



# Effect of fertilization and growth conditions on woody-tree biomass composition

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Received: 31 January 2025 / Revised: 7 April 2025 / Accepted: 14 November 2025  
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## Abstract

A biorefinery can operate with various plant materials as feed stock, (in contrast to fossil-based oil refineries). It has been defined by the International Energy Agency, Task 42, as “the sustainable processing of biomass into a spectrum of marketable bio-based products (chemicals, materials) and bioenergy (biofuels, power, heat)”. One of the challenges in operating large-scale facilities is that the biomass supply-chain is dependent on reliable availability of appropriate raw materials, continuous at all seasons while maintaining a high quality. Currently, various types of biorefineries are frequently discussed e.g. sugar-based bio refineries or thermochemical bio refineries; however, both strategies are dependent on solid and well-known characteristics of the biomass regarding their chemical and biological processes. A woody biomass supply-chain, from forest residues are influenced by factors such as: tree species, growth conditions and occasionally forest fertilization but how these factors influence the composition of the biomass are poorly understood. In this study, we used field experiments in Sweden where poplar, hybrid aspen, birch, Scots pine and Norway spruce were grown at various sites across a latitude gradient and treated with fertilizers within each site – making direct comparisons between tree species and fertilization treatment possible. The presented results demonstrate that there are minor differences in biomass composition i.e. cellulose, lignin, hemicelluloses (mannan, xylan, galactan, arabinan) and oil content, between forest site and fertilization treatment. Moreover, our results demonstrate that geographic location (northern or southern latitudes) has limited effect on the chemical composition. In addition, our results demonstrate that deciduous tree species (poplar, birch and hybrid aspen) have similar biomass composition but that the compositions are different to coniferous species. For an industrial context, our results suggest that if biomasses are a blend of coniferous and deciduous tree species, the process design must be adjusted to reach optimal usage of the lignocellulosic feedstock in full-scale industrial processes.

**Keywords** Biorefinery · Woody biomass · Lignocellulosic composition · Deciduous and coniferous tree species · Biomass supply chain

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# 1 Introduction

Increasing demand for energy, decreasing use of fossil fuels, increasing fuel prices and global warming will require an expanded search for alternative fossil-free and carbon-neutral energy supply systems where production of biofuels and chemicals are some of the key components. In this transition, biomass can serve as a renewable feedstock for producing liquid and gaseous biofuels (or chemicals). In the European Union (EU), the Renewable Energy Directive II REDII [1] sets an overall renewable energy target of 32% and a 14% target for the transport sector by 2030. Among the EU member states, Sweden stands out by using more than 20% renewable fuels for domestic transport in 2015, of which 85% are imported [2, 3]. Recently, the Swedish government has decided that, by the year 2030, CO<sub>2</sub> emissions from domestic transport, i.e. road, railway and shipping, should be reduced by 70% compared to those in 2020 [4]; in addition, Sweden is planned to be carbon neutral by 2045, followed by net negative CO<sub>2</sub> emissions [4]. This reduction in CO<sub>2</sub> emissions will require a steady biomass supply from agriculture and forestry sources. At the same time, present wood-based industry is planning for new products and an increase of their current products. Thus, there will be a future increased demand for woody biomass. One option for increasing the production of woody biomass per unit area is to plant tree species (Poplars and hybrid aspen) that have natural fast growth and to use forest management practises that will increase tree growth e.g. fertilization.

The RED II restricts the use of biomass for biofuel production if the areas could be used for food or feed production [1]. This is not the case for tree biomass production of poplars and hybrid aspen from arable land, making biomass production from tree plantations an interesting option as a resource for biorefineries in these areas. Woody biomass from the forest industry is an abundant and economically valuable resource in Sweden, covering over two-thirds of the area of the country. These forests are dominated by the softwood species Norway Spruce and Scots Pine that constitute over 75% of the growing volume, followed by hardwood, Birch of 16% and Aspen of a few percent [5].

## 1.1 Biorefineries

In contrast to existing oil refineries, a biorefinery operates with plant materials. Biorefineries have been defined by the International Energy Agency, Task 42, as “the sustainable processing of biomass into a spectrum of marketable bio-based products (chemicals, materials) and bioenergy (biofuels, power, heat)” [6]. Like petroleum, biomass can be utilized as raw material to produce a range of chemicals and energy carriers, such as ethanol, methanol, di-methyl

ether (DME) and many other compounds [7–9]. One of the challenges in operating large-scale facilities is material supply, i.e., to make sure that the availability of appropriate raw materials is secured for continuous operation all seasons and all year round [10].

If biomass is intended for use in a biorefinery plant [6, 11], parameters such as the compositions of structural carbohydrates and lignin, as well as extractives, have an influence on the possible yields of different products. The yield has a direct impact on the economic viability of the intended production process. This makes it important to have knowledge of the composition of potential lignocellulosic resources. Lignocellulosic materials differ from one species to another. However, the main constituents are basically the same, although the contents of individual carbohydrates, aromatics and other compounds vary; about 50–60% are carbohydrates, i.e. cellulose and hemicelluloses, 20–30% lignin, while the rest consists of extractives, fatty acids, ash, etc [12]. Hardwood is generally richer in xylose and arabinan, while softwood contains higher amounts of mannose.

The oil content in tree species varies between species and for trees native to boreal and temperate regions, it also shows seasonal variations with oil accumulating in the autumn in preparation for winter [13]. Oil consists of a glycerol molecule with three fatty acids attached, but fatty acids are also a major part of cell membrane lipids. Fatty acid content in tree is relatively low but still accounts for an important constituent in crude tall oil, which is a by-product from the pulp and paper industry [13].

One option to increase biomass production is to plant tree species that naturally has a high growth rate. One of the most fast growing and frequently used species in plantation forestry is the genus *Populus* [14–16]. Many of these plantations have been established on arable land [16–18] and in a few cases on forest land [19, 20]. On arable land biomass production of *Populus* (Hybrid aspen and poplars) has been shown to reach 25 m<sup>3</sup> ha<sup>−1</sup> and year<sup>−1</sup>, while at forested arable land, biomass production has been shown to be 15 m<sup>3</sup> ha<sup>−1</sup> and year<sup>−1</sup> at a plantation age of 10 years [21]. Biomass production for Norway Spruce and Scots Pine has been found to reach about 4–7 m<sup>3</sup> h<sup>−1</sup> and year<sup>−1</sup>, respectively, while for birch production it can reach 5–10.5 m<sup>3</sup> ha<sup>−1</sup> year<sup>−1</sup> [22]. Thus, there is a large potential to increase biomass supply by planting *Populus* species.

Several studies have suggested that the total available area for *Populus* plantations in Sweden is approximately 400 000 ha arable land [21, 23] and over 2.5 million ha of boreal forest land [23]. In addition to these areas, there are over 1.2 million ha of forested arable land [21]; forest land that has a high soil fertility and water holding capacity, all parameters important for a high tree growth.

Tree growth rate and total biomass production are influenced by several parameters, including site fertility and forest management practices, e.g. fertilization [24]. Fibre length and width are also influenced by growth rate. Thus, trees growing at different site conditions (high or low soil fertility) and fertilization could have an impact on biomass composition. However, knowledge regarding how site conditions and management practices influences biomass composition needs to be determined, and comparisons between tree species are needed for industrial applications.

Thus, there is a need for understanding how biomass quality is influenced by tree species, growth conditions and forest management. Therefore, in this paper, different forest management methods (fertilization) and site characteristics (arable and forest land with different soil fertility and geographic location) were investigated. In addition, the influence of biomass quality of hybrid aspen, poplar, Norway spruce and Scots pine is discussed, as well as how such differences in biomass quality can affect potential utilization in biorefineries.



**Fig. 1** Location of experiments for biomass characteristics. Agr represents experiments at agricultural land; For represents experiments at forest land. The individual numbers indicate each experiment listed in Table 1

## 2 Materials and methods

### 2.1 Experimental design for analysed biomasses properties

The method used to analyse the effect on site conditions and fertilization was based on a tree species and fertilization experiment established at two geographic regions in northern and southern Sweden in 2012–2015. In each region, three experimental sites were located (Fig. 1). At each site four blocks were designed, each containing plots with Norway Spruce, Scots pine and hybrid aspen and poplars. In addition, birch was planted at site For - 1559, For - 8252 and Agr - 1584. The plot size was 24 × 24 m and within each block aspen and poplar plots were separated from the other tree species with a 10 m corridor (Fig. 2). The stem density planted was about 1400 trees per ha corresponding to about 3 × 3 m distance between the trees. The sites were selected to represent a gradient in site index (fertility) from poor fertility T23 to fertile sites G32. Site indexes are determined according to standard procedures where the letters T or G, represent tree species where T = pine and G = Norway spruce. The numbers following the letters (T23 or G32) represent the tree height after 100 years of growth (the higher the number, the more fertile the site) (Table 1).

