

## Inter- and Intra-Specific Variation in Anatomical and Morphological Shapes and Biochemical Ratios of Sun, Intermediate and Shade Leaves from Three Deciduous Tree Species

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### Abstract

Previously we revealed significant differences in biochemical composition of sun and shade leaf litters from chestnut, oak and beech tree species that were related to mass losses. Total lignin of the leaf litters was the dominant variable affecting their decomposition rates. Proximate analysis measured an aggregation of recalcitrant compounds mostly affected microbial activity, rather than a specific biochemical constituent. It was also noted that differences in the decomposition rates of sun and shade leaves within species showed the same anomalous patterns of decomposition described by other researchers, whereby species with rapid initial mass losses had larger residual litter masses than species decomposing slowly at constant rates. We were unable to explain this phenomenon in terms of litter chemistry and suggested that this was an artefact caused by changes in the fungal community within the forest floor material used as an inoculum bed over the 2-year incubation period. In the previous study, we did not consider in detail the inter- and intra-specific variation in anatomical and morphological shapes and biochemical ratios of TFA-carbohydrates (mainly sugar constituents of hemicellulose) and phenylpropanoid derivatives (PPDs) of lignin in sun, intermediate and shade leaves which are taken as an index of lignin depolymerisation by white-rot fungi and also used to assign the proportions of plant- and microbial-derived carbohydrates by several investigators. Here, we revealed and discussed the effects of variation in anatomical and morphological shapes and biochemical ratios on litter decomposition rates. Those results can be used in future studies in order to explain the unknown phenomenon why decomposition rates subsequently decrease in the leaf litters, which initially decompose at higher rates?

**Keywords:** Litter quality, sun-intermediate-shade leaves, total TFA-carbohydrate, phenylpropanoid derivatives (PPDs) of lignin

### Geniş Yapraklı Üç Ağaç Türünün Güneş, Orta ve Gölge Yaprak Kategorilerinin Anatomik ve Morfolojik Şekillerinin ve Biyokimyasal Oranlarının Türler-arası ve Tür-içi Değişimi

#### Özet

Daha önceden yayınlanan çalışmamızda, kestane, meşe ve kayın ağaç türlerinin güneş ve gölge yapraklarının biyokimyasal yapılarındaki farklılığın, ölü örtü kütle azalmalarında önemli derecede etkili olduğunu belirlemiştik. Bu türlerin ölü örtü ayrışma oranlarını etkileyen en önemli kimyasal bileşenin lignin olduğu tespit edilmişti. Mikroorganizma faaliyetleri üzerinde önemli bir etkisi bulunan, ayrışması oldukça zor olan dirençli bileşiklerin bir toplamı olarak belirlenen kısmi analiz yöntemi (ADF-sülfirik lignin yöntemi) sonuçları, spesifik biyokimyasal bileşimlerin belirlendiği yöntemlerden (alkalin CuO oxidation ve trifluoroacetic acid (TFA)-hidroliz yöntemi) daha olumlu sonuç vermiştir. Beklenen aksine başlangıçta hızlı bir ölü örtü ayrışması gösteren ağaç türlerinin, sabit olarak ayrışan ağaç türlerine göre, ilerleyen zaman içinde orman altında daha fazla ölü örtüsüne sahip olması durumunun, aynı türün güneş ve gölge yapraklarının ayrışma sürecinde de meydana geldiği belirlenmiştir. Bu durum, ölü örtünün kimyasal yapısıyla ilişkili olarak açıklanamamıştır. Bu olayın, yaklaşık iki yıl süren laboratuvar koşullarında gerçekleştirilen ölü örtü ayrışma deneyinde altlık olarak kullanılan ölü örtü materyalinin, ayrışmayı gerçekleştiren mantar veya mikroorganizma topluluğunu değiştirmesinden kaynaklandığı varsayılmıştır. Bununla beraber, yayınlanmış çalışmada, üzerinde çalışılan üç türün, güneş, orta ve gölge yapraklarının anatomik ve morfolojik şekil değişimleri ile TFA-karbonhidrat (genel olarak yarı-selülozun şeker bileşikleri) ve lignin fenilpropan türevlerinin (PPDs) birbirleri arasındaki oransal değişimi irdelenmemiş ve ayrışmaya olan etkileri üzerinde durulmamıştır. Oysa birçok araştırmacı tarafından, ligninin fenilpropan türevlerinin bazı oranlarının beyaz çürükçül mantarları tarafından ligninin depolimerizasyonunun bir göstergesi, bazı karbonhidrat türevleri ile birbirine oranının ise bitki ve mikrobiyal kaynaklı karbonhidrat yüzdelerini belirlemede de kullanıldığı bildirilmiştir.

Bu çalışmada, güneş, orta ve gölge yaprakların anatomik ve morfolojik şekillerindeki farklılıklar ile biyokimyasal bileşen (lignin ve karbonhidratlar) türevlerinin birbirine oranlarındaki değişikliklerin ölü örtü ayrışma oranları üzerindeki etkilerinin ortaya konulması ve tartışılması amaçlanmıştır. Elde edilen sonuçlardan, başlangıçta hızlı bir ayrışma oranı gösteren ölü örtünün sonradan niçin yavaşladığına dair gelecekte yapılacak ölü örtü ayrışma çalışmalarına ve süreçlerine cevap bulmada yararlanılabilecektir.

**Anahtar Kelimeler:** Ölü örtü kalitesi, güneş-orta-gölge yaprakları, toplam TFA-karbonhidrat, Ligninin fenilpropan türevleri

## Introduction

Leaves in different locations in tree canopies are exposed to considerable variation in wind, humidity and light intensity, which affect their morphology, anatomy and physiology. The distribution, size and orientation of leaves in space determines the pattern of light availability within the canopy and controls the processes of leaf development, leaf energy balance and water use, and rates of photosynthesis (Norman and Campbell, 1989; Oquist et al., 1992; Ashton and Berlyn, 1992). Some trees, such as light-demanding canopy species or shade tolerant, understorey species, exhibit little plasticity of leaf anatomy in different parts of the canopy. Most forest trees, however, show structural differences between leaves growing in the shade and those exposed to full sunlight (Jackson, 1967; Weier et al., 1982; Roth, 1984; Raven et al., 1992). Typically, shade leaves are thinner with a larger surface per unit weight, thinner epidermis, less palisade tissue, more intercellular space and spongy parenchyma, less supportive and vascular tissues, and fewer stomata than comparable sun leaves of the same tree (Roth, 1984; Roth, 1990; Rollet, 1990). These anatomical differences are reflected by concentrations of nitrogen, cellulose, hemi-cellulose and lignin in plant tissues (Crawford, 1981; Brett and Waldron, 1990) and hence rates of litter decomposition (Heath and Arnold, 1966; Sariyıldız and Anderson, 2003a; 2003b).

There have, however, been few systematic studies of variation in the foliar chemistry of sun and shade leaves of different tree species. Hillis and Swain (1959) showed that leaves from shady parts of the Victoria plum (*Prunus domestica* var. Victoria) contained fewer phenolic substances than leaves exposed to high light intensity. King and Heath (1967) also investigated variations in the chemical

composition of the 'sun', 'intermediate' and 'shade' leaf litter from the European beech (*Fagus sylvatica* L.) and showed that the concentrations of total nitrogen, polyphenols, sugars, lignin and cellulose varied significantly between the leaf litter categories.

The biochemical components of fresh litter have been usually divided into three groups: (1) water-soluble compounds (simple sugars, amino acids), (2) polymer carbohydrates or holocellulose (hemicellulose + cellulose), and (3) acid-insoluble aromatic compounds (lignin + other phenolic compounds). Many researchers have demonstrated relationships between these initial litter quality characteristics and decomposition rates for a large number of plant species (e.g. Fogel and Cromack, 1977; Meentemeyer, 1978; Berg and Staaf, 1980; Melillo et al., 1982; Melillo et al., 1989; Berg et al., 1996 and many others). The nitrogen concentration of the litter or the C:N ratio has been identified as an important controlling factor in decomposition processes (e.g. Anderson, 1973b; Berg and Staaf, 1980; Berg and Ekbohm, 1983; McClaugherty and Berg, 1987; Taylor et al., 1989). However, other studies have emphasised the importance of initial lignin concentration in litter decomposition processes (e.g. Fogel and Cromack, 1977; Meentemeyer, 1978; Berendse et al., 1987; Berg and Tamm, 1991), the summed concentrations of lignin and cellulose (Aber et al., 1990), lignin to N ratio (Aber and Melillo, 1980; Melillo et al., 1982), lignin-cellulose index (lignin to (lignin + cellulose); LCL), and holocellulose to lignicellulose quotient (HLQ) ratios (McClaugherty and Berg, 1987). In general, it would appear that when lignin concentration increases above about 20% it can dominate litter decomposition rates

irrespective of other constituents (Sariyıldız et al., 2008; Sariyıldız and Küçük, 2005; Sariyıldız, 2015). Below this level C:N ratio, polyphenol:N ratio and simple N concentrations may be indicative of decomposition potential depending on which constituents limit microbial activities (Entry and Backman, 1995; Heal et al., 1997).

