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1                   **Can fertilization be a driver of rubber plantation intensification?**

2

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23 **Abstract**

24 Improving the productivity of existing rubber plantations is necessary to cope with the growing  
25 demand for natural rubber (NR) while limiting the environmental and social impacts linked to the  
26 expansion of rubber cultivation at the expense of natural ecosystems and food crops. The effect of  
27 fertilization on NR yield is still unclear and is poorly documented particularly in Thailand, the  
28 world leader in NR production. Hence, the main objective of this study was to re-assess the  
29 possible effects of fertilization on the yield of intensively managed rubber plantations in Thailand.  
30 Our main hypothesis was that the effect of fertilization would be higher with intensive latex  
31 harvesting practices (high tapping frequency with ethylene stimulation). To test this hypothesis,  
32 we set-up a split-plot experiment with four fertilization doses (T1, no fertilization, T2, T3, T4,  
33 respectively low, medium and high doses of NPK fertilizer) and two tapping systems (S/2 d2 with  
34 and without ethylene stimulation). Here, we present the results of the first three years on dry rubber  
35 yield, latex metabolism assessed with the latex diagnosis method, and latex and rubber properties  
36 related to their technological properties. Our results showed a positive effect of fertilization on  
37 yield from the first year, but the effect was only statistically significant ( $p < 0.05$ ) in the third year.  
38 The maximum effect of fertilization (+13%) compared to the control treatment (T1) was obtained  
39 with the highest dose of fertilizer (T4). Cumulatively over the 3-year period, the increase in yield  
40 was +5% with the T2 dose, and +8% with the T3 and T4 dose. Contrary to our main assumption,  
41 we observed no interaction between fertilization and ethylene stimulation. Latex diagnosis  
42 revealed that the effect of fertilization on yield was linked with a direct effect on latex metabolism  
43 mainly through an increase in inorganic phosphorus content (Pi). Finally, we observed no  
44 detrimental effect of the fertilizer treatments on latex and rubber quality. We can thus conclude  
45 that increasing latex yield through fertilization combined with intensive tapping does not involve  
46 any identified risks for the quality of rubber. Taken together, our results demonstrate that  
47 fertilization can help increase the yield of rubber plantations where intensive latex harvesting is  
48 practised. However, in a context of low NR prices for rubber farmers, the economic return of a  
49 +8% increase in yield is questionable. Our trial is continuing to assess the long term effects of the  
50 fertilization on yield as well as on the growth and nutritional status of the trees, and the nutrient  
51 balance of the plantation.

52 **Keywords:** Natural rubber, fertilization, ethylene stimulation, yield, latex metabolism, latex  
53 properties,

54        **1. Introduction**

55        Natural rubber (NR) extracted from rubber trees (*Hevea brasiliensis*) plays a crucial role in the  
56        socio-economic development of many tropical countries. NR is an indispensable raw material for  
57        several manufacturing industries, particularly the tyre sector which absorbs 70% of the total  
58        supply. During the last 20 years, high demand on the world market has led to the expansion of  
59        rubber plantations, particularly in South East Asia (Fox and Castella, 2013). Expansion raises  
60        questions about their environmental impacts as it triggers forest clearance (Guardiola-Claramonte  
61        et al., 2010; Hauser et al.; 2015; Hughes, 2017; Warren-Thomas et al., 2018) and about the socio-  
62        economic impact when rubber trees are planted on agricultural land where they compete with food  
63        crops (Fox and Castella, 2013; Chambon et al., 2016). One way to limit such impacts is to increase  
64        productivity per planted area by intensifying agricultural practices.

65        In most crops, intensification relies mainly on nutrient management and the use of fertilizers  
66        (Cassman, 1999; Witt et al., 2006). However, rubber tree is a crop where the harvested component,  
67        latex, is not a vegetative or reproductive organ whose biomass is directly linked to primary  
68        production. Natural rubber (NR) obtained from the latex of the trees is mainly comprised of cis, 1-  
69        4, polyisoprene, a secondary metabolite very rich in carbon (see review by Vaysse et al., 2012).  
70        Latex does not naturally exude from the trees and only tapped trees need to regenerate latex  
71        through a tapping-induced metabolism. Hence, improving tapping techniques has been the main  
72        driver of rubber plantation intensification along with the release of modern high-yielding clones.  
73        In particular, stimulation of latex metabolism with ethylene-based stimulant has made it possible  
74        to maintain or even increase the yield while reducing tapping frequency (Eschbach and Banchi,  
75        1985; Gohet et al., 1996; Thanh et al., 1996; Zhu and Zhang, 2009; Lacote et al., 2010). Lacote et  
76        al. (2010) found the maximum increase in the yield of stimulated trees ranged from +30% to +78%,  
77        compared to that of unstimulated trees, depending on the clone. Conversely, the effect of  
78        fertilization on latex yield are still unclear, and this research topic has been almost ignored for the  
79        last 40 years, as most references in the literature date from the 1970s in Malaysia (Sivanadyan et  
80        al., 1972; Pushparajah, 1973; Yogaratnam and Weerasuriya 1984) or in Côte d'Ivoire  
81        (Compagnon, 1973; Du Plessix et al., 1973). Since the 1980s, the maximum reported increase in  
82        yield due to fertilization has been 26% (Onuwaje 1983) but most other reports showed no effects  
83        below 20% or no effects at all (Murbach et al., 1999; Virgens Filho et al 2001; Gohet et al. 2013).

84 For several authors, a mature rubber plantation is a forest-like ecosystem, which is "self-  
85 sustainable" or in a "steady-state" with respect to the nutrient requirements of the trees  
86 (Krishnakumar et Potty, 1992; Sivanadiyan et al., 1995; further developed by George and Joseph,  
87 2011). Hence, the management of fertilization of a rubber plantation is different than that of annual  
88 crops. In particular, high nutrient returns to the soil through leaf fall, self-pruning and fine root  
89 turnover need to be taken into account (Samarappuli, 2000). The recycling of these nutrients is  
90 hypothesized to be enough to support the yield and the functioning of the trees thanks to the low  
91 nutrient exports through latex harvesting, which is rather low compared to the tree biomass (4-5  
92 kg/tree/year) and contains only small amounts of mineral nutrients (Bolton, 1964; Samarappuli,  
93 2000; Murbach et al., 2003). According to these authors, annual exports of nitrogen range from 5  
94 to 12 kg.ha<sup>-1</sup>.year<sup>-1</sup> while recycling of N through litter falls amount to more than 20 kg.ha<sup>-1</sup>.year<sup>-1</sup>  
95 (Murbach et al., 2003). These figures suggest that rubber tree only needs a small amount of  
96 fertilizer to support latex production and to enable a satisfactory girth increment during the mature  
97 stage. Based on experiments by Sivanadyan (1983), Watson (1989) reported that fertilization only  
98 needed to start four years after the beginning of tapping, and that N application would be sufficient  
99 if the plantation was well managed during the immature phase (e.g. with both leguminous cover  
100 and regular fertilizer applications). If required P, K and Mg fertilizers could be applied at 3 to 5-  
101 year intervals.

102 Fertilization can also change the mineral composition of the latex with possible detrimental effects  
103 on quality. For instance, divalent cations such as Mg<sup>2+</sup> are known to be detrimental to latex  
104 stability, and industrial production of latex concentrate generally includes Mg<sup>2+</sup> precipitation  
105 through phosphate addition prior to centrifugation. Collier and Lowe (1969) observed that  
106 nitrogen fertilization increased the Mg<sup>2+</sup> content of field latex while decreasing dry matter of the  
107 latex and increasing nitrogen content of air-dried sheet rubber. The higher Mg<sup>2+</sup> content reduced  
108 the mechanical stability of concentrated latex. Conversely, applying potassium or phosphate  
109 fertilizers may counteract the effect of Mg<sup>2+</sup> on latex. A long-term experiment carried out by the  
110 Rubber Research Institute of Malaysia (cited in Watson, 1989) revealed a positive effect of K  
111 fertilizers on latex stability. The decrease in Mg content after K fertilizer is applied leads to  
112 antagonism of root uptake between the two cations. A study by Philpott and Westgarth (1953)  
113 demonstrated the beneficial role of combined K and P fertilizers in latex stability, which reduced  
114 the Mg/P ratio in the latex.

115 However, in Thailand, the world's biggest producer of NR, the amount of fertilizer recommended  
116 by R&D institutions is high and, in practice, farmers sometimes apply even more than the  
117 recommended rate of NPK fertilizers in tapped plantations (Chambon et al, 2018). A recent socio-  
118 economic survey showed that farmers believe such practices are beneficial (Chambon et al., 2018).  
119 However, this assumption is not supported by field data and such practices may not only be a waste  
120 of money for the farmers, but a source of environmental pollution if the nutrients are not  
121 effectively taken up by the trees. Such contradictions may arise from the indirect effects of NPK  
122 fertilization on latex yield through the improvement of the nutrient status of the whole tree,  
123 resulting in long-term benefits. As farmers' benefits rely on good management to maintain a  
124 balance between rubber production and plant growth, it is also important to understand how regular  
125 fertilization affects tree growth. Another possible explanation for these contradictions? is that Thai  
126 rubber farmers have adapted their fertilization practices to the very intensive tapping frequencies  
127 they use (Chambon et al., 2014). According to Gohet et al. (2013), potential gain from fertilization  
128 can only be achieved through the intensification of tapping practices. As tapping drives the demand  
129 for latex regeneration, it is not surprising that no effect of fertilization can be demonstrated if the  
130 same tapping system is applied in fertilized and unfertilized plots. However, interactions between  
131 latex harvesting technologies and fertilization of tapped rubber trees are poorly scientifically  
132 documented, particularly the use of ethylene stimulation to enhance latex yield, although,  
133 Sivanadyan et al. (1972) reported a positive interaction between fertilization and ethylene  
134 stimulation, which was partly confirmed by Gohet et al (2013).