At each experimental site, fertilization treatments were performed by broadly adding 75 kg of nitrogen (N) per hectare using the commercial fertilizer SkogCan (27% N content). This was manually done repeatedly every second year after planting. Biomasses (whole stem without branches, leaves or needles) were sampled at each experimental site by selecting two representative trees (having similar heights and diameters as other trees in the plot), with no sign of damage of pathogen infection, in each block for all planted species and fertilization treatments. In some cases failure in establishment occurred (poplars at forests land); thus, biomasses could not be sampled. Missing samples are shown as NP (not present) in Table 2.

### 2.2 Sample Preparation for biomass analysis

The wood samples (fresh logs) from each location of cultivation, tree species and fertilization treatments (Fert and No-fert) were coarsely ground within a timeframe of less than two months after harvest. The samples were stored frozen at −18 °C to avoid microbiological degradation until they were thawed and utilized (a maximum of 48 h in advance). Coarse milling was performed with a mobile woodchipper (Först ST6 D, Först, Andover, UK), powered with a 42 hp diesel engine.

A total of 143 samples were selected for biomass analysis. These samples were transferred to paper bags and dried

**Fig. 2** Design of experimental block one (out of four) and of design of plot within an experimental site. Planted species are Hybrid Aspen, Silver Birch (Birch), Scots Pine, Poplar, Norway Spruce. Treatments are Fertilization (Fert) and No fertilization (No-Fert). Tree spacing are 3 × 3 m corresponding to 1100 trees per ha

Norway Spruce Fert	Norway Spruce No-Fert	Hybrid Aspen Fert	Hybrid Aspen No-Fert
Scots Pine Fert	Scots Pine No-Fert	Poplar Fert	Poplar No-Fert
Birch Fert	Birch No-Fert		

**Table 1** Experimental ID, former land use history, soil conditions, and site index (SI)

Experiment	Site type	Lat °N	Long °E	Soil type <sup>1</sup>	Soil moist class <sup>2</sup>	Preci, mm	Temp day C	SI <sup>3</sup>
Gideå (1559)	For	63.40	19.02	T/SL	ME/MO	700	1010	T23
Armsjön (1561)	For	62.15	17.42	T/SL	ME	700	1120	G24
Hemling (1560)	Ara	63.66	18.55	S/SL	ME	600	940	-
Sävsjö (1582)	For	56.98	15.48	T/SL	ME	700	1300	T22
Tönnersjö (8252)	For	56.68	13.09	S/GR	ME	1064	1500	G32
Påarp (1584)	Ara	56.42	12.71	S/FT	ME	750	1575	-

<sup>1</sup> Soil type T=till, S=sediment; Soil texture SL=sandy loamy, SA=sand, GR=gravel, FT=fine texture

<sup>2</sup> Soil moisture class ME=mesic, MO=moist, DR=dry

Site index Top height at 100 years total age [25]

T=Scots pine, G=Norway spruce. For represents forests sites and Ara arable land

**Table 2** Tree height (m) of Spruce, Pine, H-aspen, Poplar and Birch used for biomass analysis

Experiment	Spruce	Pine	H-aspen	Poplars	Birch
Gideå (1559)	1.8	1.5	3	NP	NP
Armsjön (1561)	2	2	2	NP	NP
Hemling (1560)	1.4	1.8	2	1.5	NP
Sävsjö (1582)	1.5	1.8	4	NP	4
Tönnersjö (8252)	2	2	5	NP	5
Påarp (1584)	2	2	6	8	6

NP represents samples that were not present in the experiment due to failures in establishment. For represents forests sites and Ara arable land

at 45 °C for a minimum of 72 h before they were milled to a fine powder. Milling was performed in a knife mill (SK1, Retsch GmbH, Germany). The knife mill is equipped with a 1.0 mm sieve to ensure size distribution. The samples were stored in sealed plastic bottles under dry conditions at room temperature until they were analysed for total fatty acids and structural carbohydrates and lignin.

## 2.3 Total fatty acid analysis

For determination of total fatty acid content, 100 mg DW of ground samples were transferred to glass tubes with screw caps. Acidic methylation of lipids was performed by adding 2 mL of 2% H<sub>2</sub>SO<sub>4</sub> in dry methanol (v/v) and incubating on a heating block at 90 °C for 60 min. After cooling, 300 nmol of heptadecanoic methyl ester (Larodan, Solna, Sweden) in methanol was added to each tube as internal standard. Fatty acid methyl esters were extracted into heptane by adding 1.5 mL heptane and 2 mL water, then vortexing thoroughly followed by centrifugation for 2 min at 3,000 rpm. A volume of 300 µL of the heptane phase was transferred into gas chromatography (GC) vials, which were stored at -20 °C until analysis. Separation of fatty acid methyl esters from 1 µL injections was performed on a CP-wax 58 column (FFAP-CB, 50 m, 0.32 mm inner diameter, 0.20 µm film, Varian, Palo Alto, USA) using an Agilent Technologies

**Table 3** Results from analysis of variances (ANOVA) using the general linear model for all analyzed compounds.

Source	DF	Fatty acids			AIL			ASL			Cellulose			Xylan			Galactan			Arabinose			Mannan		
		F-val	P-val	F-val	F-val	P-val	P-val	F-val	F-val	P-val	F-val	F-val	P-val	F-val	F-val	P-val	F-val	F-val	P-val	F-val	F-val	P-val	F-val	P-val	
Location	5	26.63	<0.001	22.94	0.40	0.529	<0.001	29.85	0.01	0.914	74.18	0.01	<0.001	7.85	0.31	<0.001	8.87	0.25	<0.001	53.10	<0.001	9.17	<0.001		
Fertilizer	1	0.03	0.858	0.40	0.984	0.735	<0.001	12.94	0.01	0.941	0.01	0.941	0.31	1009.15	0.618	0.577	0.25	0.618	<0.001	<0.001	0.20	0.658			
Species	4	45.01	<0.001	164.92	0.42	0.835	<0.001	3.54	0.63	0.642	135.94	0.48	<0.001	0.16	0.973	<0.001	0.33	0.893	<0.001	136.66	<0.001	624.09	<0.001		
Location*Fertilizer	5	0.14	0.984	0.42	0.984	0.735	<0.001	0.63	0.63	0.642	1.06	0.642	0.379	1.01	0.408	0.441	0.94	0.408	0.441	0.43	0.783	0.16	0.957		
Fertilizer*Species	4	0.64	0.636	0.50	0.984	0.735	<0.001	0.63	0.63	0.642	1.06	0.642	0.379	1.01	0.408	0.441	0.94	0.408	0.441	0.43	0.783	0.16	0.957		
Error	121																								
Lack-of-Fit	26	3.21	<0.001	1.42	0.112	0.112	0.112	1.98	0.009	0.009	1.49	0.085	0.085	2.98	0.022	<0.001	1.79	0.022	<0.001	2.12	0.005	4.18	<0.001		
Pure Error	95																								
Total	140																								

Results show F-values (F-val) and P-values (P-val). Factors were location, fertilizer and species, and interactions thereof

P-values below 0.01 are shown in bold numbers

AIL Acid-Insoluble lignin, ASL Acid-Soluble lignin

8860 gas chromatograph with flame ionization detector and 7693 A autosampler (Santa Clara, US). Temperature program; injector 240 °C, detector 260 °C, oven started at 150 °C increasing with 4 °C/min up to 210 °C followed by an increase with 10 °C/min up to 250 °C/min with a hold for 5 min. Fatty acid methyl esters of lengths 16–20 carbons were quantified in nmol in relation to the area of heptadecanoic acid methyl ester as internal standard. The results shown is the sum of all fatty acid methyl esters in weight%.

