	130	2.4	12*	18	32.6	0.49	7*
P 1	19	7.7	0.25	221*	20	128	1.9	10*	(10	40.2	1.52	32)[1]
P 4	19	6.8	0.17	183*	20	135	2.1	16*	18	30.1	1.45	–1
P 10	19	8.0	0.18	233*	20	121	2.8	4	18	31.8	1.06	5
Ca 1	18	4.2	0.36	75*	20	131	2.2	13*	17	33.8	1.27	11*
Ca 4	20	3.3	0.15	38*	20	145	2.9	25*	17	33.0	1.45	9
Ca 10	20	3.4	0.23	42*	20	142	1.9	22*	17	36.6	0.71	30*
CHO 1	20	6.0	0.33	150*	20	134	2.0	16*	18	35.8	0.56	18*
CHO 4	20	4.6	0.18	92*	20	134	2.4	16*	18	34.2	0.58	13*
CHO 10	19	4.6	0.23	92*	20	121	3.2	4	18	29.1	0.76	–4

N, nitrogen; P, phosphorus; Ca, calcium; CHO, carbohydrate. 1, 4, 10 are relative amounts (proportion of lowest level) of additive in each treatment

[1] Many moor-grass bags in this treatment were chewed by voles. All obviously chewed bags were rejected, but the weight loss from the remainder may still be higher than the actual decomposition loss

* $P<0.05$ (Mann–Whitney U test)

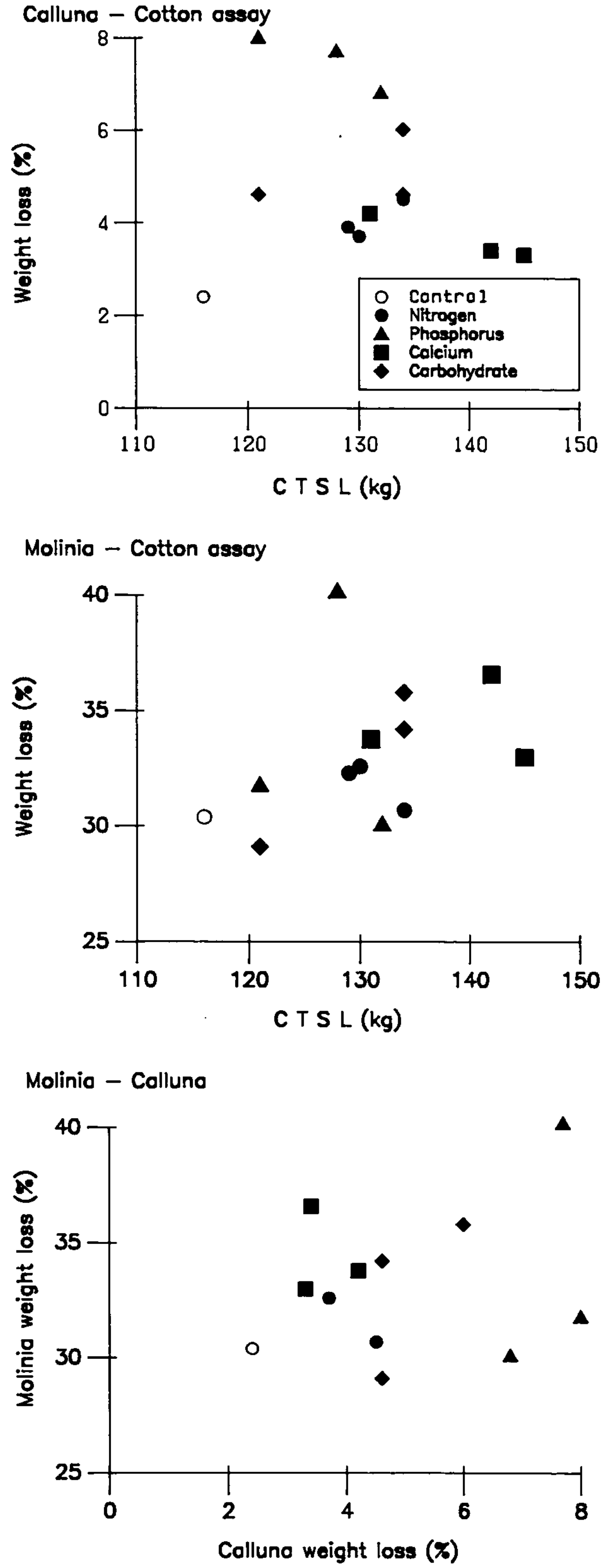

Figure 1. Relations between weight losses from heather (Calluna) *and moor-grass* (Molinia) *litters and tensile strength loss from cotton strips (CTSL) on fertilized and unfertilized plots at Glen Dye*