135 In this context, the main objective of our work was to re-assess the possible effects of fertilizers  
136 combined with ethylene stimulation on latex yield in rubber plantations in Thailand. Our starting  
137 hypothesis was that there is a positive interaction between fertilization and stimulation, as the  
138 higher metabolic demand induced by stimulation would require additional nutrient resources. To  
139 this end, we analysed yield data from a three-year experiment with four fertilizer treatments  
140 applied to stimulated and unstimulated trees. To better understand the effect of fertilization and  
141 stimulation on yield, we collected data on the latex metabolism using the latex diagnosis approach  
142 (Jacob et al., 1989, d'Auzac et al. 1997). The underlying assumption was that fertilization would  
143 have an effect on latex metabolism before having a statistically noticeable effect on yield. We also

144 measured certain latex properties to assess possible negative effects of fertilization on yield and  
145 on the quality of the latex.

## 146 **2. Material and methods**

### 147 **2.1. Site description**

148 The three-year experiment was carried out from May 2014 to April 2017 in a traditional rubber  
149 growing area at the Sithiporn Kridakorn Research Station (latitude 10°59'13.35"N, longitude  
150 99°29'22.41"E) of Kasetsart University, Prachuap Khirikhan province, Thailand (Fig.1). The  
151 research station is located at the limit between two main climatic regions of Thailand: one with an  
152 equatorial climate, which extends southward from Chumphon province to the border with  
153 Malaysia, and the other with a tropical monsoon climate with long rainy season, which covers  
154 several provinces scattered in the northern, central and the east-central parts of the country.  
155 Weather data collected at the station from 2000 to 2010 showed average annual rainfall of 1985  
156 mm (s.e. 383 mm) with 106 rainy days (s.e. 11 days) (Table 1). The rainiest months are October  
157 and November with more than 250 mm on average. The driest month is January with 64 mm on  
158 average. These climatic conditions are perfect for rubber tree cultivation with high latex yield  
159 potential (Gohet et al. 2015). Total rainfall in the two first years of the experiment (2014-15 and  
160 2015-16) was respectively 20% and 27% lower than in the 2000-2010 reference period, and 22%  
161 higher in the third year (Table 1). The number of rainy days was higher than the reference period  
162 in all three years with respectively, 112, 109 and 129 days of rain. The soil of the experimental  
163 plot, classified as Rhodic Kandudults, is deep with a sandy-loam texture. The topsoil (0-30 cm)  
164 has the following average characteristics: 7.7% clay; 71.4% sand; 20.9% loam; pH4.9 (1:1 water);  
165 0.25% C and 0.032% N (Elemental Analysis); CEC 2.1 meq/100g (pH7 ammonium-acetate);  
166 available P 12 ppm and available K 32 ppm (ICP-AES).

167 *Figure 1 and Table 1 to be inserted here*

### 168 **2.2. Experimental design**

169 The experimental plot was a ca. 9 ha rubber plantation set up in 2007 using the *Hevea brasiliensis*  
170 RRIM 600 clone, which has medium to high metabolic activity and is used in 90% of rubber  
171 plantations in Thailand (Delarue and Chambon, 2012). The plot previously contained a coconut  
172 plantation. From planting to the beginning of the experiment in May 2014, the plantation was  
173 regularly weeded but not fertilized. The trees were planted with a spacing of 8 m between tree

174 lines and 2.5 m between trees resulting in an average density of 500 trees.ha<sup>-1</sup>. Tapping the tree  
175 bark to harvest latex started at the beginning of the experiment in May 2014. Only the trees with a  
176 trunk girth of 47 cm measured 100 cm above the ground were opened on this occasion. The first  
177 tapping cut was made 150 cm from the ground by removing a thin layer of bark along a downward  
178 half spiral on the tree trunk. The trees were then tapped every two days **without any tapping rest**  
179 **in a week** until the trees completely shed their leaves in February 2015. Tapping stopped during  
180 the refoiliation period and resumed in May 2015 for another 10-month period using the same  
181 tapping system. **This tapping system is noted S/2 d2 7d7 10m(MAY-FEB)/12 with respect to the**  
182 **revised international notation for latex harvesting technology (Vijayakumar et al., 2009). We did**  
183 **not use rain guards to make it possible to tap the trees on rainy days as it is not a common practice**  
184 **in Thailand. Hence, a substantial number of tapping days were loss every year because 90% of the**  
185 **rainy days occurred during the tapping period from May to February. In the end, trees were tapped**  
186 **106, 115 and 106 days in the first, second and third year, respectively, out of the 150 tapping days**  
187 **expected with the d2 tapping frequency (i.e. 23% to 30% loss of tapping days).**

188 The trial was set up as a split plot design in randomized complete blocks with four fertilizer  
189 treatments in the main plots, two tapping treatments in the subplots and four replications (blocks).  
190 Elementary plots contained 108 trees and covered an area of 2,160 m<sup>2</sup>. Each plot was separated  
191 from adjacent plots or plantation edges by two lines of tree (16 m) or six trees (15 m) (Fig. 1). The  
192 four fertilization treatments were (T1) no fertilization, (T2) 75/45/100 g.tree<sup>-1</sup>.year<sup>-1</sup> of N,P (as  
193 P<sub>2</sub>O<sub>5</sub>) and K (as K<sub>2</sub>O) in one application in June in each year, (T3) 180/80/170 g.tree<sup>-1</sup>.year<sup>-1</sup> of  
194 N,P and K in two applications in June and October in each year, and (T4), 306/136/289 g.tree<sup>-1</sup>.year<sup>-1</sup>  
195 of N,P and K also in two applications (Table 2). Treatment T2 corresponded to the  
196 fertilization programme recommended by CIRAD in rubber estates (Gohet et al., 2013), while T3  
197 corresponded to the current recommendation in Thailand (Chambon et al., 2018). Treatment T4  
198 was based on the highest rate of fertilization found in a survey of fertilization practices used by  
199 rubber growers in Thailand (Chambon et al., 2018). The two tapping treatments differed in  
200 stimulation, no stimulation (NS) or four stimulations per year, i.e. in June, July, August and  
201 September (ST). Stimulation was done by applying ca. 0.8 gram.tree<sup>-1</sup> of a ready to use paste  
202 containing 2.5% of ethephon on 1 cm of the tapping panel located above the tapping cut.  
203 Stimulation is not usually applied when trees are tapped every two days (d2) but only when the  
204 tapping frequency is reduced (d3 and more). Our stimulated treatments thus represented a very

205 intensive system in which we expected a clearer effect of fertilization according to the hypothesis  
206 that the potential gain from fertilization can only be achieved through intensified tapping.

207 The experiment included three tapping seasons hereafter referred to as “years” (first year or year  
208 1 = May 2014-April 2015; second year or year 2 = May 2015-April 2016; third year or year 3 =  
209 May 2016-April 2017). In May 2015 and May 2016, trees that had reached 47cm of trunk girth  
210 measured 100 cm above the ground in the previous cropping seasons were opened. On average,  
211 57%, 66% and 70% of the 54 trees in each subplot were tapped in 2014-15, 2015-16 and 2016-17,  
212 respectively. There was no statistical difference in the number of tapped trees between blocks and  
213 treatments (Table 1). We did not observed any significant rate of tapping panel dryness as well.

## 214 **2.3. Measurements and data processing**

### 215 **2.3.1. Latex and rubber yield**

216 On each tapping day, tapping started at 8 P.M. and latex was collected separately from each subplot  
217 between 5 and 8 A.M. The fresh latex was weighed immediately, and a 200 mL sub-sample was  
218 mixed with 400 mL of water to determine the dry rubber content (DRC) using the Metrolac method  
219 (Smith, 1947). DRC measurements with Metrolac were calibrated against the standard NF ISO126:  
220 2005 twice a year (see ‘latex and rubber quality’ sub-section). This calibration indicated that the  
221 Metrolac method underestimated DRC by 17%. Rubber yield per subplot was obtained by  
222 multiplying fresh latex weight by calibrated DRC values. We then calculated the rubber yield per  
223 tree in  $\text{g.tree}^{-1}$  by dividing the rubber yield per subplot by the number of tapped trees in the subplot.  
224 In May at the end of each tapping season, the number of tapped trees was updated to take the newly  
225 opened trees and the trees affected by tapping panel dryness into account. At the same time, we  
226 measured the trunk girth of all the trees in the experimental plots 1.7 m above the ground.