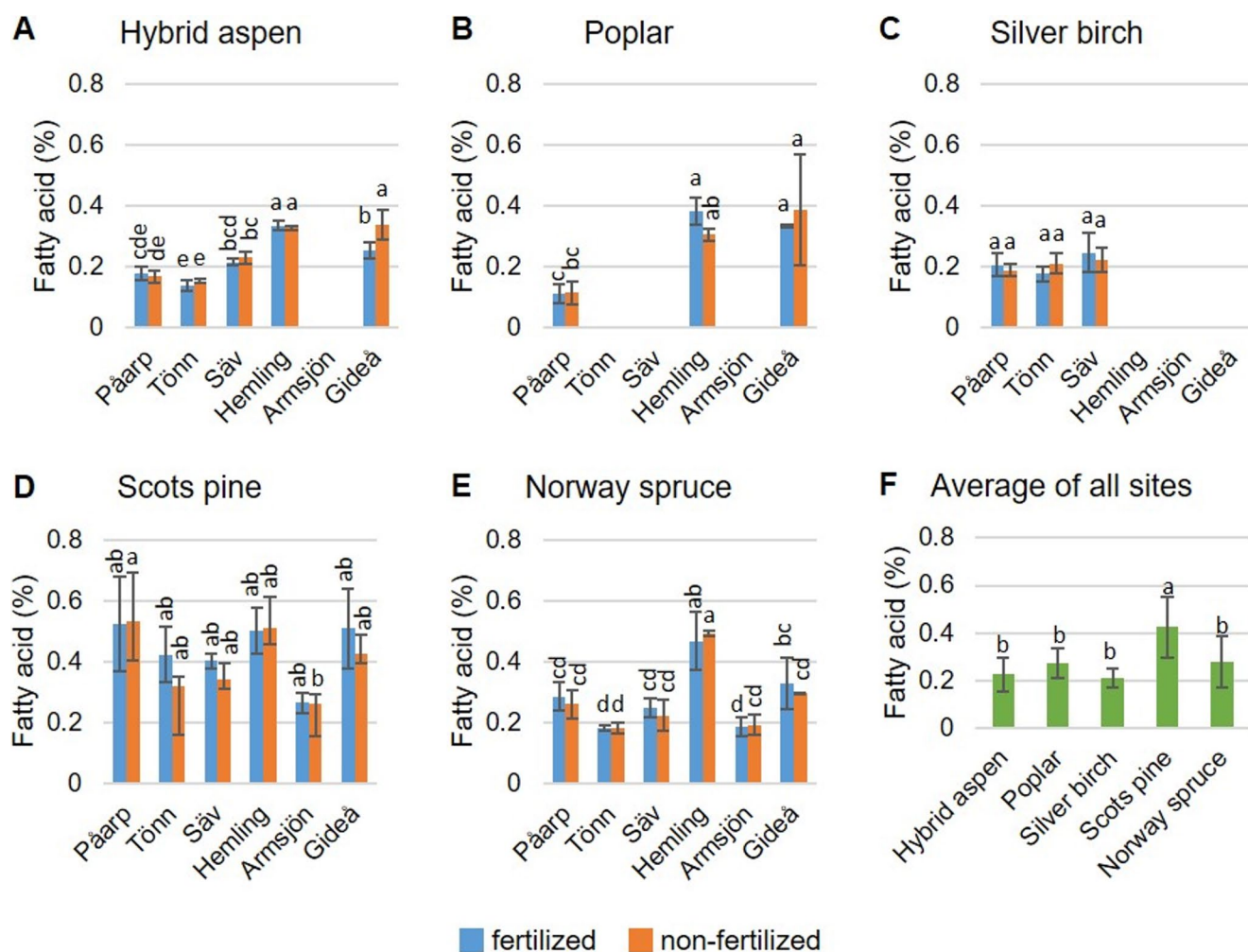
## 2.4 Structural carbohydrates and lignin analysis

Carbohydrate and lignin analyses were performed according to the standardized laboratory analytical procedures (LAP) of the National Renewable Energy Laboratory (NREL, Golden, CO, USA), titled ‘Structural Carbohydrates and Lignin in Biomass’ [26]. The procedure involves two-step acid hydrolysis to fractionate the biomass into forms that are more easily quantified. The liquid fraction after acid hydrolysis was collected to measure the acid-soluble lignin (ALS) content by an UV/VIS-spectrophotometer (UV-1800, Shimadzu, Kyoto, Japan). In addition, the hydrolysate was used for measuring the carbohydrate content utilising HPLC (Shimadzu, Kyoto, Japan). The analysis was run on a lead column (Concise Separations, Coregel CHO 782, San Jose, CA, USA) heated to 70 °C with deionized water as eluent and a flow of 0.6 mL/min, using a refractive index detector (Shimadzu). The HPLC standard sugar solution contained glucose, xylose, arabinose, mannose and galactose in relevant concentrations. The content from the solid phase was determined as acid-insoluble lignin (AIL) by gravimetric analysis.

## 2.5 Statistical analysis

Data for each of the chemical compounds was statistically analysed by analysis of variances (ANOVA) using the general linear model in Minitab 2121 (Pennsylvania, US) in which the effects of the fixed parameters tree species, geographical location, fertilizer treatment, and interactions thereof, were tested. It showed that location and species had significant effect on all compounds, while fertilizer treatment did not, and with no interaction effects seen between location and fertilizer, or between fertilizer and species. Data for each chemical compound was therefore further treated separately for each species in 1-way ANOVA, followed by post-hoc tests using the Tukey method with 95% confidence intervals to get the grouping information. Correlation plots were done using the Python (v. 3.12.6) library biokit.vis.





**Fig. 3** Total fatty acid content (% of dry weight) in tree tissues sampled from different species, locations, and fertilizer treatment (blue means fertilized, red means not fertilized). Locations are shown on the X-axes (A–E), for detailed information see Fig. 1. Results show

the mean  $\pm$  standard deviation based on 2–3 replicates. Bars that do not share a letter are significantly different according to Tukey's test at significance level  $p < 0.05$