control plots follows a similar pattern. All treatment differences in heather are significant ($P<0.05$), all but 2 in cotton, but only 6 are significant in moor-grass (Table 1).

As well as the size of response to increased soil nutrients and energy sources, the patterns of change may also be examined. To what extent do the 3 substrates respond similarly to the changed soil conditions? If all responses follow essentially the same pattern, then there should be significant correlations between the decay rates of any 2 substrates, over all treatments together. The experimental layout did not allow matching of individual samples, but means for each treatment can be correlated (Figure 1 & Table 2). The response of heather wood decomposition to all levels of P was exceptionally high, and the large increase in losses from moor-grass in treatment P 1 might be spurious (due to removal of material by voles (Muridae), see note to Table 1). Therefore, the correlations in Table 2 are calculated (i) on all points, (ii) excluding treatment P 1, and (iii) excluding all P treatments. Only cotton and moor-grass show a significant relationship in any of these analyses; even the highest correlation between them accounts for less than half of the total variance, and the 2 significant correlations both exclude treatment P 1.

Table 2. Correlations between mean decay rates (per treatment) of cotton strips and plant litters

	All treatments	Omitting P 1	Omitting all P treatments
Heather/cotton	–0.15	–0.12	0.15
Cotton/moor-grass	0.35	0.58*	0.68*
Heather/moor-grass	0.27	–0.11	0.25

* $P<0.05$ ($n = 13$)

There are, then, no simple linear relationships between heather and either of the other substrates over all treatments, with or without P, but there may be a broad correlation between cotton and moor-grass. However, if the responses of individual substrates to any one additive were to follow some kind of non-linear, possibly 'humped-shaped' curve, as described by Heal *et al.* (1981, pp619–620) for response to temperature and moisture, a search for simple linear correlations may be unjustified. Are there any other consistent patterns of responses to treatments, eg a general ranking of optimal levels of each additive among the 3 materials? In such a case, the responses could all follow the same pattern, but show no linear correlation between substrates.

In Figure 2, all responses are expressed as a percentage of the maximum response to each additive by each substrate, so that all are on a common scale, emphasizing the patterns, rather than magnitude, of the responses. There are not sufficient data to calculate true response curves, but the simplest probable ordering of responses was estimated by Jonckheere's (1954) S test on the original weight loss or CTSL for each substrate and additive, and these orders are indicated by the lines between points in Figure 2.

The level of each additive producing the largest increase in decay rates of heather wood was generally