Although the several relationships between initial litter quality and decomposition rates have been widely established, a number of authors have identified that the mechanistic basis of resource quality controls on decomposition shows important exceptions to these relationships as the decomposition proceeds with time (e.g. Anderson, 1973a; 1973b; Berg et al., 1982; Berg and Wessen, 1984; Berg and Ekbohm, 1991; Berg et al., 1993). It has been recognised that species with rapid initial mass losses have larger litter masses than species decomposing slowly at constant rates as the decomposition proceeds with time. These different patterns of decomposition were not explained by initial nitrogen, lignin or polyphenol concentrations. We still know little about why decomposition rates subsequently decrease in the leaf litters, which initially decompose at higher rates. Reasons for this phenomenon may lie in the structural configuration of lignin, hemicellulose and cellulose in the plant cell walls (Berg et al., 1984; McClaugherty and Berg, 1987; Berg et al., 1995). Reason for this phenomenon may be explained by the structural configuration of lignin, hemicellulose and cellulose in the plant cell walls (Berg et al., 1984; McClaugherty and Berg, 1987; Berg et al., 1995). Therefore the mechanisms regulating processes of decomposition requires more understanding and information on the quality, quantity and spatial configuration of the ligno-cellulose complex in the plant cell wall and microbial degradation of these constituents.

The carbohydrates extracted with TFA mainly constitute the non-crystalline constituents of the cell wall (hemicellulose and hydrated cellulose) and are indicative of the carbohydrate fraction which is more readily available to saprotrophic microorganisms (Guggenberger et al., 1995).

However, the TFA extractable carbohydrates are not only indicative of the degree of depolymerisation of cell wall polysaccharides but also development of microbial biomass in litter. Plant cell wall polysaccharides are characterised by higher proportions of the pentose sugars xylose and arabinose, whereas microbial populations synthesise dominantly the hexoses mannose, galactose and deoxysugars rhamnose and fructose (Guggenberger and Zech, 1994). Hence, the ratios of mannose + galactose-to-xylose + arabinose and of rhamnose + fucose-to-xylose + arabinose have been used to assign the proportions of plant- and microbial-derived carbohydrates by several investigators (Cheshire, 1979; Murayama, et al., 1979; Murayama, 1984; Oades, 1984).

Upon CuO-oxidation, most fresh vascular plant tissues yield ratios of vanillic acid to vanillin (Ac/Al)<sub>v</sub> and syringic acid to syringaldehyde (Ac/Al)<sub>s</sub> that have been stated to lie in the range of 0.1-0.2. An increase of acid to aldehyde ratios of the vanillyl and syringyl units from fresh to degraded lignin, which is directly related to the oxidative degradation of lignin by white-rot fungi (Hedges et al., 1988).

Here, in this present study, we report variation in morphology, anatomy and initial and final biochemical composition and ratios of different leaf categories from three deciduous tree species; sweet chestnut, (*Castanea sativa* Mill.), beech (*Fagus sylvatica* L.) and common oak (*Quercus robur* L.) growing in close proximity on the same soil type. The study was carried out as part of an extensive investigation into the effects of variation in litter quality on the rates of microbial decomposition (Sariyıldız and Anderson 2003a; 2003b). Hence, in addition to standard analyses of nitrogen and plant structural compounds, measurements were made of the phenylpropanoid constituents of lignin (Hedges and Ertel, 1982) and the sugars of cell wall carbohydrates (Guggenberger and Zech, 1994) which have been used to characterize stages of microbial decomposition in leaf litters (Cheshire, 1979; Murayama, 1984; Oades, 1984). Initial ratios of selected hexose and pentose sugars and the acid to aldehyde ratios of vanillyl and syringyl units were also

calculated to investigate whether there were any initial intra- and inter-specific variations in these ratios in the leaf litter categories from beech, oak and chestnut trees.

## **Material and Methods**

### **Sampling site and litter collection**

The sampling site was a mixed stand of chestnut, oak and beech trees in Blean Woods National Nature Reserve, south-east England, UK. The trees were coppiced 10-15 years before sampling and canopy closure was not complete. The site was about 84 m above sea level, with mean annual rainfall of 625 mm and mean annual temperature 10.5 °C. The underlying soil had a well-developed organic horizon (with mean pH 4.2) based on Stour Sands and Gravels. Further details of the site are given in Anderson (1973 a, b).

Freshly fallen litter was collected from ten, 1m quadrats located at random of the woodland floor. The main period of leaf litter fall in this area was of short duration reaching a peak at the time of sampling (Anderson 1973 a, b). The material collected showed no visible signs of fungal colonization or decomposition.

### **Determination of leaf litter categories**

The leaf litter samples were air-dried at room temperature and then sorted into the three tree species. The extreme categories of 'sun' and 'shade' leaves of the same tree species could be clearly differentiated by differences shape, colour and texture, but the mass per unit area of leaf was used to define intermediate categories. A disc was cut from the lamina of each leaf at the broadest point using a cork borer (13mm diameter) and weighed. On the basis of the disc weight each leaf was allocated to three categories for beech and oak and four categories for chestnut (which showed a wider range of leaf properties than the other two species). The relationship between disc weight and leaf anatomy for sun, intermediate and shade leaves was confirmed in histological sections by light microscopy.

### **Determination of anatomical differences between the leaf litter categories**

The relationship between disc weight and leaf type were confirmed by checking differences in anatomical structure of the leaf litter categories. Representative discs were dehydrated in an alcohol series: 70 % ethanol for 6 h, 90 % ethanol for 4 h and two changes of absolute alcohol for 4 h each. The dehydrated leaf discs were then cleared in absolute alcohol / HistoClear solution (50:50) for 24 h, in absolute alcohol / HistoClear solution (25:75) for 24 h and pure HistoClear for 24 h. Finally, the cleared leaf discs were impregnated with HistoClear / melted wax (50:50) in an oven at 40 °C, and two changes of pure wax for 1 h each. Each leaf disc was then embedded in a block of wax in a solid watch glass. After embedding the discs were sectioned using a sledge microtome. The sections were attached to the slides by warming and dewaxed with a sequence of 90, 70 and 50 % absolute alcohol respectively for 5 minutes each and finally deionised water for 5 minutes. Phloroglucinol was used as a stain for lignin, the sections were then mounted and inspected under microscope

### **Chemical analysis**

Samples of leaves from each litter category were oven dried at 85 °C and then ground in a laboratory mill to a mesh fraction less than 1mm. Analyses were carried out in duplicate.

Organic C was analysed using a Leco HF10 gravimetric carbon analyser (Leco, St. Joseph). Total N was determined by Kjeldal digestion (Allen 1989) followed by analysis of  $\text{NH}_4^+$  by the indophenol method using an auto-analyser (Bemas; Burkhard, Uxbridge, UK). Acid detergent fibre (ADF), a-cellulose and lignin were determined using the ADF-sulphuric lignin method of Rowland and Roberts (1994). The component sugars of structural polysaccharides (mainly hemicelluloses) were hydrolyzed using 4 M trifluoro acetic acid (TFA) and prepared for GC analysis according to the method of Guggenberger and Zech (1994). Carbohydrate concentrations were determined using a Shimadzu GC 14-A capillary GC, with a BPS 25m x 0.25 mm i.d.

column with standards of xylose, arabinose, fucose, mannose, galactose, glucose, sucrose and maltose (Sanger et al., 1998).

Phenylpropanoid moieties (PPDs) of uncondensed lignins were determined after alkaline CuO oxidation (Hedges and Ertel, 1982; Kogel and Bochter, 1985) using the modified procedure of Hetherington and Anderson (1998). The concentrations of PPDs were determined using a Shimadzu GC 14-A capillary GC, with a BPS 25m x 0.25 mm i.d. column with standards of p-hydroxyl (p-hydroxybenzaldehyde, p-hydroxybenzoic acid, p-hydroxyacetophenone), vanillyl (vanillin, acetovanillone, vanillic acid), syringyl (syringaldehyde, acetosyringone, syringic acid), coumaric acid and ferulic acid (Sanger et al., 1996).

Principal Component Analysis (PCA) was carried out to investigate relationships between leaf litter species and litter quality parameters. A component with eigenvalues of less than 1 was discarded (Frey and Pimental, 1978). The eigenvalues of each PC indicated the loadings of each of the variables contribute to a PC and negative values indicated inverse relationship between the value and that PC. PCA was carried out using the computer package MINITAB version 7.2 Silicon Graphics. The significance of differences in the composition of litter species and types was then analysed by ANOVA using the computer package MS Excel (version 97 SR-1).

## Results

### Differences in morphology and anatomy between species and within leaf litter categories

The disc weights ranged from 30-140 mg for chestnut, 40-130 mg for oak and 30-90 mg for beech. Three litter categories (sun, intermediate and shade) were defined for oak (40-70 mg, 80-100 mg and 110-130 mg) and beech (30-40 mg, 50 mg and 60-90 mg). The chestnut leaves showed wider variation in disc mass and were divided into four categories of shade (30-60 mg), intermediate-1 (70-90 mg), intermediate-2 (100-120 mg) and sun (130-140 mg) leaves.