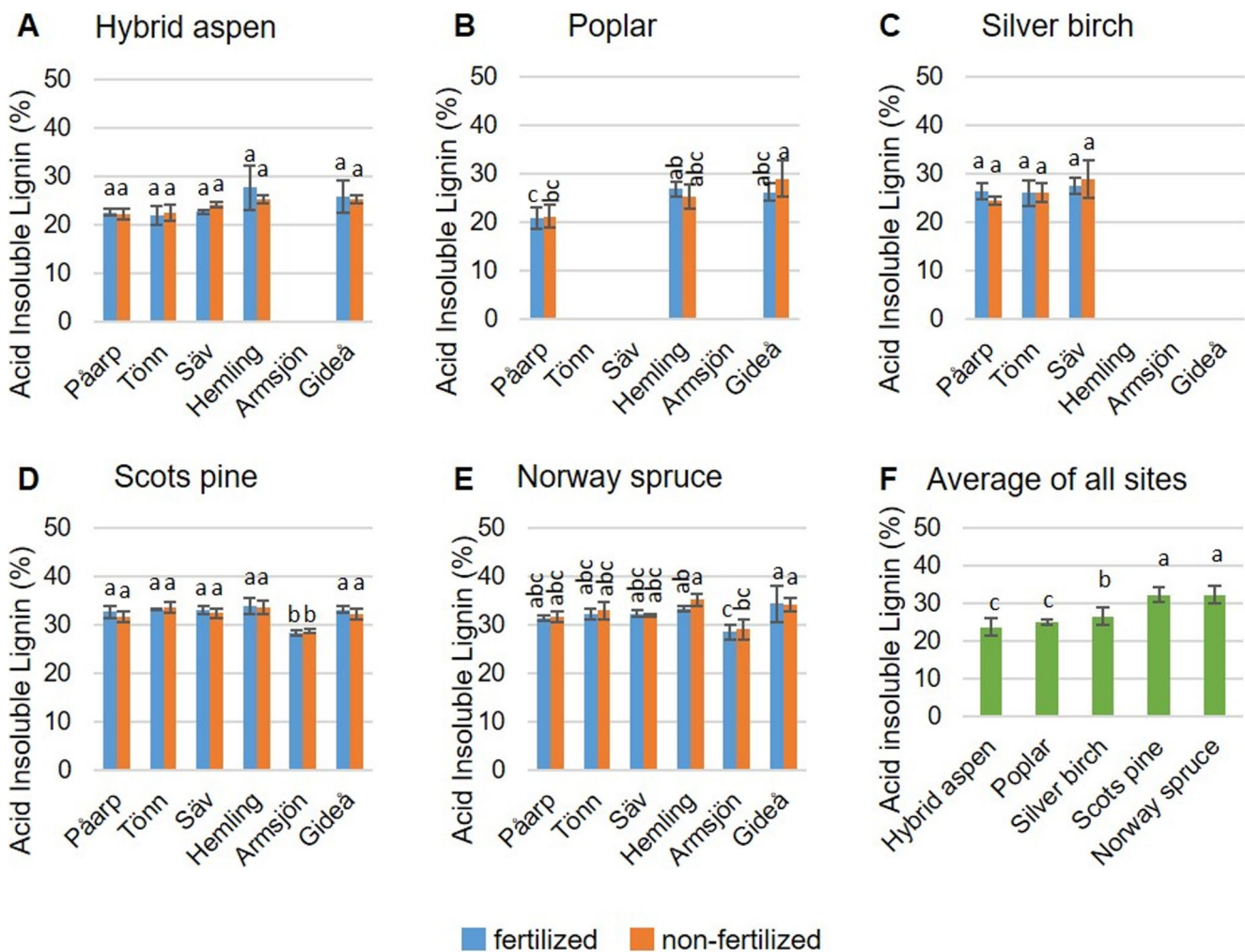
### 3 Results and discussion

This study was carried out to find out the effects – if any – of fertilization and growth location on biomass composition. It is important to note that the data gathered in this study was to an effort to provide a source for future biorefinery strategies; furthermore, access to such data is invaluable for engineers the day an actual design is carried out. To analyze the effects of tree species, geographical location, and fertilizer treatment on biomass composition of wood tissue, the contents of fatty acids, acid insoluble and soluble lignin, cellulose, xylan, galactan, arabinose and mannan were analyzed. Of these compounds, cellulose, lignin and xylan made up the major proportion of wood tissue dry matter. Analysis of variances clearly showed that location and species had a significant effect on all compounds analyzed, while fertilizer treatment had no effect (Table 3). Furthermore, there was no

interaction effect between location and fertilizer, or between fertilizer and species (Table 3). Therefore, the data was further treated separately for each species, with post-hoc tests indicating significant differences between samples. It can be noted that due to an unbalanced data set (i.e., not all species were present at all locations), it was not possible to determine if there was any interaction effect between location and species. The reason for the missing samples was caused by establishment failures of poplars on acidic soils, which is a result in line with other studies [27].

#### 3.1 Fatty acid content for tree species and growth conditions

Oil is used by trees in temperate regions as a transient energy storage during the winter when metabolic shifts occur, which lead to increased amounts of oil that is stored

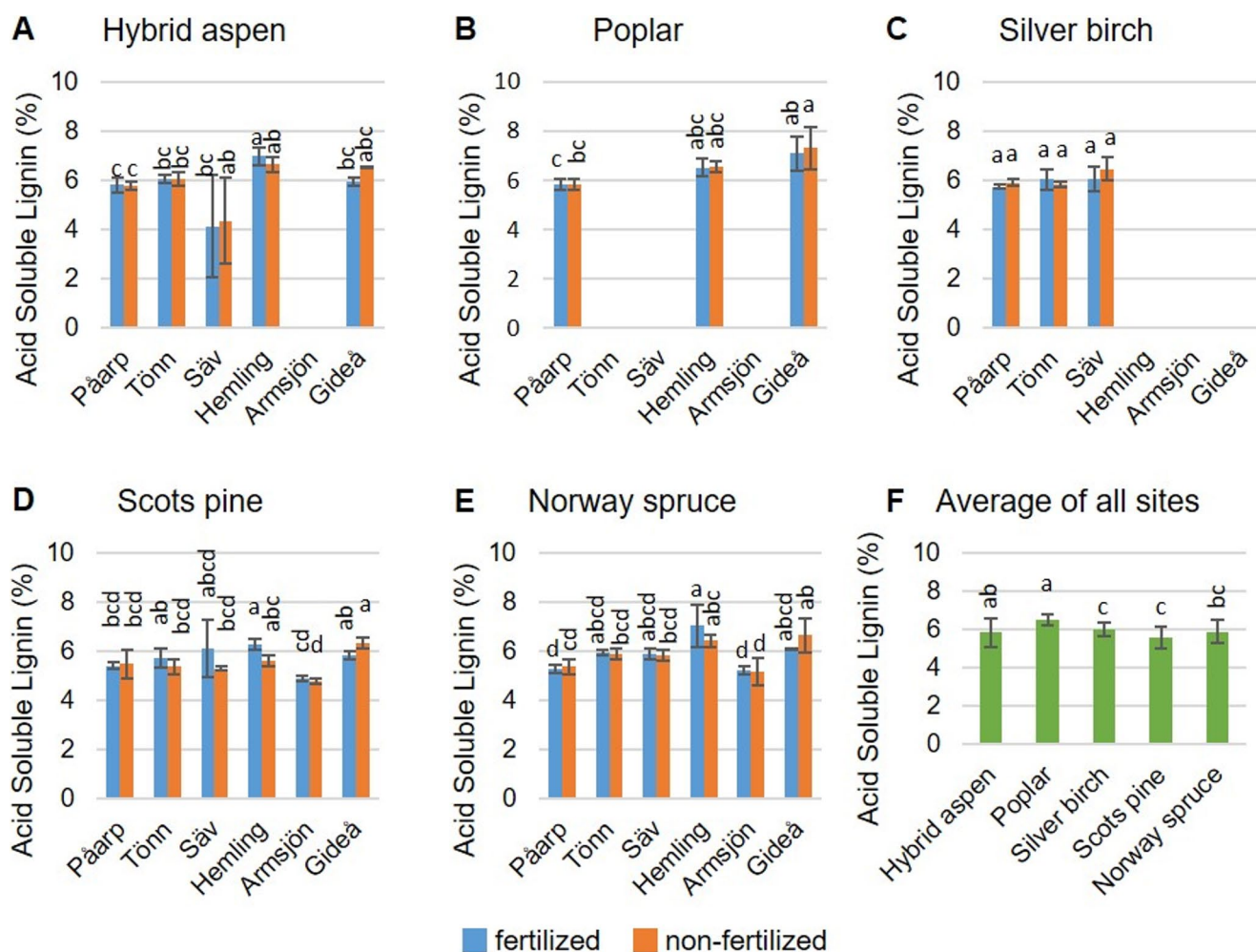


**Fig. 4** Acid-insoluble lignin content (% of dry weight) in tree tissues sampled from different species, locations, and fertilizer treatment (Blue: fertilized, Red: not fertilized). Locations are shown on the X-axes (A–E), for detailed information see Fig. 1. Results show the

mean±standard deviation based on 2–3 replicates. Bars that do not share a letter are significantly different according to Tukey's test at significance level  $p < 0.05$

in the cambial meristems and pith rays [13]. Crude tall oil is a by-product from the pulp industry which consists of fatty acids, resin acids and unsaponifiables and is produced at a yield of 30–50 kg/tonne pulp, where of approximately 60% are fatty acids [28]. The total fatty acid content in the samples in our study varied between approximately 0.1–0.5% by dry weight (Fig. 3). Significant species differences could be observed with Scots pine having the highest fatty acid content as compared to all the other species, which were not significantly different from each other (Fig. 3F). There were no clear trends with regard to the levels of fatty acids at different locations in Sweden, but for hybrid aspen and Norway spruce, the samples from Tönnersjöheden showed lower values than in samples from Hemling (Fig. 3A and E). The fatty acids consisted mainly of palmitic, oleic and

linoleic acid (16:0, 18:1, and 18:2, respectively) which are among the common fatty acids in most plants. It can be noted that even though the samples in our study were taken during the winter season, when the levels were expected to be the highest, the levels of total fatty acids were relatively low as compared to levels that were previously reported for wood tissue [13]. One explanation for this could be that the sample treatment before analysis of different compounds in our study were not optimized to avoid degradation of lipids (i.e. samples were dried at 45 °C and then stored at room temperature for a couple of months before fatty acid analysis). It could be noted that seasonal harvest time and processing and storage conditions of the raw material influence the fatty acid content, which might influence the bio-refinery output.