### 227 **2.3.2. Physiological status of the latex cells (latex diagnosis).**

228 Latex diagnosis (LD) is a method used to assess the impact of tapping on the physiological status  
229 of the latex cells (Jacob et al., 1989, D’Auzac et al. 1997). According to Jacob et al. (1989), the  
230 biochemical ability of latex cells to produce rubber is based on their sucrose (SUC), inorganic  
231 phosphorus (Pi) and thiol (RSH) contents. SUC reflects the balance between sucrose consumption  
232 by the latex cells for latex biosynthesis, and the transfer of sucrose from the apoplast to the latex  
233 cells. Pi indicates the intensity of metabolic activity in the latex cells and RSH indicates the  
234 efficiency of scavengers in counteracting oxidative stress. We applied the LD method as developed  
235 by CIRAD (Jacob et al., 1988, 1995). The latex diagnosis was performed each year in September

236 or October during the most regular and high yielding period. In each subplot, a composite sample  
237 was obtained from ten randomly selected tapped trees and ten drops of latex were collected from  
238 each tree. The LD parameters were measured using the Ashwell anthrone method (1957) for SUC,  
239 the Taussky and Shorr method (1953) for Pi, and the Boyne and Ellman method (1972) for RSH.  
240 Sucrose, thiol and inorganic phosphorus contents were expressed in millimoles per litre of latex  
241 ( $\text{mmol.l}^{-1}$ ).

### 242 2.3.3. Latex and rubber quality

243 Two sampling campaigns were carried out each year in October and June to collect latex and dry  
244 rubber samples for quality analysis. The campaigns were systematically planned around 15 days  
245 after fertilizer application. As it was not possible to obtain representative unsmoked sheet due to  
246 drying problems following the first sampling campaign in October 2014, supplementary sampling  
247 was conducted in January 2015. Several indicators of the technological properties of either latex  
248 or rubber were measured: dry rubber content (DRC), mechanical stability (MST) of the fresh latex;  
249 initial plasticity ( $P_0$ ), the plasticity retention index (PRI) and its component  $P_{30}$  (SMR bulletin N°7  
250 1992 - part B.8), and the ash content of rubber processed as unsmoked sheet (USS). Mineral  
251 content (N, P, K, Ca and Mg) was also analyzed in both latex films and rubber USS. Figure 2  
252 summarizes the processing of the latex samples collected in each plot and the methods used to  
253 measure those indicators. Latex samples were collected between 4 am and 6 am in half the 32  
254 subplots on a given tapping day, and in the other half on the following tapping day (normally 48 h  
255 later, if no rain was falling). However, MST was measured in all the subplots on the two collection  
256 days. We used the average of those two measurements as the MST value for one subplot. The  
257 standard NF ISO35:2006 method was used with the following adaptations: the sample was made  
258 of 80 mL of fresh latex instead of 80 mL of concentrated latex. The latex was not heated, and all  
259 measurements were made within two hours after collection. The rest of the latex sample was  
260 divided into three sub-samples used separately for fresh latex and rubber analysis, and DRC  
261 measurement. As mentioned above, the standard NF ISO126: 2005 was applied for the DRC with  
262 the following adaptations: the sample comprised 2 mL of fresh latex instead of 10 mL of  
263 concentrated latex. The volume and concentration of added acid were 15 mL of 2% acetic acid  
264 instead of 25-35 mL of 5% acetic acid. Mineral and ash contents of the latex were measured on  
265 latex films. The latex films were made as follows: within the two hours after latex collection, 50  
266 mL of latex were spread on a flat square recipient ( $22.5 \times 22.5 \times 3.75$  cm) and were placed in a

267 ventilated oven at 70 °C until no more white spots were visible on the surface of the sample  
268 (approx. duration 24 h). The resulting latex films were rolled and stored in plastic bags containing  
269 desiccant (5 g silica gel) prior to analysis. The mineral content expressed versus dry film weight  
270 were converted into a fresh latex reference by multiplying by the estimated total solid content of  
271 latex (TSC). The TSC was estimated by dividing measured DRC by 0.9. This numerical conversion  
272 value of the DRC/TSC ratio is regularly used in the field. It was checked experimentally in two  
273 consecutive sampling campaigns by measuring both DRC and TSC on the whole set of samples (2  
274 x 32), and the average ratio obtained was 0.90 (SD 0.03). Unsmoked sheets (USS) were made  
275 following the recommendations of the Rubber Research Institute of Thailand. First, latex was  
276 coagulated by mixing 3 L of latex with 2 L of water and 300 mL of formic acid solution 1.56%  
277 (94% diluted 60x). After 45 min, the coagulum was manually pressed, then passed 1-2 time(s)  
278 through a crusher (final thickness 10 mm), 3-4 times through a flat hand mangle and twice through  
279 a ribbed hand mangle (final thickness 2-3 mm). Finally, the USS were hung up outside in the shade  
280 to dry for one to two weeks (turned daily). The nitrogen content of both latex films and USS  
281 rubber was determined with a CHN Determinator (LECO CHN628) on a 100 mg sub-sample.  
282 Total Mg, Ca, P and K content of both latex films and USS rubber were determined with a ICP-  
283 AES (ICP Agilent 720 AES) on ashes from a 2 g sub-sample placed in a furnace at 500 °C for two  
284 hours.

285 *Figure 2 to be inserted here*

#### 286 **2.4. Statistical analysis**

287 A three-way ANOVA was performed on data collected each year to test the effect of blocks (4  
288 modalities, n=8), fertilization (4 modalities, n= 8) and stimulation treatments (2 modalities, n=16),  
289 and their respective interactions on rubber yield (in g.tree<sup>-1</sup>.year<sup>-1</sup>), LD parameters (SUC, Pi,  
290 RSH), latex and rubber quality parameters of the two sampling campaigns. Additionally, an  
291 ANOVA was performed on cumulative rubber yield (g.tree<sup>-1</sup>) at the end of the third cropping  
292 season. Tukey's HSD test was used to compare the means at the 5% probability level. The  
293 relationship between LD parameters and yield was tested every year with a multiple linear  
294 regression. Statistical analyses were performed with Xlstat software (2018.6 version, Addinsoft,  
295 Paris, France).

#### 296 **3. Results and Discussion**

297 Our study is the first assessment of the interactions between latex harvesting technologies  
298 (stimulation) and fertilization on yield of tapped rubber trees in Thailand, the world's largest  
299 producer of natural rubber. We found only one publication in English reporting on a fertilizer trial  
300 in a mature rubber plantation in Thailand (Mak et al., 2008). However, this study only compared  
301 different formulae of fertilizers applied at the same dose. We also investigated the influence of  
302 stimulation and fertilization on latex physiology and latex quality parameters for the first time.  
303 Indeed, the literature on the effect of fertilization on natural rubber production is scarce and not  
304 very recent (Gohet et al., 2013). According to Gohet et al. (2013) and Bolton (1964), one reason  
305 for the lack of studies on this topic is the technical constraints to the implementation of fertilizer  
306 trials. This kind of trial requires a large homogeneous plantation area covering several hectares,  
307 which is difficult to find in rubber smallholdings.

308 The average yield of our experimental plot (all treatments) was high with ca. 5.8 kg. tree<sup>-1</sup>. year<sup>-1</sup>  
309 in the first year and more than 7.2 kg. tree<sup>-1</sup>. year<sup>-1</sup> in the second and third year (Table 3). Annual  
310 rubber yield per tree recorded for the control treatment without fertilization nor stimulation (T1NS)  
311 ranged between 5.3 and 6.5 kg.tree<sup>-1</sup>.year<sup>-1</sup>, which is in the upper range of yield data reported in  
312 rubber production trials in Thailand using the same tapping system on the clone RRIM600 (Mak  
313 et al., 2008; Chantuma et al., 2006, 2011, 2017; Sdoodee et al., 2012; Sainoi et al., 2017). In  
314 general, the good performances of the plantation was explained by the high tapping frequency (d2)  
315 despite the loss of tapping days. Yet, the number of tapping days was similar to that used by rubber  
316 smallholders in Thailand (Chambon et al., 2014). Our results suggest that the low soil fertility  
317 (low organic matter and low N, P and K contents) was not particularly limiting. The depth of the  
318 soil and the absence of any obstacle to root growth may have compensated for the low mineral  
319 content. Roots were observed at a depth of more than 2 m (data not shown), in agreement with the  
320 results of Maeght et al. (2015). Moreover, the absence of significant tapping panel dryness (TPD)  
321 on tapped trees at the end of the third year suggests that the trees were not over-exploited. This  
322 observation was confirmed by the results of the latex diagnosis (see sub-section 3.2). The good  
323 performances of the unfertilized and unstimulated plots (T1NS, control treatment), along with the  
324 absence of major block effects on yield (Table 3), suggest that our experimental field was well  
325 managed and was located in a favourable environment. We can therefore go further with the  
326 analysis of the treatment effects on rubber yield (section 3.1), latex metabolism indicators (section  
327 3.2) and latex properties (section 3.3).