Leaves of the three tree species were very different in morphology (Figure 1). Chestnut

leaves were approximately two times larger in area than oak leaves, and about three or four times larger in area than beech leaves. There were also distinct morphological differences between leaf types (Figure 1). Sun and shade leaves of the same species varied in shape, colour and texture (thickness). Sun leaves were smaller, thicker and more deeply lobed. Shade leaves were about 1.5 times larger in area than sun leaves.

Shade leaves also differed anatomically from sun leaves by having a thinner epidermis, a less developed palisade mesophyll, a higher proportion of intercellular space, wider spacing between the veins were noted (e.g. Figure 2). Differences in thickness of mesophyll, either palisade or spongy tissues, size of the vascular system and density of the minor veins between the three trees were also observed. The thickness of mesophyll, and size of the vascular bundles, was larger in chestnut leaves, whereas beech leaves showed a higher density of the minor veins. Oak showed intermediate characteristics between beech and chestnut.

### Chemical composition of leaf categories

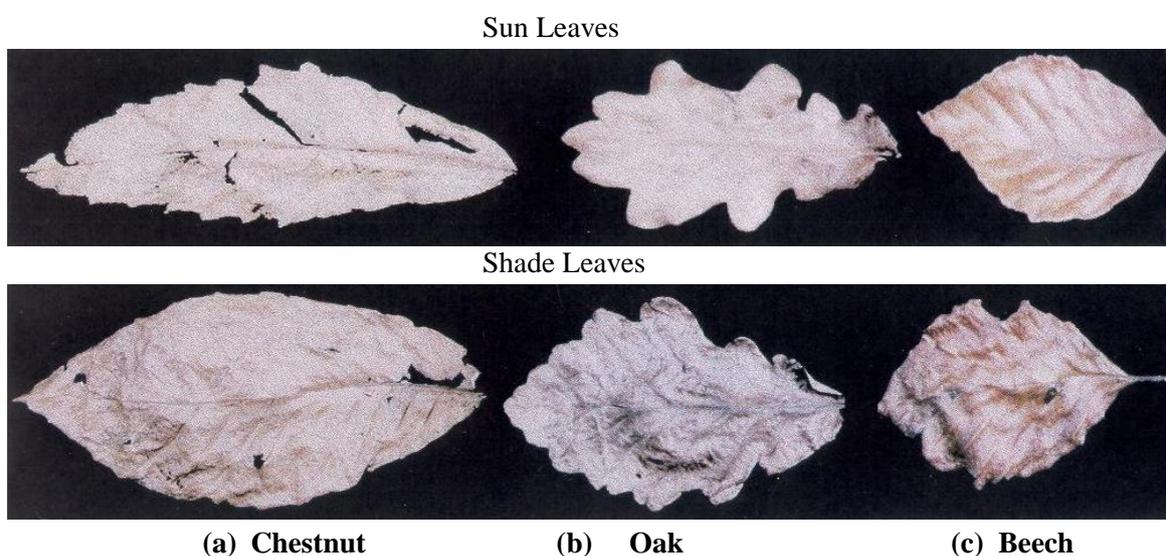
Initial total nitrogen, carbon, ADF, lignin and cellulose concentrations, and C:N and lignin:N ratios in the shade, intermediate and sun leaf categories for all three species are shown in Table 1. Results of PCA of data for leaf categories and species produced two components (PC1 and PC2) with eigenvalues of greater than 1, which explained percentage of the total variance (Table 2).

There were significant ( $p < 0.01$ ) inter-specific differences in mean N concentrations of 1.29 % for chestnut, 0.96 % for beech and 0.93 % for oak. Chestnut had the highest N concentrations in the shade (1.36%), intermediate-1 (1.32%), intermediate-2 (1.26%) and sun (1.22%) leaves. Nitrogen concentrations were lowest in oak leaves (shade, 1.00%; intermediate, 0.91%; and sun, 0.87%), with beech showing intermediate concentrations between the other two species. The trend of decreasing nitrogen concentrations from shade leaves to sun leaves was consistent for all three litter species but intra-specific differences were

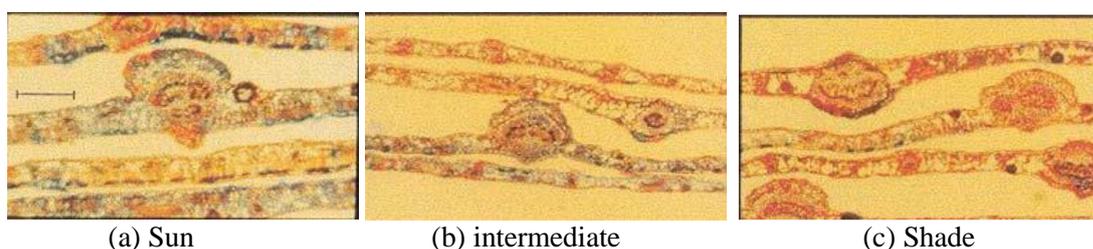
only significant ( $p < 0.01$ ,  $n = 2$ ) between sun and shade leaves.

Mean carbon concentrations were 49.6 % for chestnut, 46.3 % for oak and 46.2 % for beech. The shade, intermediate-1, intermediate-2 and sun leaves of chestnut also had the highest carbon concentrations (48.1, 48.8, 50.3 and 51.1 % respectively), whereas carbon concentrations in the shade, intermediate and sun leaves of oak and beech were approximately the same and showed the lowest carbon concentrations (about 45, 46 and 47 % respectively). Although there was a slight increase for carbon concentrations from shade leaves to sun leaves for all

species, there were no significant differences within or between species and the leaf categories. There was also a trend in C:N ratios between species ( $p < 0.001$ ) and within the leaf categories ( $p < 0.01$ ). The shade, intermediate and sun leaves of oak had the highest C:N ratios (45.5, 51.2 and 54.3 respectively), whereas the shade, intermediate-1, intermediate-2 and sun leaves of chestnut had the lowest C:N ratios (35.2, 36.9, 39.8 and 41.9 respectively). The C:N ratios showed the opposite trends to nitrogen concentrations with increasing from shade to sun leaves for all species.



**Figure 1:** Differences in morphology between sun and shade leaves of (a) Chestnut (*Castanea sativa*), (b) Oak (*Quercus robur*) and (c) Beech (*Fagus sylvatica*).



**Figure 2:** Differences in anatomy between the leaf and leaf litter categories (e.g. Chestnut (a) Sun, (b) intermediate and (c) shade leaves. Bar 10  $\mu\text{m}$ ).

**Table 1:** Initial concentrations of carbon, nitrogen, lignin, and ratios of C:N and lignin:N in shade, intermediate and sun leaves from chestnut (*Castanea sativa*), oak (*Quercus robur*) and beech (*Fagus sylvatica*).

	Chestnut				Oak			Beech		
	Shade	Int-med.1	Int-med.2	Sun	Shade	Int-med.	Sun	Shade	Int-med.	Sun
Carbon (%)	48.1 (2.05)	48.8 (0.48)	50.3 (0.23)	1.1(0.12)	5.3(1.22)	6.6(0.19)	6.9(0.19)	45.7(0.18)	46.2(0.05)	46.8(0.45)
Nitrogen (%)	1.36 (0.05)	1.32 (0.02)	1.26 (0.05)	1.22(0.12)	1.00(0.03)	0.91(0.04)	.87(0.05)	1.01(0.08)	0.96(0.01)	0.92(0.04)
Lignin (%)	17.9 (0.14)	19.1 (0.16)	21.6 (0.01)	3.1(0.26)	3.5(0.20)	6.3(0.43)	8.0(0.05)	30.4(0.24)	33.9(0.42)	38.7(0.11)
ADF (%)	46.2 (0.78)	47.8 (0.28)	48.6 (0.14)	0.0(0.20)	2.6(0.11)	4.5(0.14)	5.7(0.97)	68.5(0.72)	70.0(0.93)	71.4(0.21)
Cellulose (%)	27.0 (0.62)	25.8 (0.14)	23.5 (0.59)	1.7(0.46)	5.9(0.23)	5.6(0.11)	4.7(0.82)	35.1(0.57)	32.8(1.47)	29.2(0.38)
C : N	35.2: 1	36.9: 1	39.8: 1	41.9: 1	45.5: 1	51.2: 1	54.3: 1	45.2: 1	48.4: 1	51.1: 1
Lignin : N	13.1: 1	14.4: 1	17.0: 1	18.9: 1	23.6: 1	28.9: 1	32.4: 1	30.1: 1	35.5: 1	42.3: 1

**Table 2:** Summary of the PCA for total carbon, nitrogen, ADF, lignin and cellulose in sun, intermediate and shade leaves of chestnut, oak and beech trees. For each of the top three principal components (PC) the eigenvalue and the proportional (PrVar), cumulative variance it explains are shown with the eigenvectors and loading each variable contributes to that PC