**Fig. 5** Acid-soluble lignin content (% of dry weight) in tree tissues sampled from different species, locations, and fertilizer treatment (blue means fertilized, red means not fertilized). Locations are shown on the X-axes (A-E), for detailed information see Fig. 1. Results show the

mean  $\pm$  standard deviation based on 2–3 replicates. Bars that do not share a letter are significantly different according to Tukey's test at significance level  $p < 0.05$

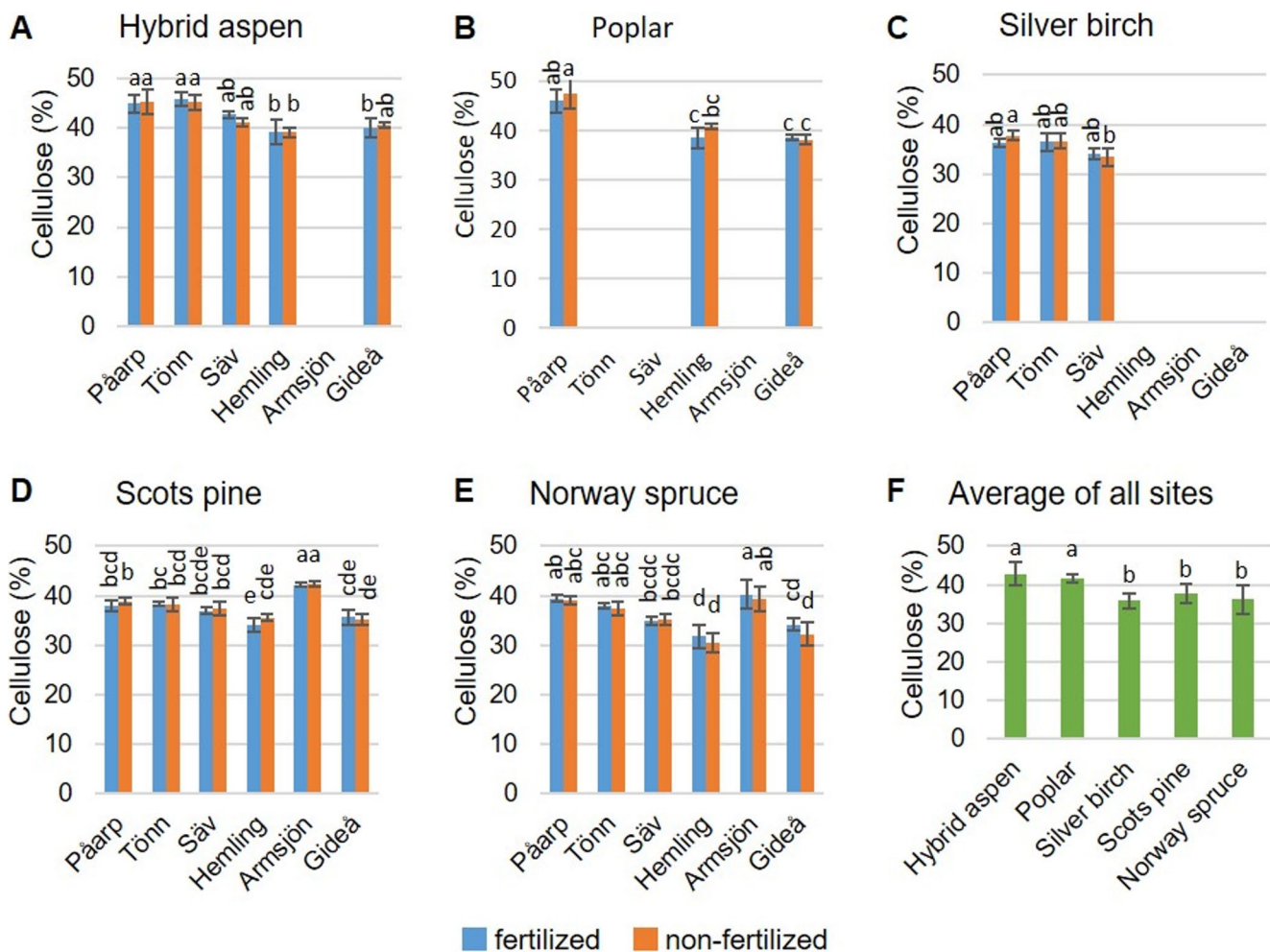
### 3.2 Composition of tree species and growth conditions

The compositions are presented in Figs. 4, 5, 6, 7, 8, 9 and 10, which show the compositions for all the tree species included in the study, compiled based on location. The overall compositions are all within the expected range, for the relevant species, which are published in the literature [29–34]. The acid-insoluble lignin (AIL) content is in general higher for the softwood species, as is the mannan content. In contrast, the xylan content is observed to be higher in hardwood species [34].

From the results of this study, it can be concluded that the location is of higher importance than the potential addition of fertilizer regarding the composition of the tree species. However, one difference that can be seen in

the structural carbohydrate data is that the Armsjön site produces Norway spruce and Scots pine with lower lignin content (Fig. 4 (AIL) and Fig. 5 (ASL)) and higher cellulose contents (Fig. 6). This is a trend that is found for the softwood species from this site. The content of AIL in Scots pine and Norway spruce are approximately the same in all samples from the different locations, except a statistically significant lower content in Scots pine from Armsjön (Fig. 6). Unfortunately, hardwood samples are lacking from this location so comparisons between some species and additional sites were not possible to analyse. There could be several reasons for this finding, e.g., soil chemistry, which may have an effect on biomass composition. However, soil classification between site “Armsjön” and site “Gideå” are similar but the differences in lignin and cellulose remain. In addition, tree heights in most





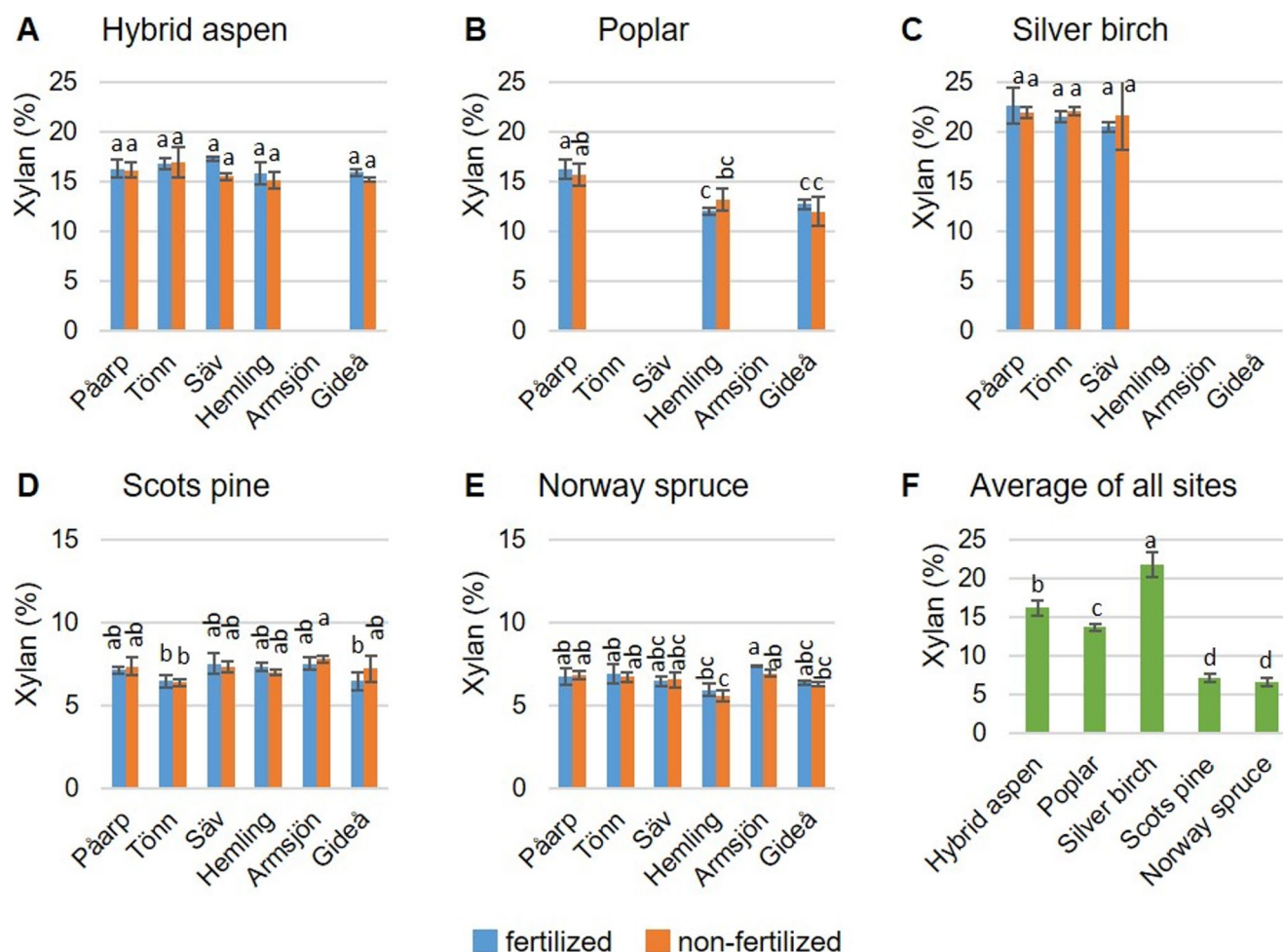
**Fig. 6** Cellulose content (% of dry weight) in tree tissues sampled from different species, locations, and fertilizer treatment (blue means fertilized, red means not fertilized). Locations are shown on the X-axes (A–E), for detailed information see Fig. 1. Results show the mean  $\pm$  stan-