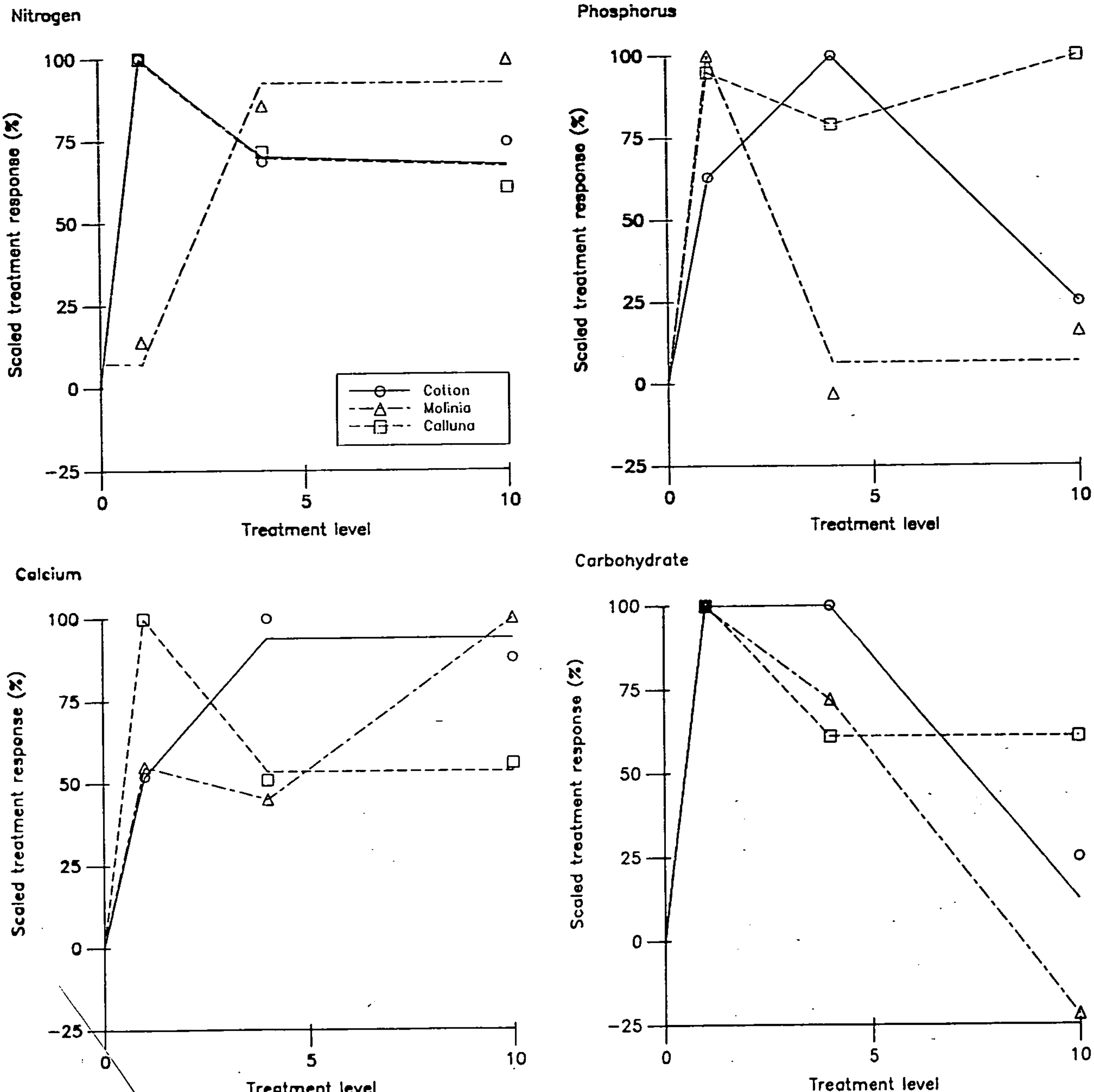

Figure 2. Mean differences from controls of weight losses (litters) and CTSL on treated plots at Glen Dye, scaled to a common range to compare shapes of responses. Lines indicate ordering of responses by Jonckheere S tests. A horizontal line joining 2 treatment levels indicates no significant difference in response between those 2 levels (eg cotton, Ca 4 to Ca 10; all substrates, N 4 to N 10). Similarly, the right-hand side of the cotton/carbohydrate line indicates no difference between CHO 10 and controls

level 1, and of cotton level 4. Moor-grass leaves had either not reached an 'optimal' level by level 10, or had a peak near level 1 followed by a more rapid decline than with either of the other 2 substrates. Optimal levels for cotton, then, are intermediate between the 2 litters, like nearly all other characteristics measured so far.

If, however, the actual shapes of the responses (as defined by the Jonckheere S tests) are compared, it is clear that in no case do all 3 substrates show the same pattern of response. In particular, 3 of the 4 sets of responses for cotton, to P, Ca, CHO additions, may be interpreted as simple 'humps', rising to a maximum response and then declining at higher levels. Responses by moor-grass include one hump (with CHO), 2 more complex patterns, probably including at least 2 turning points (with added P and Ca), and a response to N that might be the beginning of either (or neither). All 4 responses of heather are very similar to each other, and 3 are very different to both the other substrates, though its response to added N is remarkably close to that of cotton.