	Eigenvalues				Eigenvectors					ANOVA		
	PC	Value	PrVar	Cum Var	C	N	ADF	Lignin	Cellulose	F	P-value	F-crit
Chestnut	1	3.79	0.76	0.76	0.39	-0.35	0.48	0.50	-0.50	19.0	p<0.001	3.49
	2	0.84	0.17	0.92	-0.60	-0.71	0.29	-0.09	0.21	2.39	n.s	3.49
	3	0.34	0.07	0.99	0.60	-0.59	-0.41	-0.31	0.17		n.s	
Oak	PC	Value	PrVar	Cum Var	C	N	ADF	Lignin	Cellulose	F	P-value	F-crit
	1	3.57	0.71	0.71	0.39	-0.45	0.45	0.52	-0.41	19.4	p<0.001	4.26
	2	0.83	0.17	0.88	-0.29	-0.28	-0.31	-0.11	-0.25	2.89	n.s	4.26
	3	0.53	0.11	0.99	0.24	0.26	-0.29	-0.18	-0.28		n.s	
Beech	PC	Value	PrVar	Cum Var	C	N	ADF	Lignin	Cellulose	F	P-value	F-crit
	1	4.01	0.81	0.81	0.47	-0.41	0.44	0.48	-0.43	12.5	p<0.01	4.26
	2	0.63	0.13	0.93	-0.11	0.27	-0.22	0.34	0.31	3.50	n.s	4.26
	3	0.28	0.06	0.98	-0.33	0.32	0.21	0.16	0.23		n.s	

Mean ADF concentrations were 48.2 % for chestnut, 54.3 % for oak and 70.0% beech.

There was a trend of increasing ADF concentrations from shade to sun leaves for all species. The shade, intermediate and sun leaves of beech had the highest ADF concentrations (68.5, 70.0 and 71.4 % respectively), whereas the shade, intermediate-1, intermediate-2 and sun leaves of chestnut had the lowest ADF concentrations (46.2, 47.8, 48.6 and 50.0 % respectively).

Mean lignin concentrations were 20.4 % for chestnut, 25.9 % for oak and 34.3 % for beech. Lignin also showed the same trend of increasing lignin concentrations from shade to sun leaves. The shade, intermediate and sun leaves of beech had the highest lignin concentrations (30.4, 33.9 and 38.7 % respectively), whereas the shade, intermediate-1, intermediate-2 and sun leaves of chestnut had the lowest lignin concentrations (17.9, 19.1, 21.6 and 23.1 % respectively).

Mean cellulose concentrations were 24.5 % for chestnut, 25.4 % for oak and 32.4 % for beech. Cellulose concentration showed the opposite trend to ADF and lignin concentrations with highest concentrations in the shade leaves of all three species. The shade, intermediate and sun leaves of beech had the highest cellulose concentrations (35.1, 32.8 and 29.2 % respectively) compared to the shade, intermediate-1, intermediate-2 and sun leaves of chestnut (27.0, 25.8, 23.5 and 21.7 % respectively) and the shade, intermediate and sun leaves of oak (25.9, 25.6 and 24.7 % respectively). All differences in ADF, lignin and cellulose concentrations between species and within the leaf categories were significant ( $p < 0.001$ ). There was a consistent trend of increasing lignin: N ratios from shade to sun leaves.

Mean lignin: N ratios were 15.8 for chestnut, 27.8 for oak and 35.7 for beech. The shade, intermediate and sun leaves of beech had the highest lignin: N ratios (30.1, 35.5 and 42.3 respectively) compared to the shade, intermediate-1, intermediate-2 and sun leaves of chestnut (13.1, 14.4, 17.0 and 18.9 respectively) and the shade, intermediate and sun leaves of oak (18.9, 23.6 and 32.4

respectively). Differences in lignin:N ratios were significant between litter species ( $p < 0.001$ ) and within the leaf litter categories ( $p < 0.01$ ).

#### **TFA-extractable carbohydrates (sugars)**

Table 3 shows initial total and individual sugar and the ratios of ADF:total sugars and of hexose-to-pentose sugars in the leaf categories of chestnut, oak and beech species. Results of PCA of data for leaf categories and species produced two components (PC1 and PC2) with eigenvalues of greater than 1, which explained percentage of the total variance (Table 4).

Chestnut leaves contained the highest concentrations of sugars, followed by oak and beech (differences  $p < 0.001$  each case). Mean sugar concentrations were 231.9 mg/g for chestnut, 199.7 mg/g for oak and 172.1 mg g<sup>-1</sup> for beech. Sugar concentrations in each leaf category showed the same order to mean sugar concentrations with highest concentrations in chestnut leaf categories followed by oak and beech leaves. The concentrations of the individual sugars, except for xylose and arabinose concentrations, also showed the same trend as total sugars with chestnut>oak>beech. Xylose concentrations in the beech litter were higher than those in chestnut litters. However, arabinose concentrations were highest concentration in oak and the lowest in beech. Shade leaves contained significantly higher concentrations of total sugars than sun leaves for each species ( $p < 0.001$ ), but differences for individual sugars were generally small. Exceptions were xylose and mannose where chestnut leaves contained about 1.5 times higher concentrations in shade leaves than sun leaves but showed comparatively small differences in oak and beech. However, individual sugar concentrations within the leaf categories for each species were significant for xylose and arabinose ( $p < 0.001$ ), rhamnose and fucose ( $p < 0.01$ ) and mannose and galactose ( $p < 0.05$ ), but glucose, sucrose and maltose were not significant. The ratios of ADF : total sugars also showed differences between species and within the leaf categories.

**Table 3:** Initial concentrations of individual TFA-extractable sugars and ratio of hexose to pentose sugars (a) (rhamnose+fucose/xylose+arabinose), (b) (mannose+galactose/xylose+arabinose) in shade, intermediate and sun leaf litters from chestnut, oak and beech.

Sugars (mg/g)	Chestnut				Oak			Beech		
	Shade	Int-med.1	Int-med.2	Sun	Shade	Int-med.	Sun	Shade	Int-med.	Sun
Xylose	65.3 (3.81)	54.9 (0.47)	47.7 (6.52)	47.4 (1.77)	72.1 (3.01)	69.0 (2.11)	66.8 (0.68)	79.1 (0.57)	71.9 (2.99)	66.8 (4.14)
Arabinose	27.5 (0.14)	26.1 (0.86)	23.3 (0.09)	20.4 (0.39)	29.8 (1.16)	26.9 (0.15)	23.6 (0.97)	24.1 (0.85)	22.5 (0.13)	20.9 (0.13)
Rhamnose	12.8 (0.27)	11.8 (0.76)	10.6 (1.19)	10.1 (0.17)	11.6 (0.33)	10.4 (0.32)	8.63 (1.53)	8.23 (0.73)	7.58 (0.25)	6.14 (0.84)
Fucose	4.33 (0.02)	3.80 (0.29)	3.54 (0.43)	3.21 (0.07)	3.60 (0.02)	3.44 (0.22)	2.79 (0.45)	2.95 (0.11)	2.90 (0.19)	2.65 (0.02)
Mannose	17.7 (0.26)	14.6 (2.24)	13.7 (1.35)	11.8 (0.73)	9.26 (0.89)	9.08 (0.12)	7.64 (1.11)	12.5 (0.22)	11.6 (0.16)	11.2 (1.54)
Galactose	39.4 (1.57)	38.1 (0.96)	37.6 (1.05)	35.9 (1.36)	32.6 (1.43)	31.4 (0.18)	31.2 (2.53)	23.7 (0.11)	22.1 (0.39)	19.5 (0.62)
<b>Total Sugars</b>	<b>253.3</b>	<b>237.2</b>	<b>221.8</b>	<b>215.3</b>	<b>210.2</b>	<b>200.0</b>	<b>188.9</b>	<b>190.2</b>	<b>172.2</b>	<b>154.0</b>
ADF: Sugars	1.77: 1	1.89: 1	2.03: 1	2.08: 1	2.35: 1	2.61: 1	2.79: 1	3.44: 1	3.87: 1	4.41: 1
(a) (r+f)/(x+a)	0.184	0.193	0.199	0.196	0.149	0.144	0.126	0.094	0.111	0.112
(b)(m+g)/(x+a)	0.615	0.651	0.723	0.704	0.411	0.422	0.429	0.351	0.357	0.375

**Table 4:** Summary of the PCA for total and individual TFA-extractable sugars in sun, intermediate and shade leaves of chestnut, oak and beech trees. For each of the top three principal components (PC) the eigenvalue and the proportional (PrVar), cumulative variance it explains are shown with the eigenvectors and loading each variable contributes to that PC.