dard deviation based on 2–3 replicates. Bars that do not share a letter are significantly different according to Tukey’s test at significance level  $p < 0.05$

experimental sites are similar so differences in tree size are not likely to cause these differences alone. As the cause for this difference currently remains unknown, the result with lower lignin and higher cellulose content at site “Armsjön” must be regarded with caution. The variation in cellulose content in the samples from the five locations is larger than are the corresponding lignin contents. However, the trends regarding location are not obvious. For hardwood, a more southern location seems to be beneficial to yield a higher cellulose content (Figs. 1 and 6). In contrast, this trend is not observed regarding softwood; however, a difference can be perceived for the two northern locations, Hemling and Gideå, where the cellulose content in Norway spruce is lower, in comparison with the other locations. The xylan contents vary less for the different harvesting locations (Fig. 7).

Regarding the galactan content, which makes up a smaller part of both hardwood and softwood (Fig. 8), it can be noted that the location is of minor importance for all wood species. In contrast, the arabinan content is slightly more influenced by the location of the plantation. A guarded interpretation of these results is that the more northern the location, the higher the arabinan content; however, the Armsjön site produced softwood species with notably less arabinan content than the other sites (Fig. 9). In softwood, the content of mannan is slightly lower in wood originating from the location Hemling, which is in the most northern and inland location. However, the mannan contents in Scots pine and Norway spruce are clearly higher than in the hardwood species (Fig. 10).

In terms of utilizing the different wood species in a biotechnology-based biorefinery, there are only minor differences

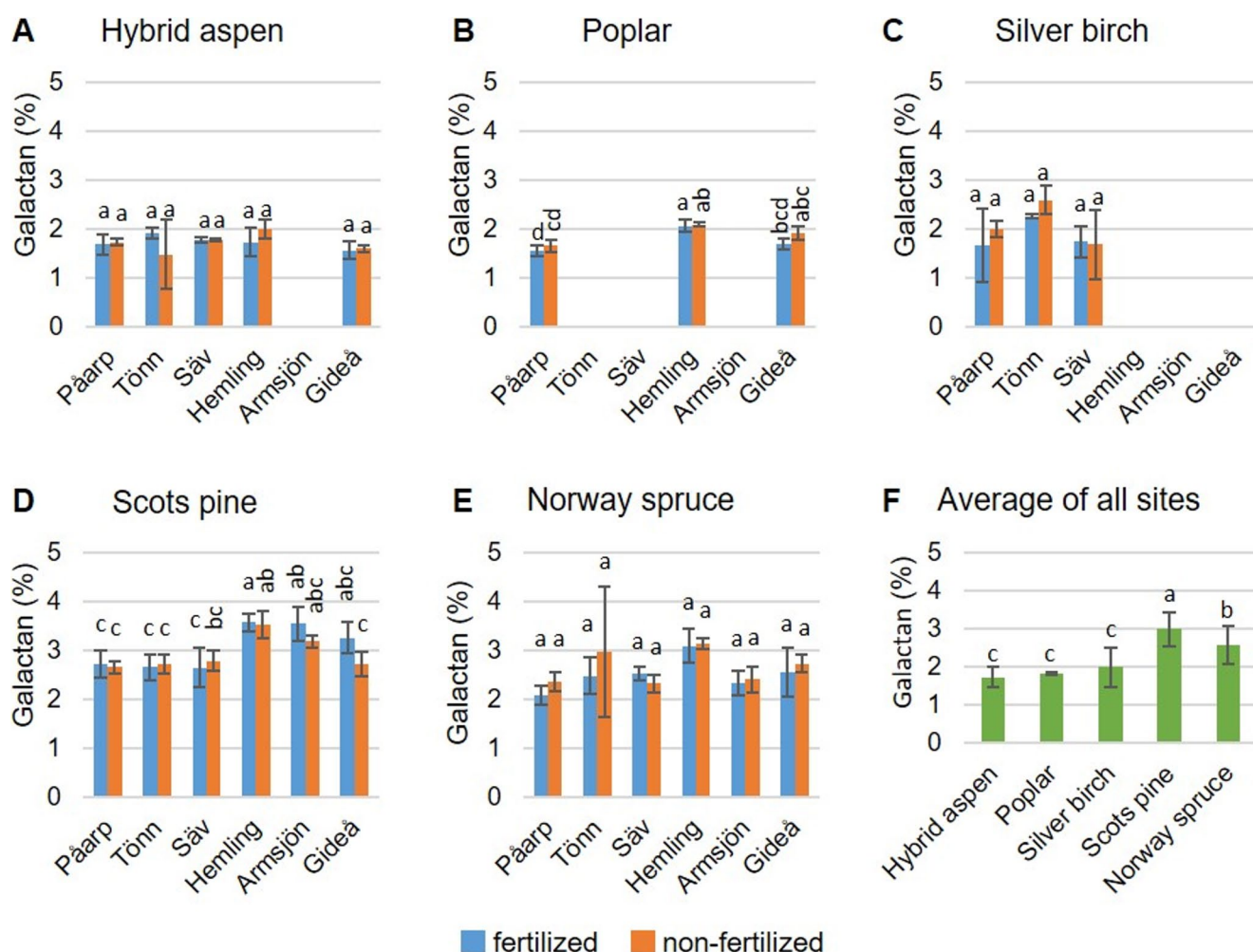


**Fig. 7** Xylan content (% of dry weight) in tree tissues sampled from different species, locations, and fertilizer treatment (blue means fertilized, red means not fertilized). Locations are shown on the X-axes (A–E), for detailed information see Fig. 1. Results show the mean  $\pm$  stan-

dard deviation based on 2–3 replicates. Bars that do not share a letter are significantly different according to Tukey's test at significance level  $p < 0.05$

between the output of carbohydrates from the different sites. Based on the compositional data alone, it would be possible to produce almost similar amounts of materials from one hectare at any of the sites. However, addition of fertilizer is important for annual growth; this results in a higher productivity and a higher yield per area, but our results suggests that there are minor changes in biomass composition. Altogether, this suggests that from a forest management perspective, biomass productions can be optimised with one purpose: high production at each land unit. However, biomass production varies between species, with *Populus* species (Hybrid aspen and poplars) it can reach  $25 \text{ m}^3 \text{ ha}^{-1}$  and  $\text{year}^{-1}$  at arable land, but at forested arable land, biomass production is lower, about  $15 \text{ m}^3 \text{ ha}^{-1}$  and  $\text{year}^{-1}$  at a plantation age of 10 years [21]. However, at forest sites, poplar is problematic to cultivate due to the acidic soil conditions [27, 35]. Here, a better choice of tree species would probably be hybrid aspen or

birch as for these species' biomass production would probably be about  $4\text{--}10 \text{ m}^3 \text{ h}^{-1}$  and  $\text{year}^{-1}$ . The findings in this study suggest that site conditions (except Armsjön) have limited effects on the biomass composition; thus, a wood biomass supply chain does not require the need of dividing where a tree species are produced but instead separating or with known mixtures of biomasses of known tree species. It is important to note that the biomass composition of the trees originating from different locations may influence the overall result after further processing. For example, a lower cellulose content will result in less glucose, which affects the overall yield of the desired product. In the same manner, a variation in lignin content will cause a similar problem, if lignin is to be used for a specific product. The ratio of the constituents may be important when decisions about the process design are to be made. If the process aims at generation of fermentation products, such as ethanol or lactic acid, it