328 **3.1. Effects of stimulation and fertilization on rubber yield (Table 3)**

329 Our results show a positive effect of fertilization on yield from the first year, but the effect was  
330 only statistically significant ( $p=0.023$ ) in the third year. This year, we observed that yield increased  
331 with the dose of fertilizers: +8% than the yield of T1 for T2, +10% for T3 and +13% for T4 in the  
332 third year. After three years, the cumulative yield of T3 and T4 treatments was 8% higher than T1.  
333 This effect was only significant at the 10% level ( $p=0.071$ ). Stimulation by applying ethephon on  
334 the tapping cut four time a year had a strongly significant positive effect ( $p<0.0001$ ) on rubber  
335 yield the three years of the experiment. The strongest effect of stimulation was observed in the  
336 third year with a 20% increase in yield, whereas in the first two years, the effect was similar (+12-  
337 13%). After three years of tapping, the cumulative yield of stimulated trees was 15% higher than  
338 that of unstimulated trees. It is noteworthy that the yield of stimulated and fertilized treatments  
339 (ST or T2, T3, T4) increased the third year compared to the second year, while it decreased for  
340 control treatments (NS or T1). We did not find significant interactions between fertilization and  
341 stimulation treatments.

342 The maximum effect of fertilization on rubber yield (+13%) we observed was similar to that  
343 reported by Gohet et al. (2013) and Murbach et al. (1999), two of the most recent works on the  
344 effect of fertilization on modern high-yielding varieties with intensive tapping systems. Yield  
345 increases of +20% and more were obtained in works conducted before the 1990s in less intensive  
346 rubber cultivation systems (Sivanadyan et al. 1972; Pushparajah, 1973; Yogaratnam et al., 1984;  
347 Onuwaje et al., 1983). However, in our specific conditions, the response to fertilization may have  
348 been restricted because the yield of the unfertilized control was already high, despite the apparent  
349 low soil fertility. Increasing yield through external inputs is of course harder when the base line  
350 yield is already high. In other words, there were probably no major nutrient limitations in the  
351 rubber plot before the beginning of the experiment. Nevertheless, the response of rubber yield to  
352 fertilizer addition was quite rapid. We observed a positive but not significant effect from the first  
353 year that became significant at  $p<0.05$  in the third year. The effect on cumulative yield over the  
354 three years of the experiment was significant at the 10% probability threshold. According to Bolton  
355 (1964), we could have expected a slower response of yield to fertilizer. Bolton argued that  
356 additional fertilizer would first affect tree growth before increasing yield because of the low level  
357 of nutrients exported by latex harvesting compared to the total amount of nutrients required for

358 tree growth. However, we observed no effect of the fertilization treatments on trunk radial growth  
359 (Table 3).

360 As expected, stimulation with an ethylene based product had the strongest effect on yield. Unlike  
361 fertilization, stimulation significantly increased rubber yield since the first year of the experiment.  
362 The effect of stimulation we observed with 4 ethephon applications per year (+15% in cumulated  
363 yield over the three-year experiment) was much lower than the effects reported by Lacote et al.  
364 (2010) that ranged from +29% to +85%. This result can be explained by the more intensive tapping  
365 frequency in our study (d2, meaning tapping every other day) than in the study of Lacote et al.  
366 (2010), which was with a d4 (tapping once in 4 days). Although stimulation is not recommended  
367 with d2 tapping frequency, we choose to include a stimulated d2 tapping system in our experiment  
368 to test the hypothesis that fertilization can have a bigger effect on yield when trees are stimulated  
369 because of their increased nutrient requirements, or that only an increase in tapping intensity can  
370 exploit the higher yield potential enabled by fertilization (Sivanadyan et al. 1972, Gohet et al.  
371 2013). Our results do not support this hypothesis as we did not find any significant interaction  
372 between the stimulation and the fertilization treatments. In other words, yield response to fertilizer  
373 application was similar with and without stimulation. In particular, we observed that fertilization  
374 contributed, as stimulation did, to maintain or even increase rubber yield the third year while yield  
375 of the control (unfertilized) treatment decreased compared to the second year.

376 Altogether, these results demonstrate that fertilization, independently of the use of ethylene  
377 stimulation, can increase the yield of rubber plantation under high tapping frequency system  
378 (tapping once every 2 or 3 days) such as those used by smallholders in Thailand. As ethylene  
379 stimulation is not recommended with these tapping systems, improving the nutrition of the trees  
380 can thereby be a main driver of rubber plantation intensification. However, the maximum effect,  
381 and the most significant, of fertilization in our experiment was obtain with the highest dose of  
382 fertilizer (T4 treatment, Eq. to 306/136/289 g.tree<sup>-1</sup>.y<sup>-1</sup>, Table 2). This treatment corresponds to the  
383 highest dose of inorganic fertilizers reported by rubber smallholders in Thailand (Chambon et al.  
384 2018). This dose is 1.7 times higher than the standard fertilizer recommendation in Thailand and  
385 4 times higher than the CIRAD recommendation for industrial estates. Chambon et al. (2018)  
386 calculated that this dose of fertilizer with a 10% increase in yield would only be profitable if the  
387 price of rubber is more than USD4.kg<sup>-1</sup>, while the price had rarely exceeded USD 2.kg<sup>-1</sup> over the  
388 last 10 years. Additionally, the environmental impacts, i.e. the risks of nutrient leaching to

389 groundwater and N<sub>2</sub>O emissions to the atmosphere, with such a high fertilization rate must be high  
390 (Zhou et al., 2016).

391 In the next sub-section, we analysed the effect of fertilization and stimulation on the indicators of  
392 latex metabolism we measured. We collected these data in order to better understand the effect of  
393 fertilization on rubber yield. The underlying assumption was that fertilization would have an effect  
394 on latex metabolism before having a statistically noticeable effect on yield in relation to better  
395 latex regeneration.

396 *Table 3 to be inserted here*

### 397 **3.2. Effects of stimulation and fertilization on latex metabolism**

398 Latex diagnosis (LD) is a method used to assess the impact of tapping on the physiological status  
399 of the latex cells (Jacob et al., 1989, D'Auzac et al. 1997). Both fertilization and stimulation  
400 treatments had significant effects on the LD indicators, but the effects were different among the  
401 years and among the indicators. Inorganic phosphorus content (Pi) was the indicator most affected  
402 by the treatments. Pi increased with both stimulation and fertilization (Table 4). These effects were  
403 statistically significant in both the second and third year but not in the first year. Fertilization had  
404 a stronger effect on Pi than stimulation with an average increase of 16% increase with high levels  
405 of fertilization (T3 and T4) compared to control plots (T1) in 2015-2016, and of about 30% in  
406 2016-2017. Low fertilization (T2) had little or no effect. There was also a slight interaction  
407 between stimulation and fertilization treatments in the second and third year (significant in the  
408 third year). Without stimulation, Pi increased with the dose of fertilizers but, with stimulation, only  
409 T3 had a significantly higher Pi than control (T1). This means that the effect of fertilization on Pi  
410 was stronger in non-stimulated trees. The sucrose content of the latex (SUC) decreased with  
411 stimulation the three years and with fertilization on years 2 and 3 (Table 4). These effects were  
412 only significant for stimulation on year 1 and 2. Indeed, we observed a decrease in the effect of  
413 stimulation on SUC over time (-38% in 2014-2015, -18% in 2015-2016 and -12% in 2016-2017).  
414 There was no consistent effect of either stimulation or fertilization on thiol content (RSH).  
415 Stimulation had a strong negative effect on RSH in the first tapping season (-24%), but no effect  
416 in the second and third years. Fertilization had a positive effect on RSH in general but with no  
417 clear significant trend linked with the dose of fertilizer. The high variability of this parameter (up  
418 to 21% within the same treatment) partly explains the lack of statistical significance. For a better  
419 understanding of the effect of latex diagnosis parameters on yield, we performed multiple linear

420 regressions between the three latex diagnosis parameters and the latex yield expressed in g.tree-  
421 1.year-1 in each year. The results in Table 5 show that the p-value of the model decreased from  
422 the first to the third cropping season. This means that the fitness of the multiple linear regression  
423 to predict yield improved year after year. In 2014-2015, the three latex parameters explained only  
424 30% of the yield variation whereas they explained nearly 60% in 2016-2017. SUC had a significant  
425 effect on yield in the second and the third year (p-value<0.05). Pi had a significant effect on yield  
426 only in the third year but this effect was stronger than for SUC.

427 Our starting hypothesis was that stimulation and fertilization would have a positive effect on latex  
428 yield through improved latex regeneration. The latex diagnosis (LD) indicators confirmed this  
429 hypothesis. Taken together, the results of the LD showed that latex metabolism was well activated  
430 after the first year of tapping and was well balanced in the two following years. In agreement with  
431 Jacob et al. (1989), the increase in sucrose content (SUC) along with a decrease in inorganic  
432 phosphorus (Pi) and thiol (RSH) content we observed in the third year suggest that the risk of over-  
433 exploitation of the trees was low. Similarly, the negative correlation between SUC and yield, and  
434 the positive correlation between Pi and yield revealed by the multiple linear regression between  
435 LD indicators and rubber yield are consistent with the results of previous studies showing that  
436 SUC decreases and Pi increases with an increase in tapping intensity (Tupy, 1985; Gohet et al.,  
437 1996; Jacob et al., 1998). In particular, these correlations and their underlying mechanisms have  
438 been well documented in the case of increases in yield obtained by ethylene stimulation (Amalou  
439 et al. 1992; Dusotoit-Coucaud et al., 2009; Lacote et al., 2010).