	Eigenvalues				Eigenvectors								ANOVA		
	PC	Value	PrVar	Cum Var	Xylose	Arabinose	Rhamnose	Fucose	Mannose	Galactose	Glucose	Total	F	P-value	F-crit
Chestnut	1	6.79	0.68	0.68	0.34	0.33	0.38	0.38	0.37	0.33	0.32	0.37	15.4	p<0.001	3.49
	2	2.42	0.24	0.92	0.23	0.16	-0.02	0.06	0.07	-0.19	-0.49	-0.17	1.35	n.s	3.49
	3	0.34	0.03	0.96	-0.40	0.44	-0.14	-0.05	0.27	0.48	-0.48	0.21		n.s	
Oak	PC	Value	PrVar	Cum Var	Xylose	Arabinose	Rhamnose	Fucose	Mannose	Galactose	Glucose	Total	F	P-value	F-crit
	1	6.11	0,61	0,61	0,32	0,36	0,39	0,39	0,34	0,31	0,35	0,39	11,4	p<0.01	4,26
	2	2,39	0,24	0,85	0,33	0,13	0,06	-0,05	-0,32	0,33	-0,06	0,16	2,51	n.s	4,26
3	2,21	0,12	0,97	0,61	0,31	-0,06	0,03	-0,18	-0,39	-0,41	0,07		n.s		
Beech	PC	Value	PrVar	Cum Var	Xylose	Arabinose	Rhamnose	Fucose	Mannose	Galactose	Glucose	Total	F	P-value	F-crit
	1	5,98	0,60	0,60	0,38	0,36	0,36	0,32	0,16	0,39	0,39	0,41	18,4	p<0.001	4,26
	2	2,08	0,21	0,81	-0,15	0,06	-0,26	0,04	0,61	-0,09	0,21	0,02	2,02	n.s	4,26
3	1,15	0,12	0,93	-0,16	-0,05	0,11	-0,23	0,24	0,17	0,07	-0,02		n.s		

Mean ADF: total sugar ratios were 1.94, 2.58 and 3.91 for chestnut, oak and beech respectively. There was also a consistent trend of increasing ADF:total sugar ratios from shade to sun leaves for all species. The ratios of hexose to pentose sugars also showed similar trends to ADF:total sugars ratios with chestnut>oak>beech, and within species slightly increasing ratios from shade to sun leaves for all species.

#### **Phenylpropanoid moieties from lignin**

Table 5 shows the concentrations of initial CuO extractable phenylpropanoid moieties (PPD), ratios of lignin: PPD, vanillyl: syringyl and acid to aldehyde ratios of vanillyl and syringyl in the leaf categories of chestnut, oak and beech species. Results of PCA of data for leaf categories and species produced two components (PC1-PC2) with eigenvalues of greater than 1, which explained percentage of the total variance (Table 6).

Mean concentrations of total PPDs either between species or between each leaf category showed the opposite trends to total sugar concentrations with beech>oak>chestnut. Mean concentrations of PPDs were 62.6 mg g<sup>-1</sup> for chestnut, 75.7 mg/g for oak and 103.0 mg g<sup>-1</sup> for beech. The concentrations of PPDs in the different leaf categories of oak were higher than those of chestnut, but lower than those of beech. The concentrations of total PPDs were also statistically significant between species and within the leaf categories (p<0.001). The concentrations of individual PPDs were also highest in beech and lowest in chestnut (Table 6). Vanillyl and syringyl were dominant moieties, constituting 95 % of total PPDs, but the ratios of two moieties varied significantly between species (p<0.001, beech>oak>chestnut) and within the leaf categories (p<0.05, sun>intermediate>shade). The concentrations of individual PPD were significantly different between species (vanillyl p<0.001; syringyl p<0.01) and within the leaf categories (vanillyl, syringyl p<0.001). The concentrations of total and individual PPDs in the sun leaves were significantly higher than those in the shade leaves for all species, whereas the lignin: PPD ratios showed the reverse trend with

highest ratios in the shade leaves. However, the vanillyl : syringyl ratio showed the opposite trends to lignin: PPD ratio with increasing from shade to sun leaves for all three tree species. The ratios of acid to aldehyde of vanillyl (Ac/Al)<sub>v</sub> was low in leaf litter categories compared with the ratios of acid to aldehyde of syringyl (Ac/Al)<sub>s</sub> for all species. Both ratios did not show any considerable variation between chestnut and oak, whereas beech had the lowest ratios compared to chestnut and oak.

#### **Changes in sugar and PPD concentrations during decomposition TFA-extractable sugar concentrations**

Final sugar concentrations in the three leaf litter species and the leaf litter categories after 24 months decomposition in microcosms is shown in Table 7. Mean final concentrations of sugars were 146.9 mg/g for chestnut, 161.8 mg g<sup>-1</sup> for oak and 176.8 mg/g for beech. After 24 months, there was a general decrease in extractable sugar concentrations, but the proportional losses varied from about 36.7 % in chestnut, 19.0 % in oak and no significant changes in beech. The various litter categories for the three tree species formed a continuous sequence of decreasing sugar losses from chestnut shade leaves to beech sun leaves.

Mean concentrations of the individual sugars (Table 7) followed the same general trend in losses as the total concentrations with the proportional largest changes in chestnut and relatively small losses in beech (Figure 3). In the chestnut shade leaves, rhamnose and glucose showed the highest losses (about 53 and 45 % of initial concentrations respectively), followed by mannose, galactose and arabinose (about 36 % losses) while xylose and fucose showed comparatively small losses (<25 % losses). The magnitude of these mass losses decreased across the series of litter qualities from shade to sun leaves, and from chestnut, though oak to beech leaves, so that there were insignificant changes in individual sugar in beech sun leaves. Because of these differences in individual sugars, the final ratios of hexose to pentose also varied in chestnut and oak leaf litter categories, but had contrasting trends.

**Table 5:** Initial concentrations of vanillin, vanillic acid, syringaldehyde and syringic acid, and ratios of vanillyl: syringyl and (a) acid to aldehyde ratios of vanillyl and (b) syringyl in shade, intermediate and sun leaf litters from chestnut, oak and beech.