6 *Discussion*

In having only 2 litter types, the data from this study

Plate 6. Insertion of cotton strip in the field (Photograph G Howson)

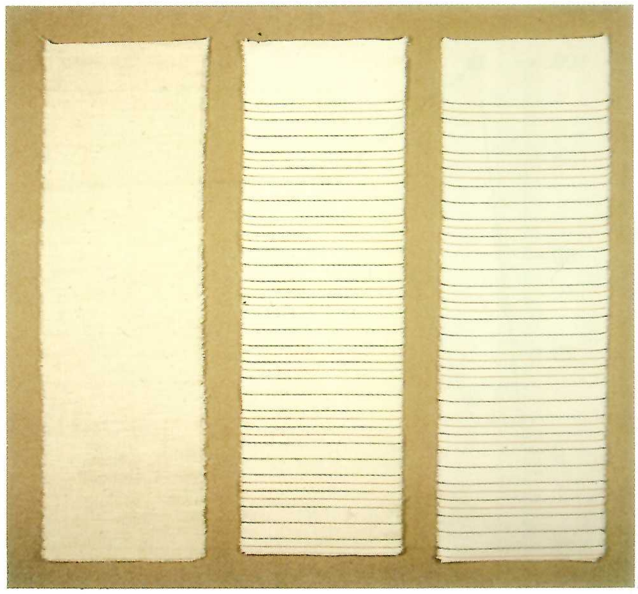

Plate 7. The original unbleached calico cloth and the Shirley Soil Burial Test Fabric (1976 green, and 1981 blue) used for cotton strip assay (Photograph P A Coward)