**Fig. 8** Galactan content (% of dry weight) in tree tissues sampled from different species, locations, and fertilizer treatment (blue means fertilized, red means not fertilized). Locations are shown on the X-axes (A–E), for detailed information see Fig. 1. Results show the mean  $\pm$  stan-

dard deviation based on 2–3 replicates. Bars that do not share a letter are significantly different according to Tukey's test at significance level  $p < 0.05$

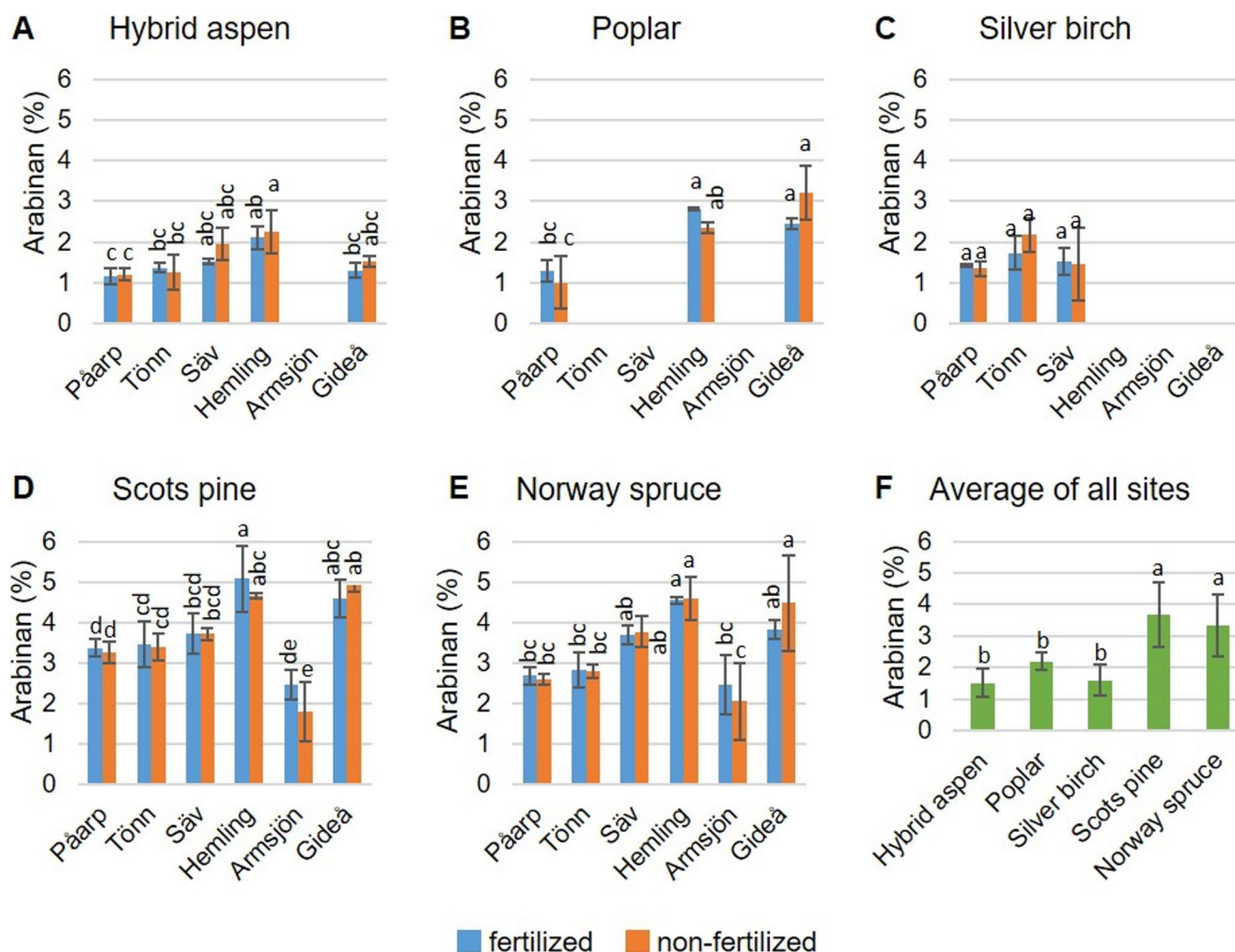
is common to use an enzyme cocktail, which converts the polymers into monomeric sugars. However, if the intended product is generated from, e.g., arabinoxylans or galactoglucomannans, then the ratio between the individual sugars has an impact on the final product.

The performance of the process may also be affected by the presence of compounds that were not analysed in the current study, e.g., various extractives. In addition, our results suggest that fertilization has limited effect on biomass composition, suggesting that application of fertilizers can be used to increase biomass production without consideration of biomass composition. In fact, forest fertilization is one of the cost-effective methods to increase growth of the Swedish forest, but it should be noted that the analysed samples are collected from relatively small trees, thus, fertilization effects later in the rotation period could occur.

There are large land areas available where highly productive biomass plantations (*Populus*) could be established reaching approximately 400 000 ha arable land [21, 23] and over 1.2 million ha of forested arable land [21]. It should be mentioned that the use arable land for these plantations will not compete with food and feed production as the land can not meet the requirement for high productivity and extensive and costly transportation of equipment to harvest and manage the sites.

However, the largest potential for producing large volumes of biomass is probably forested arable land (often monocultures with Norway spruce and with low biodiversity values). Therefore, replacing these stands with a fast-growing broadleaf will probably have a positive effect on biodiversity and biomass productivity. As these tree species have a high biomass production, pressure of





**Fig. 9** Arabinan content (% of dry weight) in tree tissues sampled from different species, locations, and fertilizer treatment (blue means fertilized, red means not fertilized). Locations are shown on the X-axes (A–E), for detailed information see Fig. 1. Results show the mean  $\pm$  stan-

dard deviation based on 2–3 replicates. Bars that do not share a letter are significantly different according to Tukey's test at significance level  $p < 0.05$