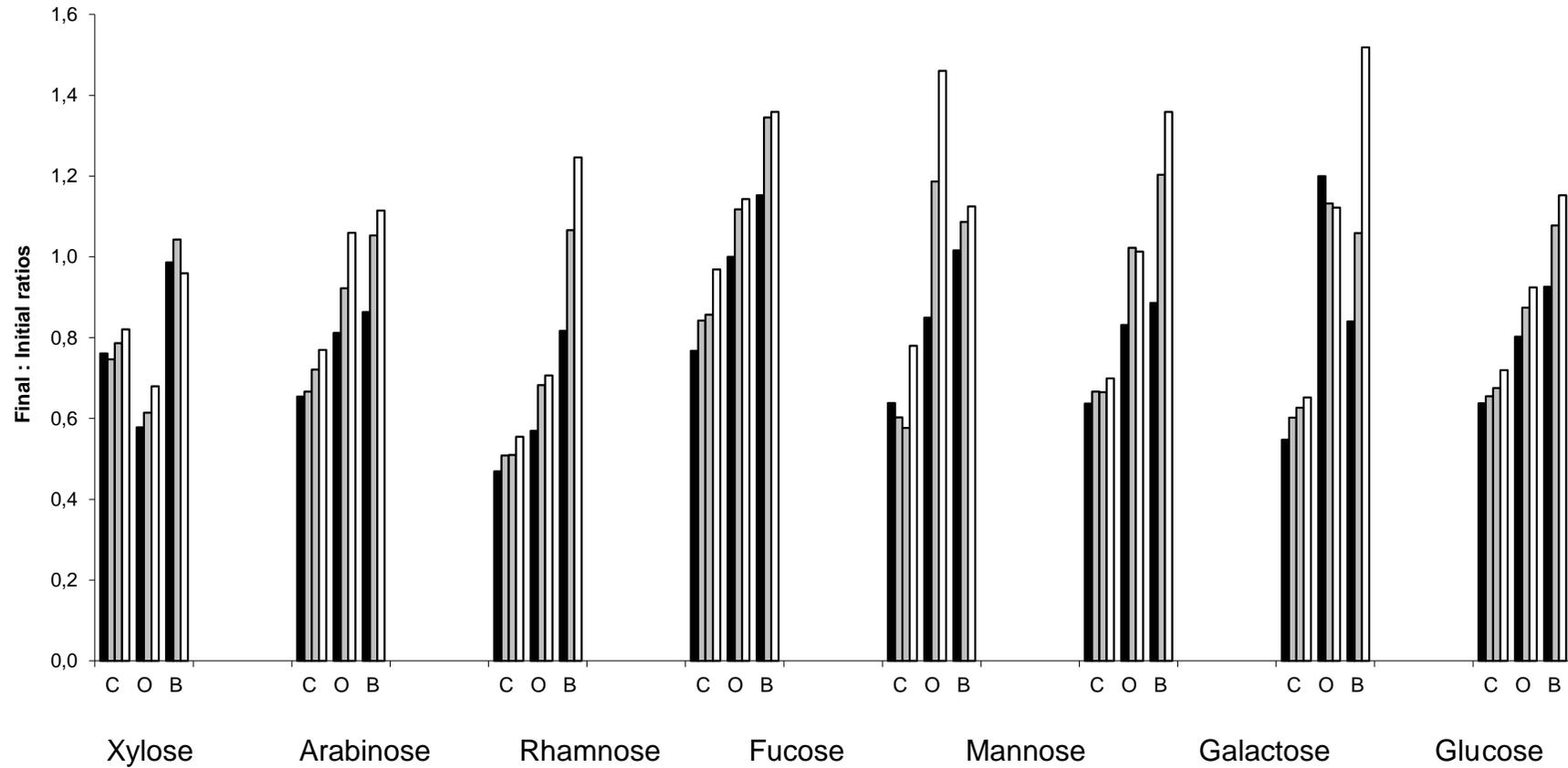
CuO-extractable PPDs (mg/g)	Chestnut				Oak			Beech		
	Shade	Int-med.1	Int-med.2	Sun	Shade	Int-med.	Sun	Shade	Int-med.	Sun
Vanillin	26.9 (0.93)	34.4 (1.32)	43.0 (1.04)	55.0 (0.20)	37.6 (0.72)	49.0 (0.24)	73.8 (0.66)	58.8 (0.22)	73.9 (0.87)	91.5 (1.03)
Vanillic acid	0.96 (0.17)	0.89 (0.05)	1.20 (0.09)	1.13 (0.03)	0.80 (0.03)	1.13 (0.42)	1.40 (0.17)	0.72 (0.31)	0.71 (0.05)	0.84 (0.44)
<b>Total Vanillyl</b>	<b>30.5 (0.72)</b>	<b>38.0 (1.39)</b>	<b>48.1 (1.02)</b>	<b>60.1 (0.28)</b>	<b>40.5 (0.85)</b>	<b>52.9 (0.33)</b>	<b>78.8 (0.98)</b>	<b>61.6 (0.53)</b>	<b>76.4 (0.78)</b>	<b>94.9 (1.57)</b>
Syringaldehyde	6.67 (0.38)	7.94 (0.99)	9.66 (0.47)	11.9 (1.68)	7.11 (0.45)	7.97 (0.69)	12.3 (0.30)	11.1 (1.13)	12.6 (0.03)	15.1 (0.01)
Syringic acid	1.20 (0.08)	1.57 (0.09)	2.40 (0.03)	2.26 (0.12)	1.30 (0.23)	1.99 (0.32)	2.31 (0.27)	1.68 (0.20)	2.07 (0.02)	2.45 (0.12)
<b>Total Syringyl</b>	<b>11.3 (0.87)</b>	<b>13.4 (1.02)</b>	<b>16.2 (0.67)</b>	<b>19.2 (2.26)</b>	<b>11.1 (0.10)</b>	<b>13.7 (1.71)</b>	<b>19.9 (0.97)</b>	<b>16.9 (1.47)</b>	<b>18.3 (1.87)</b>	<b>22.4 (0.41)</b>
<b>Total PPDs</b>	<b>45.2</b>	<b>54.2</b>	<b>68.3</b>	<b>82.8</b>	<b>54.1</b>	<b>69.4</b>	<b>103.5</b>	<b>84.2</b>	<b>100.4</b>	<b>124.5</b>
Lignin : PPD	3.96: 1	3.52: 1	3.16: 1	2.79: 1	4.34: 1	3.78: 1	2.71: 1	3.61: 1	3.37: 1	3.11: 1
Vanillyl : Syringyl	2.69: 1	2.83: 1	2.97: 1	3.13: 1	3.64: 1	3.87: 1	3.95: 1	3.65: 1	4.17: 1	4.23: 1
(a) (Ac/Al) v	0.036	0.026	0.028	0.021	0.021	0.023	0.019	0.012	0.011	0.009
(b) (Ac/Al)s	0.181	0.198	0.148	0.189	0.183	0.149	0.188	0.151	0.164	0.162

**Table 6:** Summary of the PCA for total and individual CuO-extractable phenylpropanoid moieties (PPD) in sun, intermediate and shade leaves of chestnut, oak and beech trees. For each of the top three principal components (PC) the eigenvalue and the proportional (PrVar), cumulative variance it explains are shown with the eigenvectors and loading each variable contributes to that PC.

	Eigenvalues				Eigenvectors					ANOVA			
	PC	Value	PrVar	Cum Var	p-hydroxyl	Vanillyl	Syringyl	p-coumaric	Ferulic	Total	F	P-value	F-crit
Chestnut	1	9.60	0.64	0.64	-0.15	-0.37	-0.32	-0.11	-0.22	-0.39	13.5	p<0.01	3.49
	2	3.51	0.23	0.87	-0.46	0.13	0.18	-0.47	0.19	0.13	2.43	n.s	3.49
	3	1.41	0.09	0.97	-0.15	0.13	-0.17	-0.19	-0.53	0.05		n.s	
Oak	1	12.8	0.86	0.86	-0.30	-0.30	-0.31	-0.20	-0.05	-0.36	12.5	p<0.01	4.26
	2	1.29	0.09	0.94	-0.15	-0.10	0.10	-0.11	0.85	-0.06	1.89	n.s	4.26
	3	0.78	0.05	0.99	0.09	0.08	0.04	0.15	0.22	0.07		n.s	
Beech	1	9.68	0.65	0.65	-0.31	-0.30	-0.31	-0.20	-0.11	-0.31	11.6	p<0.01	4.26
	2	2.48	0.17	0.81	-0.15	0.01	0.16	-0.25	0.31	0.03	1.08	n.s	4.26
	3	0.36	0.16	0.97	0.09	0.22	0.03	-0.43	-0.51	0.17		n.s	

**Table 7:** Final concentrations of total and individual TFA-extractable sugars and ratio of hexose to pentose sugars (a) (rhamnose+fucose/xylose+arabinose), (b) (mannose+galactose/xylose+arabinose) in shade, intermediate and sun leaf litters from chestnut, oak and beech after 24 months decomposition experiment.

Sugars (mg/g)	Chestnut			Oak			Beech			
	Shade	Int-med.1	Int-med.2	Sun	Shade	Int-med.	Sun	Shade	Int-med.	Sun
Xylose	49.7 (2.07)	41.0 (0.52)	37.5 (1.08)	38.9 (2.04)	41.7 (1.49)	42.4 (2.47)	45.4 (1.14)	78.0 (1.58)	75.0 (0.89)	64.1 (0.25)
Arabinose	18.0 (0.19)	17.4 (0.63)	16.8 (0.64)	15.7 (0.69)	24.2 (0.75)	24.8 (0.29)	25.0 (0.82)	20.8 (2.84)	23.7 (0.65)	23.3 (1.30)
Rhamnose	6.0 (0.29)	6.0 (0.17)	5.4 (0.04)	5.6 (0.33)	6.6 (0.31)	7.1 (0.50)	6.1 (0.23)	6.7 (1.21)	8.1 (0.18)	7.6 (0.88)
Fucose	3.3 (0.13)	3.2 (0.10)	3.0 (0.04)	3.1 (0.11)	3.6 (0.07)	3.8 (0.25)	3.2 (1.39)	3.4 (0.42)	3.9 (0.20)	3.6 (0.24)
Mannose	11.3 (2.44)	8.8 (0.55)	7.9 (0.72)	9.2 (0.68)	7.9 (0.21)	10.8 (0.24)	11.1 (0.49)	12.7 (0.26)	12.6 (0.13)	12.6 (0.30)
Galactose	25.1 (0.41)	25.4 (0.71)	25.0 (1.33)	25.1 (1.07)	27.1 (0.92)	32.1 (0.45)	31.6 (1.37)	21.0 (2.80)	26.6 (0.85)	26.5 (2.31)
Total sugars	155.8	146.5	141.1	144.3	161.0	166.5	165.6	173.6	182.5	174.2
(a) (r+f)/(x+a)	0.137	0.158	0.155	0.159	0.155	0.162	0.132	0.102	0.122	0.128
(b)(m+g)/(x+a)	0.538	0.586	0.606	0.628	0.531	0.638	0.607	0.341	0.397	0.447



**Figure 3:** The ratios of final:initial sugar concentrations in shade (black columns), intermediate (shaded columns) and sun leaves (white columns) from chestnut (C), oak (O) and beech (B) trees. Final sugar concentrations were determined after 24 months decomposition in microcosms under controlled laboratory conditions.

For chestnut leaf litter categories, there was a decrease with time, whereas there was an increase for oak leaf litter categories. However, there was no significant variation for beech between initial and final ratios.

#### **PPD concentrations**

Concentrations of PPD in the three leaf litter species and the leaf litter categories after 24 months decomposition in microcosms are shown in Table 8.