440 In addition, our results provide original insights into the combined effect of stimulation and  
441 fertilization on latex metabolism and yield. The main effect of fertilization on latex metabolism  
442 was on Pi. The maximum effect of fertilization on Pi was even stronger than that of stimulation  
443 after the first year. In the third year, both treatments had a highly significant positive impact on  
444 Pi with +33.5% for T4 compared to T1, and +24.7% for stimulated trees compared to unstimulated  
445 ones. The multiple linear regression between yield and LD indicators showed that Pi became the  
446 main explanatory factor in the third year. Positive correlation between rubber yield and Pi is well-  
447 known (Amalou et al., 1992). The metabolism of rubber particles involves active biochemical  
448 mechanisms which require a lot of energy and then fast cycling of ATP and NADPH, releasing  
449 free Pi into the cell cytoplasm. The positive effect of stimulation on Pi is in line with the results of  
450 Lacote et al. (2010). Gohet et al. (2013) also reported a +20% significant increase in Pi in response

451 to a combination of intensive stimulation and a high dose of fertilizer. However, the effect of  
452 fertilization on Pi in our study was stronger and was significant in both the stimulated and  
453 unstimulated treatments. Moreover, the effect of fertilization on Pi was stronger on unstimulated  
454 trees.

455 As expected, stimulation had a significant negative effect on SUC (Lacote et al., 2010). The  
456 decrease in SUC content in stimulated trees can be explained by activation of rubber biosynthesis,  
457 which consumes more sucrose (Tupy and Primot, 1976). Surprisingly, the positive effect of  
458 fertilization on yield did not result in a significant reduction in SUC with the dose of fertiliser.  
459 However, this result does not mean that fertilization had no effect on SUC. On the contrary, as a  
460 decrease in SUC was expected, this could mean that fertilization enhanced the supply of SUC to  
461 latex cells to cope with the increased latex productivity. The positive, but not significant, effect of  
462 fertilization on SUC observed in the first year is consistent with this hypothesis. This hypothesis  
463 is also in line with the results of Gohet et al. (2013) who observed a significant increase in SUC in  
464 response to high dose of fertilizer combined with intensive stimulation. In their study, SUC was  
465 low (<5 mMol) even in the control treatment. In our study, SUC levels were rather high in all  
466 treatments particularly in the third year (Chantuma et al., 2006). Thus, another possible explanation  
467 for the absence of response of SUC to fertilization is that sucrose was not limiting for rubber  
468 production in our experimental conditions. This would explain the high yields obtained with the  
469 control treatment right from the onset of the experiment. This would also explain the increase in  
470 SUC in the third year in all treatments and the decline in the effect of stimulation on SUC over  
471 time (-38% in 2014-2015 with  $p < 0.0001$ , -18% in 2015-2016 with  $p = 0.001$  and -12.3% in 2016-  
472 2017 with  $p > 0.05$ ).

473 Our analysis demonstrated that fertilization had a positive impact on latex metabolism by 1)  
474 increasing inorganic phosphorus (Pi) content and hence the energy available to regenerate rubber  
475 particles, 2) supporting the supply of sucrose (SUC) in the latex despite the increased output of  
476 latex. These results suggest that the effect of fertilization on yield was directly related to the effect  
477 of fertilization on latex metabolism, likely through an effect on the enzymatic activity of the latex  
478 cells. Our analysis showed that SUC and Pi were the LD parameters the most correlated with yield  
479 and that they are good predictors of the latex yield in response to fertilization.

480 Next, we presented the results on the effect of the treatments on latex and rubber properties. As  
481 we observed significant effects on LD indicators, we could wonder whether these properties, which  
482 are linked to the quality of latex and rubber, were also affected by the experimental treatments.

483 *Table 4 and 5 to be inserted within this sub-section*

#### 484 **3.4. Effect of stimulation and fertilization on latex and rubber properties.**

485 We quantified six indicators of the technological properties of latex and rubber: the dry rubber  
486 content (DRC) and the mechanical stability (MST) of the fresh latex; the initial plasticity ( $P_0$ ), the  
487 plasticity retention index (PRI) and its component  $P_{30}$ , and the ash content of rubber processed as  
488 unsmoked sheet (USS) (Table 6). Simultaneously, we analysed the mineral content (N, P, K, Ca,  
489 Mg) of fresh latex and dry rubber (Table 7).

490 Over the three-year period, we observed a decrease in MST (from 61 to 50 s.), PRI (from 104 to  
491 91) and rubber USS ash content (from 0.33 to 0.27%) and an increase in  $P_0$  (from 34 to 42). We  
492 found no significant effects of fertilization on the technological properties of the latex and USS  
493 rubber. Stimulation had a significant negative effect on DRC ( $p=0.001$ , ST/NS=-3.50%) and a  
494 significant positive effect on PRI ( $p=0.004$ , ST/NS=+3.10%) in the third year only. Although not  
495 significant, stimulation and fertilization tended to destabilize the latex in the two first cropping  
496 years (-5.9% and -12.4% MST with stimulation; -12.6% and -15.6% MST for T4 treatment).

497 Over the three-year period, Mg and N contents in both fresh latex and USS rubber tended to  
498 increase whereas P, K and Ca contents decreased. The only significant effect of fertilization on  
499 mineral contents was on the N content of fresh latex in the third year (+6.3% on average,  $p=0.046$ ).

500 The significance of this difference was not conserved in USS rubber, even though USS rubber  
501 from T4 contained 4.5% more nitrogen than USS rubber from T1. The P content of latex also  
502 tended to increase with fertilization during the third cropping year, but the effect was not  
503 statistically significant (T4/T1 +13.9%,  $p=0.122$ ). Several effects of stimulation on mineral  
504 contents were observed. N, P, K and Mg contents of USS rubber from trees growing in the  
505 stimulated plot were higher, and this effect became significant in the third cropping year.  
506 Logically, ash content of USS rubber from stimulated plots was up to 5% higher than that of non-  
507 stimulated ones (Table 6).

508 To our knowledge, this study is the first to provide a full set of data on latex and rubber properties,  
509 including mineral contents and several indicators of technological properties such as MST or PRI,  
510 in response to stimulation and fertilization treatments. The results revealed no significant effects

511 of fertilization on the technological properties of either fresh latex or USS rubber. However,  
512 although not significant, the reduced stability of the latex in the fertilized treatments compared to  
513 controls observed in the first two years is in accordance with the findings of Collier and Lowe  
514 (1969). The only significant effect of fertilization on latex mineral content and properties, an  
515 increase in N content, was observed the third year. Although not significant, latex P content  
516 showed the same tendency. These results are in agreement with data on rubber yield and latex  
517 metabolism and support the conclusion that fertilization significantly modified the latex properties  
518 three years after the beginning of the experiment. Longer-run observations will be necessary to  
519 confirm this conclusion.

520 Significant effects of stimulation on latex properties also appeared in the third year. Increased  
521 mineral contents (except Ca) with stimulation are consistent with reports in the literature (D'Auzac  
522 et al., 1989). It is noteworthy that stimulation affected the mineral contents of USS rubber and not  
523 those of fresh latex. This can be attributed to a concomitant decrease in dry rubber content (DRC),  
524 which is a well-documented effect of stimulation (Coupé et Chrestin, 1989). This phenomenon  
525 was particularly clear in the October measurements, which were preceded by four applications of  
526 stimulant (data not shown). USS rubber is indeed the dry matter of latex from which the  
527 hydrophilic matter is removed during USS processing (from 8% to 10% of total dry matter). In  
528 fact, our measurements showed that significant amounts of minerals were lost during this process.  
529 Vaysse et al. (2017) calculated that about 33% of the nitrogen contained in latex was lost during  
530 USS processing. Losses of K (ca. 90% of initial content in latex), Mg (ca. 80%) and P (ca. 60%)  
531 were even greater. Only Ca content was preserved. This finding highlights the complex  
532 relationships between the mineral composition of fresh latex and dry rubber, and their possible  
533 effects on rubber quality. However, in our study, the observed differences in the mineral content  
534 of USS rubber had no direct effect on the technological properties of USS rubber. In particular,  
535 the increase in ash content of USS rubber induced by stimulation led to a content that was less than  
536 half the maximum accepted by the Technically Specified Rubber standard (0.6% for TSR5 grade,  
537 ISO 2000:2003, TSR standard is used as reference as no ash content standard is available for sheet  
538 rubber). Similarly, stimulation had significant effects on P<sub>0</sub> and PRI but technologically speaking,  
539 the averaged differences (less than 2 points "P<sub>0</sub>" and less than 3 points "PRI" respectively, see  
540 supplementary Table 4) are not significant (Bateman and Sekhar, 1966).

541 Finally, the absence of an effect of both fertilization and stimulation treatments on the  
542 technological properties of NR is a positive result. Indeed, it proved that, while having a positive  
543 effect on production, none of the treatment had a detrimental effect on quality.