Brown earth Peaty gley Podzol

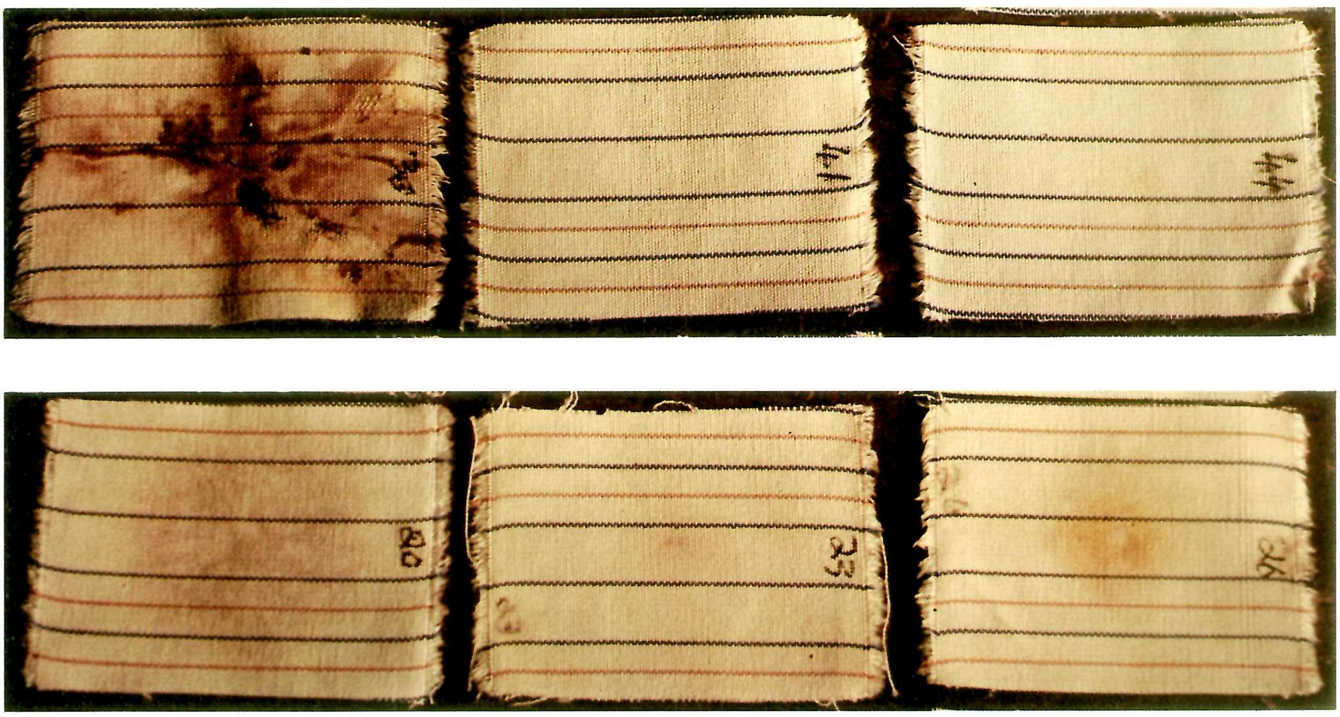

Plate 8. Pigmentation of cotton produced after growth of fungi with 3 soils:
i. Chrysosporium pannorum *ii.* Chrysosporium merdarium
(Photographs J Gillespie)

Plate 9. Mississippi floodplain mixed hardwood sites used for cotton strip assay
i. flooded ii. non-flooded
(Photographs E Maltby)

can only provide a limited comparison between results from the cotton strip assay and from litter decomposition as measured by weight loss. However, in the absence of any other comparable information obtained by simultaneous use of the 2 methods, it is important to make this preliminary examination of any relationships between the 2 measures of decomposition processes in order to highlight some of the problems involved. Comparison of the 2 measures on a seasonal basis is also discussed in French (1988a).

6.1 Substrate quality and decomposition

Using similar criteria to Heal and French (1974), the 3 substrates can be ranked in order of overall 'quality', and their decay rates compared with their quality rank. Cotton strips are essentially devoid of mineral nutrients, but they contain no lignin or other direct microbial inhibitors, and are not physically or structurally especially difficult to decompose. Moor-grass leaves have only moderate lignin content, are low in inhibitory compounds, relatively high in mineral nutrients (1.5–2.0%) and present few physical or structural barriers to decomposers. Their general 'quality' is thus rather higher than that of cotton. Conversely, heather stems have a high lignin content (>25%), a considerable quantity of other inhibitory substances (eg tannins), fairly low nutrient content (total nutrients <1%), are physically hard and structurally intransigent. These are all features of low-quality substrates. The order of substrate quality is therefore: heather<cotton<moor-grass. The decay rates of the 3 substrates are in the same order as their quality ranking, and their overall responses to soil additives, as to be expected, in the reverse order. Their differing patterns of response to different levels of additives may also be related to their differing physical and chemical compositions, and the degree to which these require specialized micro-organisms for their breakdown.

Despite the limitations of the data, it is possible to smooth the responses derived from the Jonckheere tests, taking into account the decomposition mechanisms which are possibly operative, to give a (partly speculative) series of full response curves (ie interpolated and smoothed between actual data points) which are fully consistent with the available data (Figure 3). The one case where the response of cotton is not a simple hump (indicating an optimal level for the full microflora decomposing a single simple substrate) is with addition of N. Widden *et al.* (1986) have shown that there is selection of microflora by some of my soil treatments, and also selection by cotton strips for particular cellulolytic organisms. My own field observations (French 1988c) suggest selection of different groups of organisms on cotton at different levels of added N, but not of P or Ca, where it seems that only the intensity and not the direction of microbial selection varies between treatment levels. The responses of cotton strips to added N within the 3 sample periods (which were summed for comparison with litter weight losses) form a series from an immediate response to low levels of N which quickly declines, to little or no initial response to high levels, but the response increasing with time (French 1988c). This pattern suggests that low levels of N increase the activity of the native microflora, without significant selection, the increase ceasing as the added N is used up. However, higher levels of N, in combination with the presence of cotton cellulose, alter the microflora through selection for a group which can efficiently use both cellulose and the extra N. This subgroup, however, takes some time to build up in the overall population, so it shows no effect for several months after N addition. Combining these 2 patterns gives a curve of the type shown in Figure 3.