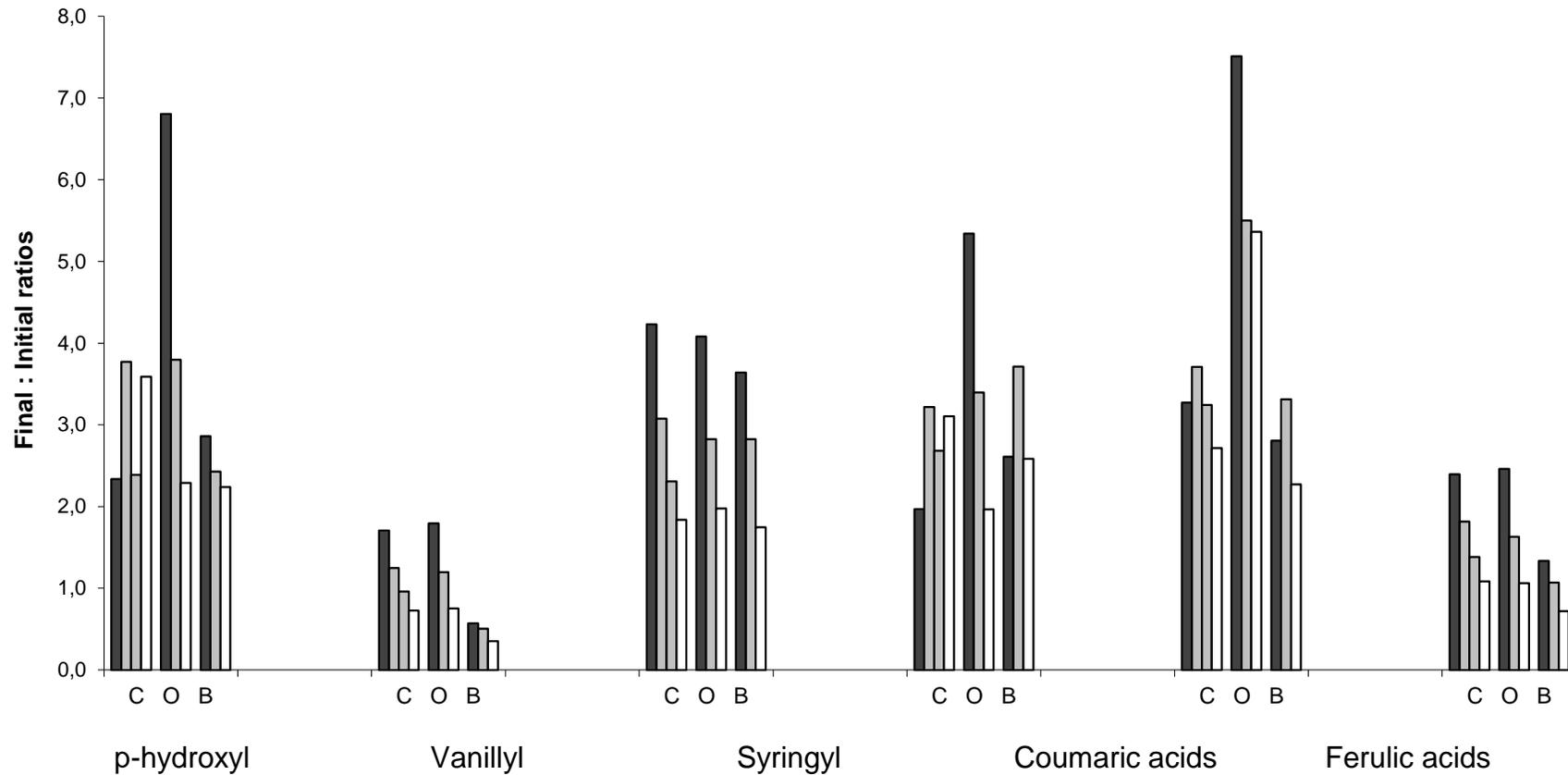
Mean final concentrations were 97.7 mg g<sup>-1</sup> for chestnut, 118.7 mg g<sup>-1</sup> for oak and 103.1 mg g<sup>-1</sup> for beech indicating an increase in the extractability of PPDs in chestnut and oak. There were also differences in the concentrations of individual compounds within and between litter species (Table 8). The ratios of final-to-initial concentrations are shown in Figure 4 to enable assessment of relative changes in PPD concentrations. Ratios less than 1 indicate lower concentrations of extractable PPD after 24 months than fresh litter while ratios >1 reflect increased lignin concentrations, or greater susceptibility to the extraction procedure as a consequence of microbial activities.

For the final-to-initial total PPD concentrations, there was a consistent pattern of higher PPD concentrations in

shade than sun leaves for chestnut and oak after 24 months (Figure 4). The same relative trend for leaf types was evident for beech but reflecting a decrease in PPD concentrations in sun leaves after incubation. The differences between initial and final concentrations were not significant, but reflect trends shown by vanillyl constituents which, because of high overall concentrations, influenced trends in total PPD concentrations. Vanillyl concentrations in the leaf litter categories were initially 3 or 4 times higher than syringyl concentrations for all species, but after 24 month incubation, the large differences between vanillyl and syringyl concentrations were reduced for chestnut and oak. In contrast, beech leaf litters contained higher syringyl concentrations than vanillyl concentrations. The acid to aldehyde ratios of vanillyl and syringyl units also showed an increase for all trees. In contrast to initial ratios, these ratios showed considerable differences between species and within the leaf litter categories. Beech had the highest ratios, whereas chestnut showed the lowest ratios. Oak had medium ratios between beech and chestnut. There was also consistent pattern of increasing these ratios from shade to sun leaf litters for all species.

**Table 8:** Final concentrations of vanillyl and syringyl, and ratios of vanillyl:syringyl and (a) acid to aldehyde ratios of vanillyl and (b) syringyl in shade, intermediate and sun leaf litters from chestnut, oak and beech after 24 months decomposition experiment.

CuO-extractable PPDs (mg/g)	Chestnut			Oak			Beech			
	Shade	Int-med.1	Int-med.2	Sun	Shade	Int-med.	Sun	Shade	Int-med.	Sun
Total Vanillyl	52.0 (2.49)	47.4 (0.88)	46.2 (0.91)	43.7(0.60)	72.6(2.08)	63.2 (0.43)	59.4(2.68)	35.2 (1.24)	38.6 (4.93)	33.5 (0.59)
Total Syringyl	47.8 (3.62)	41.2 (1.48)	37.4 (0.25)	35.3(0.37)	45.3(0.22)	38.7 (0.35)	39.3(1.24)	61.5 (4.08)	51.7 (0.91)	39.1 (2.89)
Total PPDs	108.3	98.3	94.4	89.7	133.0	113.0	110.0	112.5	107.4	89.4
Vanillyl: Syringyl	1.09: 1	1.15: 1	1.24: 1	1.24: 1	1.60: 1	1.63: 1	1.51: 1	0.57: 1	0.75: 1	0.86: 1
(Ac/Al) <sub>v</sub>	0.099	0.118	0.115	0.120	0.113	0.123	0.133	0.127	0.148	0.183
(Ac/Al) <sub>s</sub>	0.204	0.215	0.229	0.289	0.223	0.232	0.265	0.254	0.263	0.319



**Figure 4:** The ratios of final:initial PPDs in shade (black columns), intermediate (shaded columns) and sun (white columns) from chestnut (C), oak (O) and beech (B) trees. Final PPDs were determined after 24 months decomposition in microcosms under controlled laboratory conditions.

### Discussion and Conclusion

Smaller leaf size, thicker leaf blades and closer vein spacing found in sun leaves are consistent with findings in other taxa exhibiting sun-shade variation observed by the other researchers, e.g. Eschrich et al. 1989; Barbacka et al., 1998; ). Sun leaves are smaller, with more deeply lobed margins, and have more stomata, thicker mesophylls, and thicker cuticular membranes when compared with shade leaves. The difference between sun and shade leaves is based primarily on anatomical features of the mature leaf (Ashton and Berlyn, 1994). A leaf with two prominent layers of palisade parenchyma belongs to the category of sun leaf; a leaf with only one layer of palisade cells is regarded as a shade leaf.

Differences in the chemical composition of sun and shade litters can be related to variation in morphology, sclerophylly, leaf thickness, amounts of photosynthetic tissues, spongy parenchyma and degree of vascularisation which reflect well-documented, physiological adaptations of living leaves (Sarkanen and Hergert, 1971; Adler, 1977; Crawford, 1981; Brett and Waldron, 1990; Kürschner, 1997). However, with the exception of the work of King and Heath (1967), we are unaware of any comprehensive studies on intra- and inter-specific composition of leaf litters from trees growing in close proximity on the same soil type; i.e. where variation in litter quality is not confounded by differences in environment or soil fertility.

Differences in the biochemical composition of sun and shade leaves could be related to variation in leaf thickness, palisade mesophyll thickness, less intercellular space and spongy parenchyma and conductive tissues. Chloroplasts contain approximately half of the total protein in leaves and half of the soluble protein in a leaf is invested in the enzyme Rubisco (Salisbury and Ross, 1985). The cell wall most of the cellulose, hemicellulose and lignin with higher concentrations of lignin in vascular tissues (Sarkanen and Hergert, 1971; Adler, 1977; Crawford, 1981; Brett and Waldron, 1990). Hence, with two layers of the chlorophyll-bearing palisade cell sun leaves should have higher concentrations of

nitrogen and cell wall constituents per unit area than shade leaves. However, in this present study, the determination of these litter quality parameters in sun and shade leaves was on weight basis rather than per unit leaf surface. Because sun leaves were heavier than shade leaves, determinations expressed per unit mass resulted in higher concentrations of N and cell wall constituents in shade leaves than sun leaves. However the higher concentrations of lignin and PPDs in sun leaves than in shade leaves could be attributed to greater vascular development for transporting photosynthate and xylem sap.