544 *Table 6 and 7 to be inserted here*

#### 545 **4. Conclusion and perspectives**

546 Our study is the first experimental work to investigate the effects of increased doses of mineral  
547 fertilizers combined with stimulation of trees on the yield, latex metabolism and technological  
548 properties of latex and dry rubber in a rubber plantation in Thailand. Our results show a positive  
549 and significant effect of fertilization on yield three years after the beginning of the experiment.  
550 The effect of fertilization was similar with and without ethylene stimulation. The hypothesis that  
551 the effect of fertilization on latex yield would only be higher under more intensive tapping was  
552 thus not confirmed. In practice, the effect of fertilization and stimulation were additive. We also  
553 demonstrate that the effect of fertilization on yield was linked to a direct effect on latex metabolism  
554 mainly through an increase in inorganic phosphorus content (Pi). We observed no significant effect  
555 of the fertilizer treatments on latex and rubber properties. Therefore, increasing latex yield by  
556 fertilization combined with intensive tapping did not have any identifiable risks on the  
557 technological properties of rubber. Thus, our study suggest that fertilization can be an efficient  
558 driver of the intensification of rubber plantation. In order to validate this result on the long-term,  
559 our study is continuing. In addition to direct effects on yield and the latex regeneration processes,  
560 we are analysing the effect of fertilization on the growth and the nutritional status of the trees. We  
561 are also collecting data in order to quantify the actual mineral balance of the different fertilization  
562 treatments and to assess their respective environmental impacts through nutrient leaching or N<sub>2</sub>O  
563 emissions. Similarly, we are studying the feasibility of recycling the water used for USS processing  
564 which contains large amounts of minerals. Finally, the profitability of the fertilization practices  
565 must be assessed with respect to the expected effect on yield and the price of natural rubber.  
566 Altogether, these new data will contribute to a multi-criteria evaluation of fertilization practices in  
567 rubber plantation that can help farmers to decide whether or not using fertilizers in their rubber  
568 plantations.

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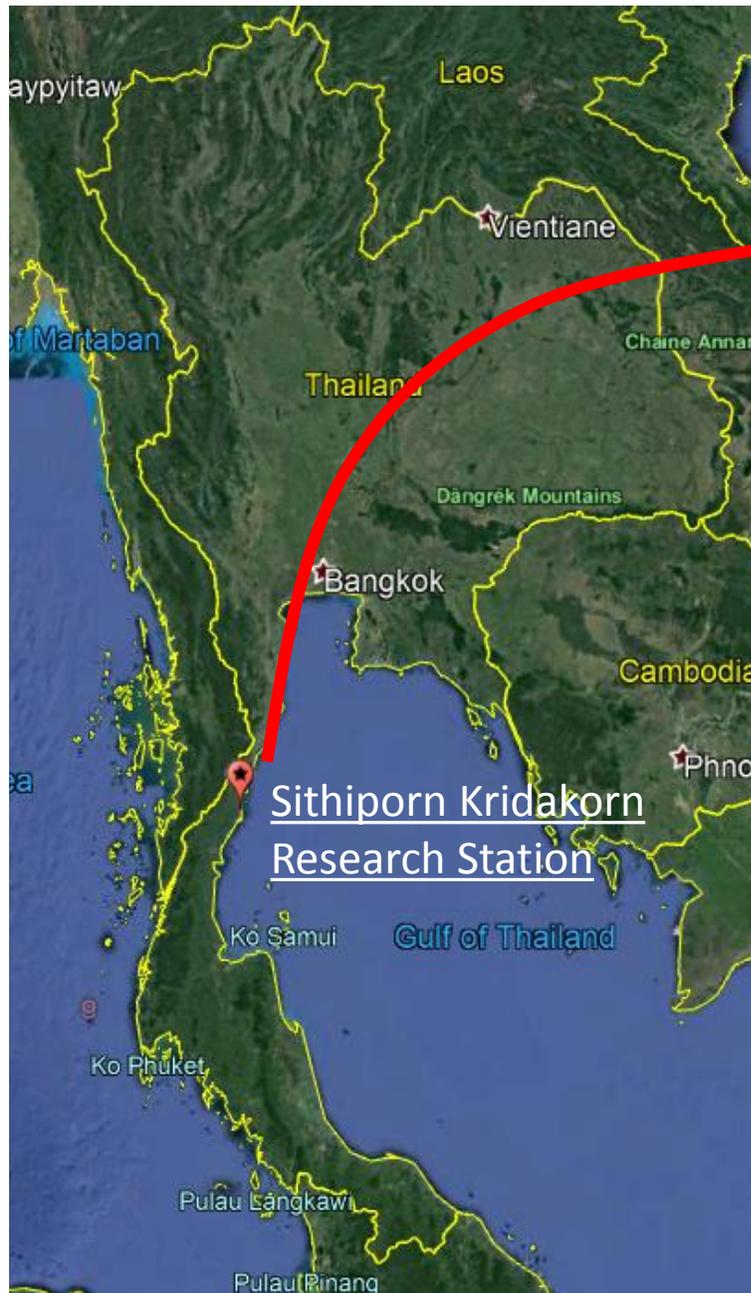
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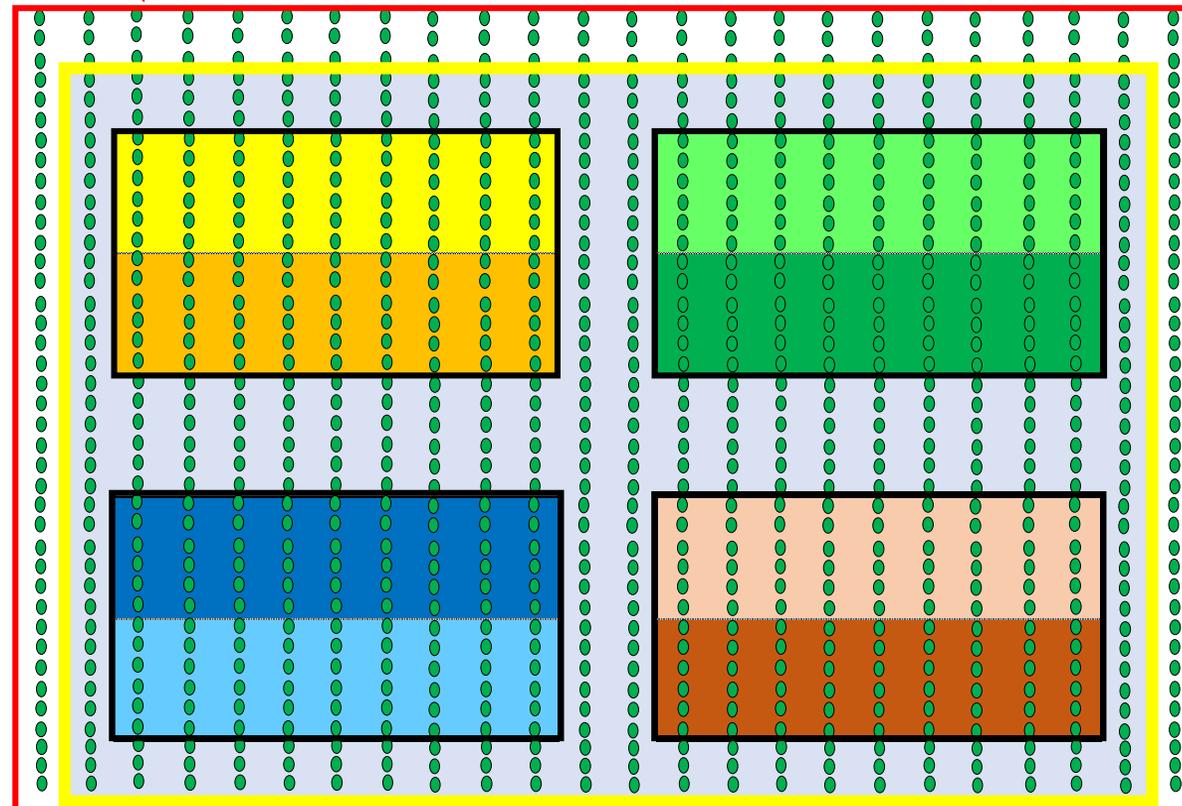
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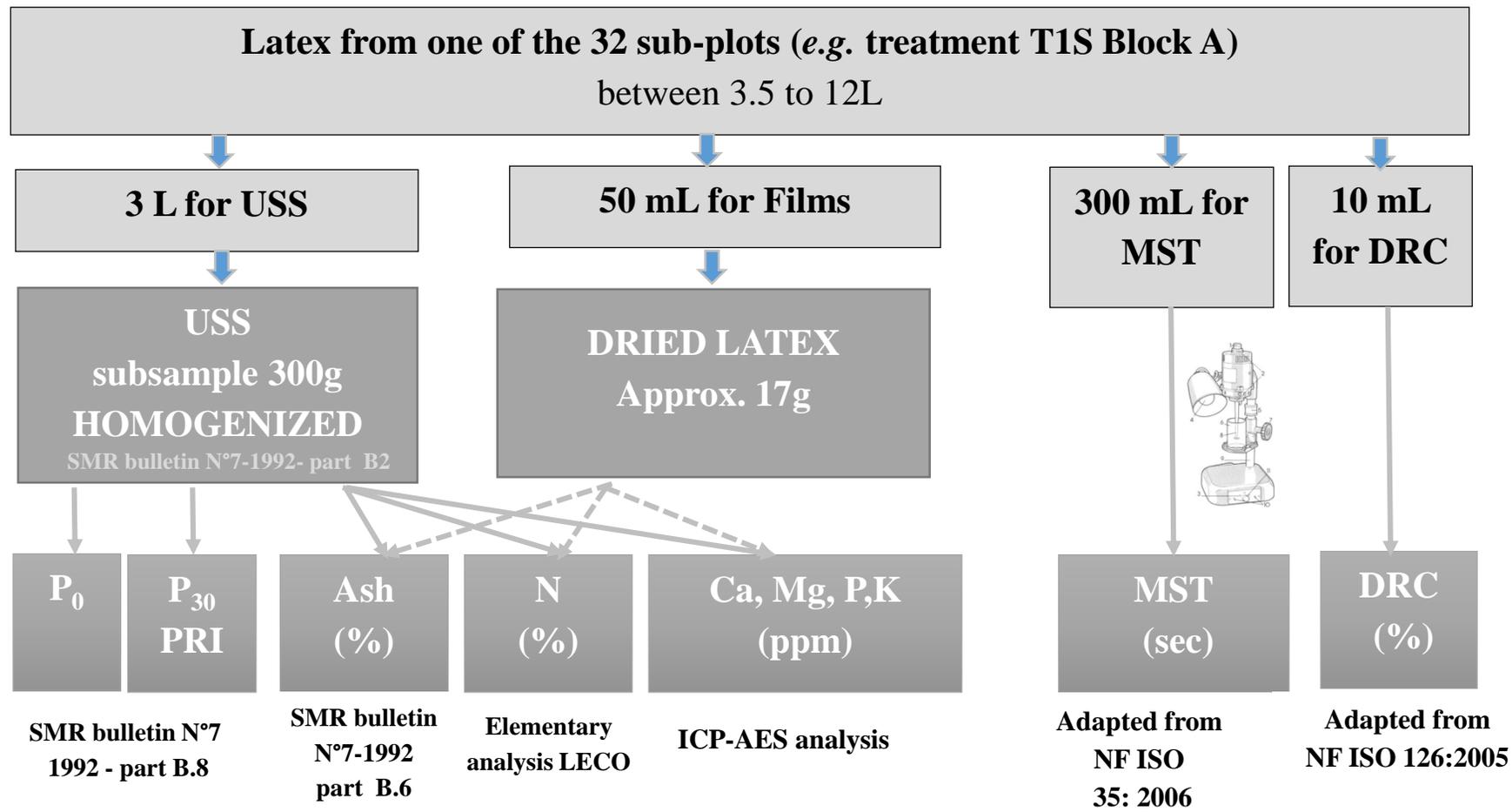
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781