Heather will select strongly for ligninolytic organisms, so mechanisms of decomposition are likely to be similar. Selection by substrate could conflict with selection by treatment, as many lignin decomposers are intolerant of, or do not compete well in, high nutrient conditions, while others, including many basidiomycetes, have particular requirements for combinations of nutrients (and other environmental conditions). Hence, at any one treatment level, there is likely to be only a limited proportion of the total microflora able to decompose heather wood under those conditions. The strongest influence on decay rates here is likely to be the physical and chemical intransigence of the heather wood, and it is notable that all responses of heather in Figure 3 follow essentially the same pattern. This is not the case with the other 2 substrates; with them, it is practically impossible to fit a single shape of curve to the data for all 4 additives.

Moor-grass leaves, with a more balanced mixture of constituents than either heather or cotton, would not be expected to show any simple limits, or exert any major microbial selection pressure, and generally this substrate shows a more complex array of responses than heather or cotton.

The detailed relationships between substrate quality and patterns of decomposition are discussed further elsewhere (French 1988c). Perhaps more important in the context of this paper is the distinction between the simple 'humps' characteristic of the responses of cotton (a simple, almost pure, single substrate) and the more complex or varied responses of the 2 litters (which both contain a mixture of substrates). Yet again, however, the cotton is intermediate, this time in its degree of variation, between the complete consistency of heather's responses and the very varied responses of moor-grass. In many ways, heather wood is a more distinctive substrate (cf also the correlations between decay rates, Table 2) and, generally, the 2 litters are no more similar than either of them and cotton.