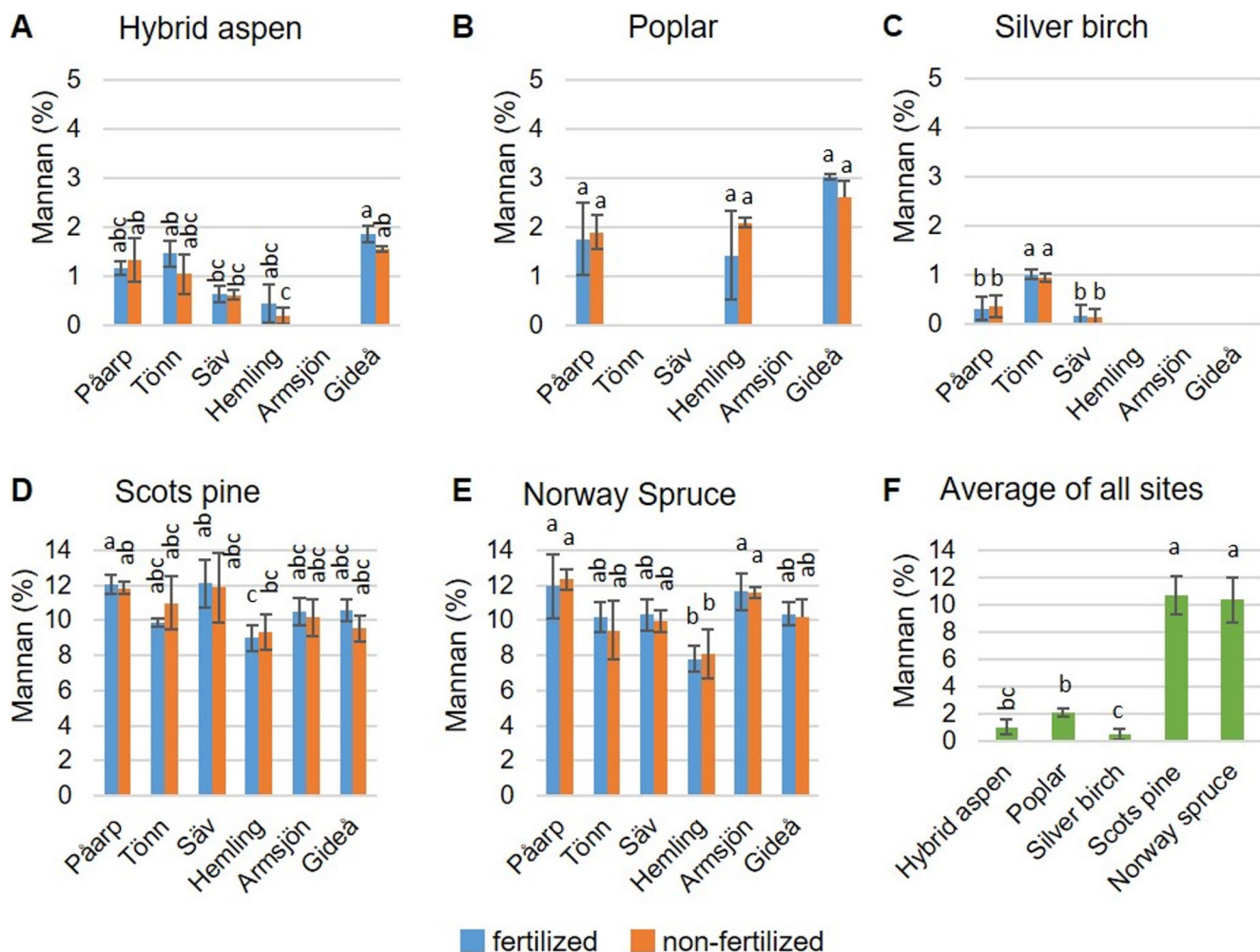
harvesting natural forests might therefore decrease. Interestingly a newly published paper Böhlenius et al. [21] suggested that biomasses from these plantations, if established, could support 16 bio-fuel industries, each with a capacity of 160 000 ton (dry weight) annually with the main location in the southern part of Sweden. Biorefineries require vast amounts of all-year-round material supply, which must not be transported too far away from the biorefinery location to avoid high material costs, while maintaining a sustainable chain of production [36]. Therefore, it is of great advantage if the material supply to the biorefinery is not limited to specific wood species. If the product(s) can be generated from a blend of woody materials, the dependency of a single tree species is alleviated [36]. This may be possible, if the main product(s) is not relying on a certain relationship between xylose and arabinose, or glucose, galactose and mannose, which may be

the situation, e.g., if gels or films are to be manufactured, where the components must be available at certain ratios to assure a consistent product quality.

However, our pairwise correlations between different chemical compounds in deciduous and coniferous species (Fig. 11) suggest that that cellulose content is strongly negatively correlated to the acid-insoluble lignin content in deciduous trees. The same correlation could be seen in coniferous species, but in addition the cellulose content was also strongly negatively correlated to acid-soluble lignin as well as to arabinan content. In both deciduous and coniferous species, a strong positive correlation was observed between fatty acid and arabinan content. Further, arabinan content in conifer species was also strongly positively correlated to both acid-soluble and acid-insoluble lignins.

However, if the wood is intended for various fermentation processes, such as bioethanol or lactic acid production,





**Fig. 10** Mannan content (% of dry weight) in tree tissues sampled from different species, locations, and fertilizer treatment (blue means fertilized, red means not fertilized). Locations are shown on the X-axes (A–E), for detailed information see Fig. 1. Results show the mean  $\pm$  standard deviation based on 2–3 replicates. Bars that do not share a letter are significantly different according to Tukey's test at significance level  $p < 0.05$

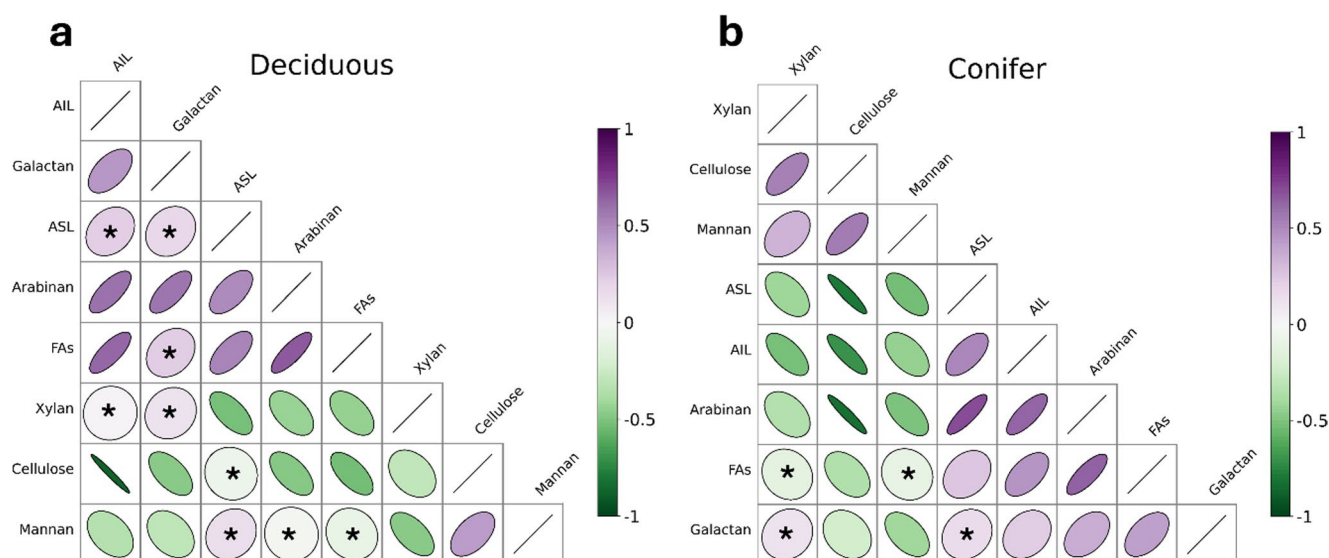
where the raw material is hydrolysed to monomeric sugars, the blend of various tree species may not be critical. Nevertheless, it is of vital importance that the production procedure is robust for various raw material blends to avoid unexpected issues in the process; most chemical processes are designed for certain residence times in the reactors, and a specified range of expected product concentrations prior to the final downstream upgrading steps. If this does not hold true, the final product may be outside of the required product specification range.

## 4 Conclusions

This study is one of few studies that investigates how biomass composition is influenced by growth conditions and forest management methods aimed at increasing biomass

supply for industrial purposes, such as biofuel and chemical production.

- The results suggest that biomass properties are not influenced by geographic locations, i.e. longitude (north to south) but site conditions could have an impact.
- Growth enhancing treatments with fertilizers had no effect on biomass composition.
- Deciduous tree species have similar biomass composition; however, since the compositions are different to coniferous species, full-scale industrial processes must be properly designed and/or adjusted to reach optimal usage of the biomasses utilised. These results have an industrial relevance, and they can be utilized for the aforementioned process-design. The results from this study may have the possibility to be generalized if similar studies are carried out in other regions and/or countries.



**Fig. 11** Pairwise correlations between the content of different compounds in tree biomass based on the full data sets from different locations and fertilizer treatments. Data for all deciduous species in (a) and all conifer species in (b). Colour key indicates the scale of positive

(purple) and negative (green) correlation. Correlations without “\*” are significant ( $p \leq 0.05$ ) according to Spearman’s rank correlation coefficient. AIL, acid-insoluble lignin, ASL; acid-soluble lignin

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Ida Lager: writing—review and editing, writing—original draft- analysis of oil content – data analysis and visualization - funding acquisition.

Ola Wallberg: funding acquisition – design of the original experiment – supervision – data analysis.

Mats Galbe: writing—review and editing, writing—original draft - data analysis and visualization.

Christian Roslander: formal analysis of wood properties – sample preparation.

Stephen Burleigh: writing—review and editing, writing—original draft - data analysis and visualization.

Henrik Böhlenius: writing—review and editing, writing—original draft - data analysis and visualization - funding acquisition – design of the original experiment.

**Funding** Open access funding provided by Swedish University of Agricultural Sciences. The project was funded by the Swedish energy agency project number 47992-1.

## Declarations

**Competing interests** The author declares that there is no competing interests.

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