	Fertilization	Stimulation
	T1	NS
	T1	ST
	T2	NS
	T2	ST
	T3	NS
	T3	ST
	T4	NS
	T4	ST





**Table 1.** Some features of the three tapping seasons: cumulative rainfall and rainy days, and mean temperature compared to the 2000-2010 statistics; number of tapping days, average number of tapped trees and trees affected by tapping panel dryness per subplot (n=16). Numbers in brackets are standard errors of the mean.

Year	Rainfall (mm)	Rainy days (n°)	Temperature (°C)	Tapped trees (n°)	Tapping days (n°)	TPD trees (n°)
Reference period (2000-2010)	1985 (383)	106 (11)	26.6 (1.3)	NA	NA	NA
2014-2015	1598	112	27.3	31 (2.7)	106	0
2015-2016	1452	109	27.4	36 (2.7)	115	0
2016-2017	2428	129	26.7	38 (3.1)	106	1 (1.5)

**Table 2.** Details of fertilization treatments

Treatment code	NPK Fertilization		Comments
	Early rainy season	Late rainy season	
T1	0	0	Control
T2	500g/tree 15/9/20 NPK	0	Eq. to 75/45/100 g.tree <sup>-1</sup> .y <sup>-1</sup>
T3	500g/tree 21/7/14 NPK	500g/tree 15/9/20 NPK	Eq. to 180/80/170 g.tree <sup>-1</sup> .y <sup>-1</sup>
T4	850g/tree 21/7/14 NPK	850g/tree 15/9/20 NPK	Eq. to 306/136/289 g.tree <sup>-1</sup> .y <sup>-1</sup>

**Table 3.** Average annual and cumulative dry rubber yield (g.tree<sup>-1</sup>), and average trunk girth (cm) at the end of the third year in each treatment (fertilization x stimulation). Letters in brackets show significant differences among the calculated means (Tukey’s test, p<0.05). The rows “Stimulation effect”, “Fertilization effect”, “Block effect” and “Fert.xStim effect” give the results of the ANOVA of the main treatment, sub-treatment, block and interaction effect. p-values in bold are statistically significant effects. Percentages represent the ratio of the yield of a given treatment to the control (ST to NS for stimulation treatment, T2, T3 or T4 to T1 for fertilization treatment).

Treatment	Rubber yield 2014-15 (g.tree <sup>-1</sup> .year <sup>-1</sup> )	Rubber yield 2015-16 (g.tree <sup>-1</sup> .year <sup>-1</sup> )	Rubber yield 2016-17 (g.tree <sup>-1</sup> .year <sup>-1</sup> )	Cumulative Rubber yield 2014-17 (g.tree <sup>-1</sup> )	Trunk girth April 2017 (cm)
All means	5794	7238	7239	20272	53.5
Stimulation effect	<b>p&lt;0.0001</b>	<b>p&lt;0.0001</b>	<b>p&lt;0.0001</b>	<b>p&lt;0.0001</b>	p=0.661
NS	5447 (b)	6830 (b)	6573 (b)	18850 (b)	53.6
ST	6142 (a)	7647 (a)	7905 (a)	21693 (a)	53.3
ST/NS	<b>+13%</b>	<b>+12%</b>	<b>+20%</b>	<b>+15%</b>	0%
Fertilization effect	p=0.131	p=0.297	<b>p=0.023</b>	p=0.071	p=0.868
T1	5552	6973	6731 (b)	19257	53.3
T2	5779	7239	7240 (ab)	20258	53.4
T3	5957	7369	7394 (ab)	20720	53.3
T4	5890	7372	7590 (a)	20851	53.9
T2/T1	+4%	+4%	+8%	+5%	0%
T3/T1	+7%	+6%	+10%	+8%	0%
T4/T1	+6%	+6%	<b>+13%</b>	+8%	+1%
Block effect	p=0.234	p=0.821	p=0.912	p=0.988	p=0.458
Fert x Stim effect	p=0.934	p=0.903	p=0.788	p=0.948	p=0.447

**Table 4.** Average annual values of sucrose, inorganic phosphorus (Pi) and thiol contents of the latex in each treatment (fertilization x stimulation). Letters in brackets show significant differences among the calculated means (Tukey’s test, p<0.05). The rows “Stimulation effect”, “Fertilization effect”, “Block effect” and “Fert.xStim effect” give the results of ANOVA of the main treatment, sub-treatment, block and interaction effect. p-values in bold are statistically significant effects. Percentages represent the ratio of the yield of a given treatment to the control (ST to NS for stimulation treatment, T2, T3 or T4 to T1 for fertilization treatment).

Treatment	Sucrose content (mM)			Pi content (mM)			Thiol contents (mM)		
	2014-15	2015-16	2016-17	2014-15	2015-16	2016-17	2014-15	2015-16	2016-17
All mean	9.7	9.6	13.3	14.1	16.4	12.0	0.21	0.36	0.21
Stimulation effect	<b>p&lt;0.0001</b>	<b>P=0.001</b>	p=0.066	p=0.070	<b>p=0.008</b>	<b>p&lt;0.0001</b>	<b>p&lt;0.0001</b>	p=0.738	p=0.609
NS	11.9 (a)	10.5 (a)	14.2	13.5	15.5 (b)	10.7 (b)	0.24 (a)	0.37	0.21
ST	7.4 (b)	8.6 (b)	12.4	14.7	17.3 (a)	13.3 (a)	0.18 (b)	0.36	0.21
ST/NS	<b>-38%</b>	<b>-18%</b>	-12.3%	<b>9%</b>	<b>11%</b>	<b>24.7%</b>	<b>-24%</b>	-1%	-2.5%
Fertilization effect	p=0.253	p=0.182	p=0.543	p=0.929	<b>P=0.008</b>	<b>p&lt;0.0001</b>	p=0.350	<b>p=0.018</b>	p=0.187
T1	8.9	10.3	14.5	13.8	15.2 (b)	10.3 (b)	0.21	0.35 (b)	0.20
T2	9.3	9.5	12.6	14.2	15.3 (b)	11.0 (b)	0.20	0.39 (a)	0.22
T3	9.9	9.6	13.0	14.3	17.8 (a)	13.2 (a)	0.22	0.37 (ab)	0.21
T4	10.6	9.0	13.0	14.0	17.4 (ab)	13.7 (a)	0.23	0.35 (b)	0.23
T2/T1	4%	-7%	-13%	3%	0%	7.1%	0%	<b>13%</b>	12%
T3/T1	10%	-7%	-10%	4%	<b>17%</b>	<b>29%</b>	5%	6%	6%
T4/T1	18%	-12%	-10%	1%	15%	<b>33%</b>	9%	2%	16%
Block effect	p=0.614	p=0.789	p=0.983	p=0.285	p=0.595	p=0.628	p=0.692	p=0.751	p=0.803
Fert.xStim. effect	p=0.679	p=0.203	p=0.871	p=0.765	p=0.657	<b>p=0.025</b>	p=0.521	p=0.777	p=0.279

**Table 5.** Parameters of the multiple linear regressions between latex yield (in g.tree<sup>-1</sup>.year<sup>-1</sup>) and the three biochemical indicators of the latex diagnosis (sucrose content (SUC), thiol contents (THI) and inorganic phosphorus content (Pi)) for each year. p-values in bold highlight significant effects of the model or variables.