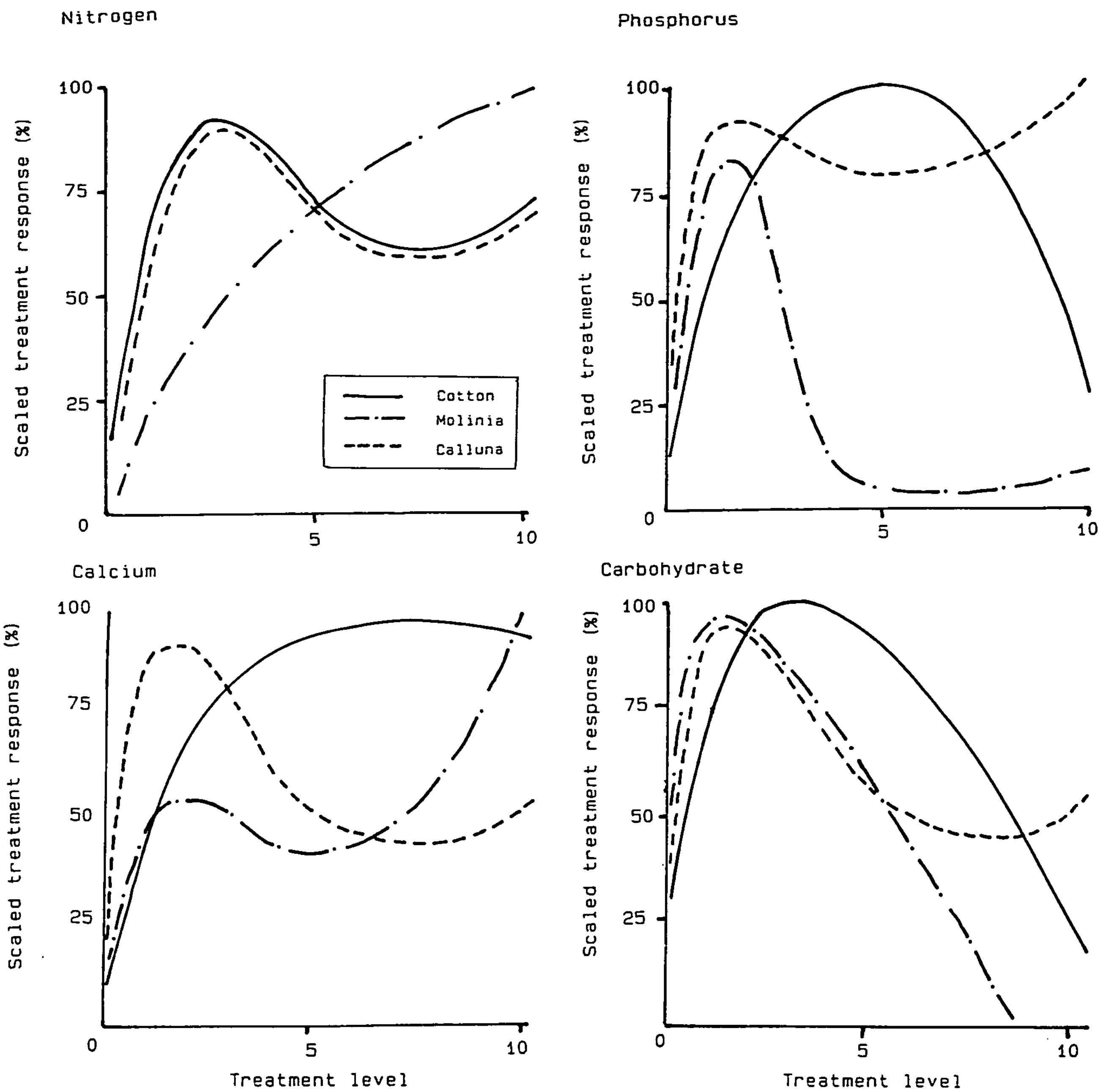

Figure 3. Smoothed response curves, showing probable patterns of change in decomposition rates of cotton and of plant litters with soil additives, derived from the data in Figure 2

6.2 International Biological Programme (IBP) decomposition studies – a wider comparison

The results presented here suggest that, on average, cotton strips can be used as a broad general comparative index of decomposer activity (eg in relation to environmental factors), but that detailed extrapolation to the behaviour of assemblages of more 'natural' substrates may not be possible. This qualification, from my results, also applies to the use of any single substrate as a general indicator of the behaviour of others. In the IBP tundra biome studies (Heal *et al.* 1974), both natural litters and cotton strips were used in a wide range of sites (albeit not always in strictly comparable situations), and the relation between their decay rates and site characteristics can be compared. Both cotton and natural litters showed similar responses to temperature and moisture (Heal & French 1974; Heal *et al.* 1974, 1981), except at very high moisture levels, where decomposition of litters often continued after cotton strip decay had ceased. The response surface was also flatter with litters, partly because of the greater variation due to substrate quality. Responses to edaphic conditions were less comparable, but CTSL and litter weight losses showed very similar trends when superimposed on a principal component analysis of climate and soil variables. From these studies, then, there appears to be a general agreement of results derived from CTSL and litter bag weight losses, when they are compared on a broad scale. The experiments discussed here for Scottish moorland indicate the probable limits of that agreement when the scale of comparison is narrower.

7 *The use of the cotton strip assay*

At Gisburn Forest, tree growth, nutrient release and nutrient uptake by trees were all related to CTSL in soils (Brown 1988). On a broader scale, the IBP studies discussed above showed strong parallels between decomposition of cotton and plant litters in their response to environmental variation. Other studies have given similar results, involving inter-relationships between cotton strips, other decomposition measurements, and nutrient release and turnover. Yet Howard (1988) concludes that the breakdown of pure cellulose added to soil cannot provide an index of litter decomposition rate, release of litter nutrients, or 'general biological activity'. This apparently contradictory conclusion is justified by his reference to poor conceptual models of 'soil physiological systems', and an argument from a particular definition of cellulose decomposition through a *reductio ad absurdum* of the chain of connections from CTSL through cellulose decomposition, litter decay and soil organic matter decomposition, to nutrient release. Howard's own arguments are themselves open to criticism; for example, he ignores the many alternative chains connecting CTSL to nutrient release, such as CTSL being a measure of physical penetration or 'opening up' of the substrate, which may be the process most limiting access to, and hence release of, nutrients and other resources contained therein, with no need to invoke carbon loss rates in any direct form. Nevertheless, his critical examination of the models (actual or implied) involved in much of the use of cotton strips should be considered carefully by those using the method. This consideration is especially important if the cotton strip assay is used as an indirect index of other processes, when the kind of model assumed, in relation to the purpose of the measurement, may be critical to interpretation of results.

8 *Acknowledgements*

I thank Sir William Gladstone, Bt, for permission to work at Glen Dye. Many people assisted with field or laboratory work, or provided useful advice and criticism during the study.

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