The results of presented here are generally consistent with those of King and Heath (1967) for *F. sylvatica* in that shade leaves had higher nitrogen and cellulose concentrations than sun leaves, whereas sun leaves had high lignin concentrations; mainly as a consequence of larger amounts of vascular tissue required for translocation of water and photosynthate. Sun leaves also have two layers of palisade tissue containing chloroplasts which constitute about half of the protein in leaves as Rubisco (Salisbury and Ross 1985). However, because these analyses were made on a gravimetric basis, rather than per unit area, the percentage concentrations of N in sun leaves was reduced by the greater mass of structural compounds than in shade leaves.

Data for the hydrolysable sugars indicate variation in the composition of hemicelluloses in different leaf types and species, which have not previously been documented. Similarly, concentrations of phenylpropanoids indicate novel differences in lignin composition. The alkaline CuO oxidation does not release phenylpropanoids from condensed lignins, which are a major constituent of 'Klason' lignin determined by conventional proximate analyses. The ratio of lignin: PPDs may therefore be an indication of the degree of lignin depolymerisation and potential microbial degradability. Irrespective of the exact chemical composition of these fractions the lignin: PPD ratios showed significant variation between species with shade leaves having lower relative concentrations of extractable lignin derivatives than sun leaves.

Differences in the relative concentration of syringyl and vanillyl moieties, which comprise the main constituents of Gymnosperm lignins, also indicate significant variation in the composition of lignins within and between species. We are unable to interpret the functional basis of these differences but they could affect microbial decomposition of these tissues.

The pattern of litter decomposition, where species with rapid initial mass losses have larger litter masses than species decomposing slowly at constant rates, was observed for the sun and shade leaves (Sarıyıldız and Anderson 2003 (a,b)). The decomposition rates of the shade leaves were initially higher than the sun leaves. However, as the decomposition proceeded with the time the decomposition rates of sun leaves were similar to, or greater than the shade leaves. The question is risen here that why the decomposition rates ceased in the shade leaves which initially decomposed at higher rates. Because within species total sugar and PPD concentrations initially clearly reflected differences in decomposition rates between sun and shade leaves, the changes in total and individual sugars and PPD concentrations were determined at the end of the 2-year study in order to try and explain this phenomenon. The results showed that there was a general decrease in sugar concentrations, but the proportional losses varied from about 36.7 % in chestnut, 19.0 % in oak and no significant changes in beech. The various litter categories formed a continuous sequence of decreasing sugar losses from chestnut shade leaves to beech sun leaves. In the chestnut shade leaves, rhamnose and glucose showed the highest losses (about 53 and 45 % of initial concentrations respectively), followed by mannose, galactose and arabinose (about 36 % losses) while xylose and fucose showed comparatively small losses (<25 % losses). These changes reflect differences in the decomposition rates of the constituent hemicelluloses. The magnitude of these mass losses decreased across the series of litter qualities from shade to sun leaves, and from chestnut, through oak to beech leaves, so that there were insignificant changes in the

hemicellulose constituents of beech sun leaves.

As for total PPDs in the present study, there was a consistent pattern of higher PPD concentrations in shade than sun leaves for chestnut and oak after 24 months, though sun leaves initially contained higher concentrations of PPD than shade leaves. The same relative trend for leaf types was evident for beech but reflecting a decrease in PPD concentrations in sun leaves after incubation. The higher PPD concentrations in shade than sun leaves after 24 months could have been attributed to two factors: (1) differences in N concentrations, (2) differences in the quality of lignin and its biodegradability by micro-organisms. Although N concentration did not appear to influence the rate of litter decomposition, the differences in PPDs loss rates between shade and sun leaves showed a negative correlation with their initial N concentrations. The same negative relationship between total lignin loss rate and initial N concentrations was also observed by other authors (e.g. Hermann et al., 1977; Berg et al., 1982; Berg and Ekbohm, 1991 and Rutigliano et al., 1996).

In terms of lignin quality and biodegradability by micro-organisms, the different rates of decomposition between the vanillyl and syringyl moieties, which comprised about 91-95 % of the total PPDs could have been another factor resulting in the different PPD loss rates between sun and shade leaves. Although some researchers have shown that the phenylpropanoid moieties have significantly different rates of decomposition by soil fungi and bacteria in vitro (e.g. Kirk et al., 1975; Faix et al., 1985; Seelenfreund et al., 1990; Vasudevan and Mahadevan, 1991; Rodriguez et al., 1994) there have been few studies in the literature that investigated the chemical quality of lignin-polymer, in relation to litter decomposability by the soil microbial biomass in situ. Previous studies (e.g. Kirk et al., 1975; Highley, 1982; Hedges et al., 1988) indicated that syringyl rings in lignin molecules were more reactive overall to white-rot fungi attack than less methoxylated vanillyl rings. Ander et al. (1984) in vitro also noted that the vanillyl moiety had a significantly slower rate of decomposition by

white-rot fungi than syringyl. However, whether this pattern is due to primarily to a greater intrinsic reactivity of doubly methoxylated syringyl rings or to their different crosslinking pattern or locations in tissues is not well known (Goni et al., 1993). In the present study *in situ*, the result showed the opposite trend with the vanillyl moieties in leaf litter decomposed higher rates than syringyl and influenced trends in the total PPDs. This appears to be the first time that the difference in lignin decomposition rates is related to the higher decomposition rate of vanillyl moieties by micro-organisms in leaf litters. The results also indicate that the nature of the ring linkages within lignin polymers is not the only factor that appears to determine their overall degradative lability. Because of the different decomposition rates of these moieties by micro-organisms and the formation of new and stable complexes in relation to elevated concentrations of lignin and N as explained above the chemical quality of sun and shade leaf litters changed over time and influenced the ongoing processes of decomposition.

In conclusion, morphological and anatomical changes in leaves of the same tree under different environmental factors due to the tree canopy structure results in significant variations in the chemical composition of sun, intermediate and shade leaf litters. The proportion of these leaf types will vary with the structure of the canopy and stand development and affect rates of litter decomposition (Swift et al., 1979; Sariyıldız and Anderson, 2003 (a, b). Conclusions about the generality of the relationships between tree species and litter type presented here are constrained by the lack of plot replication. However, they are consistent with the results of an extensive study of variation in the chemistry of leaves and litter from beech, oak and chestnut trees in replicate woodlands on a wide range of different soil types (Sariyıldız, 2000; Sariyıldız and Anderson 2005).

This study investigated the effects of variation in the leaf litter quality of sun and shade leaf litters from the same three trees on their decomposition rates indicating the importance of the quantity and quality of lignin in affecting decay rates of sun and

shade leaf litters. It is not only because lignins are recalcitrant as a consequence of their aromatic composition and structure, but also because they can physically inhibit the activity of carbohydrates by masking substrate surfaces of hemicellulose and cellulose. Thus, the disappearance of the early part of these fractions in sun and shade leaf litters was strongly influenced by their initial lignin concentrations (and complexing with structural carbohydrates). Shade leaf litters with lower initial lignin showed the greater degree of decomposition of sugars, whereas sun leaf litters with high initial lignin concentrations showed the lower degree of decomposition of sugars. As labile compounds were being degraded in shade leaf litters by micro-organisms with the enhancing effect of higher N concentrations, the PPD concentrations showed an increase, with the simultaneous formation of more recalcitrant compounds with higher N concentrations. This resulted in the slow degradation of both PPD, and lignified cellulose and the decomposition of shade leaf litter was retarded. However, low available N concentration in sun leaf litters favoured the slow growing micro-organisms that were able to decompose lignin. This in turns changed the chemical quality of sun and shade leaf litters over time compared to their initial chemical composition. Under these conditions, the preference of these micro-organisms to decay the phenylpropanoid moieties from lignin was towards the vanillyl moieties and strongly influenced the total PPDs loss rates between sun and shade leaf litters. As far as the relationships between intra-specific variation in the litter quality and decay rates, and understanding of interactions of the biochemical constituents of litter at the cellular level, where microbial decomposition take place, were concerned, determination of the quality, quantity and spatial configuration of the ligno-cellulose complex in the plant cell wall appeared to provide further insight into the understanding the mechanisms regulating processes of decomposition. It may have been that the short time of the decomposition experiment and variable conditions obscured the fine details. Thus, the relationship between inter-specific variation in the litter quality and

decay rates was well described by proximate analysis of organic fractions, mainly lignin. The long-time decomposition experiments are, therefore, required to test the usefulness of new litter quality parameters and methods for analysing plant litter quality as it relate to decomposition.

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