	2014-15	2015-16	2016-17
p value model	<b>0.016</b>	<b>0.004</b>	<b>&lt; 0.0001</b>
R <sup>2</sup> model	0.304	0.378	0.595
p value SUC	0.534	<b>0.006</b>	<b>0.001</b>
p value RSH	0.223	0.416	0.180
p value Pi	0.775	0.052	<b>&lt; 0.0001</b>
Coefficient norm. SUC	-0.187	-0.484	-0.462
Coefficient norm. RSH	-0.368	0.134	-0.169
Coefficient norm. Pi	0.048	0.321	0.617

**Table 6.** Technological properties of fresh latex and USS rubber. Main results of the ANOVA. Columns “Stimulation effect”, “Fertilization effect”, “Block effect” and “Fert.xStim effect” give the results of ANOVA of the main treatment, sub-treatment, block and interaction effect. p-values in bold are statistically significant. Percentages represent the ratio of the yield of a given treatment to the control (ST to NS for stimulation treatment, T2, T3 or T4 to T1 for fertilization treatment). Data per treatment are presented in Supplementary Tables S1 to S4

Treatment	Year	All mean	Stimulation effect (p-value)	ST/NS	Fertilization effect (p-value)	T2/T1	T3/T1	T4/T1	Block effect (p-value)	Fert.xStim. effect (p-value)
MST Latex (sec)	2014-15	61	0.369	-5.9%	0.303	1.6%	-10.0%	-12.6%	0.645	0.637
	2015-16	58	0.059	-12.4%	0.322	-4.4%	-1.4%	-15.6%	0.535	0.889
	2016-17	50	0.167	3.2%	0.568	3.7%	2.9%	5.7%	0.089	0.301
DRC latex (%)	2014-15	33.3	0.662	-0.9%	0.730	-1.1%	-2.9%	-2.3%	0.581	0.992
	2015-16	32.0	0.669	-1.0%	0.877	-2.6%	-1.2%	-0.5%	0.932	0.806
	2016-17	37.9	<b>0.001</b>	-3.5%	0.346	0.4%	-1.0%	-2.1%	<b>0.035</b>	0.479
P <sub>0</sub> USS	2014-15	34	0.257	1.6%	0.925	1.3%	0.7%	0.7%	0.759	0.839
	2015-16	38	0.429	-1.0%	0.398	-0.1%	-2.7%	-1.8%	<b>0.026</b>	0.489
	2016-17	42	<b>0.047</b>	-2.7%	0.268	-1.6%	-2.9%	-3.4%	<b>0.020</b>	0.300
P <sub>30</sub> USS	2014-15	36	<b>0.023</b>	2.5%	0.002	3.8%	6.0%	5.8%	<b>0.002</b>	0.396
	2015-16	37	0.814	-0.2%	0.126	2.0%	-0.9%	-1.3%	<b>0.009</b>	0.560
	2016-17	38	0.859	0.3%	0.849	-0.3%	-1.8%	-1.6%	0.193	0.973
PRI USS	2014-15	104	0.545	0.8%	0.050	2.4%	5.1%	5.1%	0.106	0.787
	2015-16	98	0.349	0.9%	0.350	2.0%	1.9%	0.6%	0.118	0.096
	2016-17	91	<b>0.004</b>	3.1%	0.664	1.4%	1.2%	1.7%	0.140	0.086
Ash USS (%)	2014-15	0.33	0.347	-2.4%	<b>0.016</b>	-0.4%	-5.1%	-10.9%	<b>&lt;0.0001</b>	0.551
	2015-16	0.27	0.077	5.1%	0.948	-1.1%	1.1%	-0.2%	0.840	0.909
	2016-17	0.27	0.034	5.7%	0.346	1.4%	5.5%	5.0%	0.484	0.452

**Table 7. Nutrient contents of fresh latex and USS Rubber.** Main results of the ANOVA test. See table 6 for details. Data per treatment are given in Supplementary Tables S1 to S4.

Treatment	Year	All means	Stimulation effect (p-value)	S/NS	Fertilization effect (p-value)	T2/T1	T3/T1	T4/T1	Block effect (p-value)	Fert.xStim. effect (p-value)
<b>N latex (ppm)</b>	<b>2014-15</b>	2251	0.142	5.00%	0.754	4.30%	1.10%	4.00%	0.110	0.890
	<b>2015-16</b>	2483	0.469	1.10%	0.269	-3.40%	-0.40%	0.20%	0.892	0.475
	<b>2016-17</b>	2649	0.699	0.70%	<b>0.046</b>	5.10%	6.30%	7.50%	0.751	0.715
<b>P latex (ppm)</b>	<b>2014-15</b>	633	<b>0.049</b>	4.90%	0.838	-1.80%	1.30%	-0.60%	<b>0.042</b>	0.817
	<b>2015-16</b>	489	0.221	4.90%	0.592	-4.40%	3.00%	0.50%	0.187	0.676
	<b>2016-17</b>	580	0.057	8.10%	0.122	9.00%	11.80%	13.90%	0.951	0.251
<b>K latex (ppm)</b>	<b>2014-15</b>	1716	0.732	-0.80%	0.877	-1.90%	-2.50%	-1.30%	0.964	0.641
	<b>2015-16</b>	1528	0.676	-1.20%	0.127	-2.80%	6.20%	4.50%	0.255	0.841
	<b>2016-17</b>	1483	0.570	3.40%	0.745	4.10%	7.70%	7.90%	0.453	0.458
<b>Ca latex (ppm)</b>	<b>2014-15</b>	20	0.902	-1.10%	0.802	-11.00%	-4.80%	-8.90%	0.800	0.119
	<b>2015-16</b>	11	0.733	2.90%	0.863	-9.30%	-6.00%	-3.30%	0.848	0.819
	<b>2016-17</b>	12	0.865	-1.00%	0.448	3.00%	-0.90%	-9.10%	<b>&lt;0.0001</b>	0.401
<b>Mg latex (ppm)</b>	<b>2014-15</b>	325	<b>0.040</b>	11.00%	0.191	-13.20%	-4.20%	10.10%	<b>0.013</b>	0.905
	<b>2015-16</b>	406	0.384	6.80%	0.972	3.20%	4.70%	1.20%	0.824	0.985
	<b>2016-17</b>	553	0.121	8.80%	0.363	6.50%	-6.00%	-4.40%	<b>0.023</b>	0.631
<b>N USS (ppm)</b>	<b>2014-15</b>	4597	<b>0.025</b>	8.70%	0.079	-0.80%	-1.90%	10.20%	0.280	0.0656
	<b>2015-16</b>	5148	0.244	2.80%	0.984	0.10%	1.10%	0.70%	0.946	0.953
	<b>2016-17</b>	4598	<b>0.005</b>	5.00%	0.272	1.20%	2.70%	4.50%	0.491	0.969
<b>P USS (ppm)</b>	<b>2014-15</b>	692	0.083	4.80%	0.522	3.10%	5.20%	0.90%	0.970	0.939
	<b>2015-16</b>	625	0.099	5.20%	0.876	1.30%	3.40%	2.50%	0.493	0.524
	<b>2016-17</b>	610	<b>0.004</b>	7.40%	0.133	0.50%	4.50%	7.20%	0.279	0.574
<b>K USS (ppm)</b>	<b>2014-15</b>	682	0.471	3.60%	0.378	9.70%	7.70%	12.80%	<b>0.013</b>	0.283
	<b>2015-16</b>	423	0.797	1.00%	0.240	4.60%	0.20%	10.60%	0.110	0.617
	<b>2016-17</b>	455	<b>0.010</b>	8.90%	0.084	-2.10%	3.00%	9.30%	<b>&lt;0.0001</b>	0.290
<b>Ca USS (ppm)</b>	<b>2014-15</b>	30	0.380	10.50%	0.620	-12.70%	2.40%	-6.40%	0.174	0.129
	<b>2015-16</b>	38	0.574	-4.70%	0.992	-4.50%	-1.50%	-4.30%	<b>&lt;0.0001</b>	0.449
	<b>2016-17</b>	32	0.318	12.60%	0.985	-3.40%	-6.30%	-2.70%	0.590	0.215
<b>Mg USS (ppm)</b>	<b>2014-15</b>	196	0.105	7.90%	0.977	1.90%	2.60%	0.60%	<b>0.026</b>	0.529
	<b>2015-16</b>	206	0.286	3.70%	0.608	-0.90%	-4.20%	2.10%	0.172	0.813
	<b>2016-17</b>	232	<b>0.004</b>	10.70%	0.302	-2.20%	-1.90%	6.00%	<b>0.021</b>	0.346